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RESEARCH ARTICLE | *Neural Control*

Effects of inspiratory muscle-training intensity on cardiovascular control in amateur cyclists

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Martins de Abreu R, Porta A, Rehder-Santos P, Cairo B, Donisete da Silva C, De Favari Signini É, Sakaguchi CA, Catai AM. Effects of inspiratory muscle-training intensity on cardiovascular control in amateur cyclists. *Am J Physiol Regul Integr Comp Physiol* 317: R891–R902, 2019. First published October 9, 2019; doi:10.1152/ajpregu.00167.2019.—Chronic effects of inspiratory muscle training (IMT) on autonomic function and baroreflex regulation are poorly studied. This study aims at evaluating chronic effects of different IMT intensities on cardiovascular control in amateur cyclists. A longitudinal, randomized, controlled blind study was performed on 30 recreational male cyclists undergoing IMT for 11 wk. Participants were randomly allocated into sham-trained group (SHAM, $n = 9$), trained group at 60% of the maximal inspiratory pressure (MIP60, $n = 10$), and trained group at critical inspiratory pressure (CIP, $n = 11$). Electrocardiogram, finger arterial pressure, and respiratory movements were recorded before (PRE) and after (POST) training at rest in supine position (REST) and during active standing (STAND). From the beat-to-beat series of heart period (HP) and systolic arterial pressure (SAP), we computed time domain markers, frequency domain indexes in the low frequency (0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) bands, an entropy-based complexity index (CI), and baroreflex markers estimated from spontaneous HP-SAP sequences. Compared with SHAM, the positive effect of MIP60 over the HP series led to the HF power increase during REST (PRE: 521.2 ± 447.5 ms^2; POST: 1,161 ± 878.9 ms^2) and the CI rise during STAND (PRE: 0.82 ± 0.18; POST: 0.97 ± 0.13). Conversely, the negative effect of CIP took the form of the decreased HP mean during STAND (PRE: 791 ± 71 ms; POST: 737 ± 95 ms). No effect of IMT was visible over SAP and baroreflex markers. These findings suggest that moderate-intensity IMT might be beneficial when the goal is to limit cardiac sympathetic hyperactivity at REST and/or in response to STAND.

arterial pressure; autonomic nervous system; baroreflex; breathing exercise; complexity; heart rate variability; sport medicine

INTRODUCTION

Inspiratory muscle training (IMT) is considered a supplementary tool to promote the performance of athletes via the reduction of perceived breathlessness and attenuation of peripheral muscle fatigue, which are the main limitations in physical exercise practice (21, 25). Moreover, since IMT can evoke post-training modifications of respiratory patterns and these changes affect autonomic regulation, IMT has been investigated as a method to improve vagal control directed to the sinus node and lower arterial blood pressure (15, 24). Autonomic cardiovascular control is frequently studied noninvasively through the analysis of spontaneous fluctuations of heart period (HP) and systolic arterial pressure (SAP), referred to as HP and SAP variability (30a). HP variability analysis was found to be useful to prescribe exercise intensity, monitoring modifications of cardiac autonomic control during training and measuring the chronic effect of exercise training programs (2, 27, 65). For example, a reduction of the magnitude of HP changes and, more specifically, a diminution of the respiratory sinus arrhythmia (RSA) were associated with fatigue and training overload (18). The assessment of the SAP variability, especially in the low frequency (LF, from 0.04 to 0.15 Hz) band, in connection of the characterization of cardiac baroreflex complements the analysis of HP variability (36, 37). Although it is well known that aerobic exercise and endurance training shifts sympathovagal balance toward vagal enhancement and sympathetic inhibition in athletes (1, 56), the effects of IMT on cardiovascular control are still unknown in this population (15).

In patients with cardiovascular disease, low-intensity IMT, namely at 30% of maximal inspiratory pressure (MIP), seems to be valuable because it increases RSA, as monitored via the high frequency (HF) power (from 0.15 to 0.5 Hz) of the HP series (15), thus supporting an increase in vagal modulation and a reduction in the sympathetic one. However, the improvement of the cardiac autonomic control in athletes might be limited given their higher basal vagal drive compared with sedentary or pathological individuals, or it might necessitate a higher-intensity IMT to become significant. The application of different IMT intensities over a population featuring a high basal vagal control could help in elucidating the basic mechanisms underlying the IMT effect at sinus node level and the IMT intensity necessary to observe sizable modifications of cardiac autonomic regulation. The effect of a high-intensity IMT on cardiac autonomic regulation is less frequently assessed. In healthy elderly subjects, it was observed that HP

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variability parameters during a session of high-intensity IMT were smaller than those measured during IMT sessions of lighter intensities (2), thus indicating that the relation between the IMT intensity and modifications of the autonomic function is not proportional during IMT session. In addition, data about the chronic effects of IMT on vascular and baroreflex regulations are even more scanty on both healthy and pathological groups.

The aim of this study is to compare the effects of 11 wk of IMT applied at different intensities (i.e., intermediate and maximum magnitudes) on the cardiac autonomic control of amateur cyclists through HP variability analyses at rest in supine position (REST) and during active standing (STAND). We hypothesize that IMT of moderate intensity improves cardiac autonomic function and its response to STAND in amateur cyclists, whereas at highest intensity, these positive effects might not be present and even negative influences might appear. Although the assessment of the impact of IMT on cardiac autonomic function is the primary end point of this study, secondary end points are the evaluation of the influence of IMT on vascular regulation via SAP variability analysis and the characterization of cardiac baroreflex via spontaneous HP-SAP sequence technique.

METHODS

Study design and ethical procedures. This is a longitudinal, randomized, controlled blind study in agreement with the recommendations given in Schulz et al. (52). Randomization, masking procedures, and level of blinding were described in Rehder-Santos et al. (48). This study was conducted on 30 male recreational cyclists (age: from 20 to 40 yr) who were randomly allocated into 3 groups according to IMT type: *1*) a sham group (SHAM, $n = 9$) performing an IMT of very limited intensity; *2*) a group performing an IMT of moderate intensity at an intermediate fraction of MIP set at 60% (MIP60, $n = 10$); *3*) a group trained at the critical inspiratory pressure (CIP, $n = 11$) performing an IMT of high intensity. The training protocol was registered in ClinicalTrials.gov (NCT02984189), and the study was approved by the Human Research Ethics Committee of the Federal University of São Carlos (UFSCar) (Protocol: 1.558.731). The study adhered to the principles of the Declaration of Helsinki for research studies involving humans. All participants provided written informed consent to participate in the study.

Eligibility criteria, exclusion rules, and sample size assessment. The size of the population was suggested by Ferreira et al. (24), who found a significant IMT effect on cardiac vagal control in patients with hypertension with a group composed of <10 subjects. The participants were recruited through public calls advertised at UFSCar, local broadcast media, and social networks. Subjects were eligible if they were apparently healthy and practiced cycling for at least 6 uninterrupted months and at least 150 min per week. In addition, the participants were enrolled if they did not present abnormalities in the cardiovascular and respiratory systems. We excluded cyclists with alterations in the electrocardiogram (ECG) at REST and during the physical exercise test on bicycle ergometer, subjects who were obese with body mass index larger than 30 kg/m^2, subjects with cardiovascular risk factors, smokers or former smokers with less than 1 yr of interruption, habitual drinkers, subjects who used drugs or medicines that could interfere with cardiovascular control and autonomic function, and subjects who performed any type of IMT during the last 12 mo. Throughout the IMT period, participants were instructed not to alter their lifestyle and type of physical training. Volunteers who did not complete the 3 weekly IMT sessions or the 11 full weeks of IMT, who modified their physical activities, or who started using any supplement or medication during IMT were excluded.

Experimental procedure and protocol. All the experimental procedures were performed at the Cardiovascular Physical Therapy Laboratory, Department of Physical Therapy, UFSCar, São Carlos, Brazil, in a room where temperature was maintained between 21°C and 24°C and relative humidity between 40% and 60%. The tests were always performed in the evening. In addition, participants were advised not to ingest stimulant drinks, avoid heavy meals and strenuous physical exercise, sleep well the night before, and dress in comfortable clothes and shoes. The experimental protocol lasted 13 wk. In the first week, some tests were performed to characterize the participants. Then, IMT started during the second week and continued for 11 wk. The assessment of the cardiovascular control and baroreflex from variability recordings was performed before training (PRE) within the first week and after training (POST) within the thirteenth week.

Characterization of population. During the first, fifth, ninth, and thirteenth weeks, participants underwent anamnesis, conventional 12-lead ECG at REST, clinical treadmill exercise test, cardiopulmonary test to evaluate peak oxygen uptake (peak $\dot{V}O_2$), evaluation of MIP, maximal expiratory pressure (MEP) and respiratory frequency (RF), and incremental respiratory muscle endurance test. Data obtained during the evaluation sessions carried out during the first, fifth, and ninth weeks were utilized to set the initial inspiratory muscle load and readjust it in the remaining IMT period.

Peak $\dot{V}O_2$ was evaluated via an incremental exercise protocol on an electromagnetic braking cyclergometer (CORIVAL V3, Lode BV, The Netherlands) (4). The protocol consisted of a 6-min evaluation at REST and 3 min in free-load warm-up and a gradual increase of load until the exercise was stopped, followed by a 6-min active recovery and 1-min passive recovery. The load increment was calculated for each participant according to the procedure proposed by Wasserman et al. (66). Moreover, the participants were instructed to maintain a rate between 60 and 80 revolutions/min throughout the protocol. The test lasted from 8 to 12 min. The metabolic and ventilatory variables were collected on breath-by-breath basis through a gas analyzer (ULTIMA MedGraphics, St. Paul, MN) and processed through specific software (Breeze Suite 7.1, MedGraphics). The peak $\dot{V}O_2$ was defined as the highest value of $\dot{V}O_2$ obtained in the last 30 s of the incremental exercise (4).

MIP and MEP were evaluated using a digital manovacuometer (MVD-300, Globalmed, Porto Alegre, Brazil). The MIP was determined from the residual volume during maximal inspiratory effort, whereas the MEP was determined from the total lung capacity during maximal expiratory effort. These maneuvers were performed against an occluded airway with a small air passage (2 mm). A maximum of 5 maneuvers was performed, with a 30-s interval between each maneuver (51), and the highest pressure of the 3 measurements with the lowest dispersion was used to define MIP and MEP. Respiratory muscle weakness was detected when MIP and MEP were below 60% of the value predicted according to Neder et al. (34). All the maneuvers were performed by the same investigator.

Different types of IMT. The subjects performed IMT for 1 h, 3 days per week, for 11 wk, using a linear inspiratory loading device (PowerBreathe, Ironman K5, HaB). The protocol was composed of a warm-up phase lasting 5 min, during which each participant performed a constant loading protocol at 50% of their training load, followed by 3 consecutive IMT sessions of 15 min. The second and third IMT sessions were preceded by 1-min recovery. In the case of SHAM, MIP60, and CIP, the inspiratory resistance (expressed in cmH_2O) was set, respectively, to 6 cmH_2O, 60% of MIP, and between 80% and 90% of MIP. The fraction of MIP utilized in the CIP training was optimized according to Rehder-Santos et al. (48) in such a way to allow the athlete to conclude the session without experiencing muscle fatigue. During training, the subjects were instructed to maintain the RF at 12 acts per minute, and this rate was reinforced by a verbal command of the physiotherapist.

Signal acquisition and beat-to-beat variability series extraction. We acquired ECG (lead MC5) via a bioamplifier (BioAmp FE132,

ADInstruments), noninvasive continuous finger arterial pressure (Finometer Pro, Finapres Medical Systems), and respiratory movements through a thoracic belt (Marazza, Monza, Italy). Signals were sampled at 1,000 Hz (Power Laboratory 8/35, ADInstruments). Subjects were initially maintained at REST for 10 min to stabilize the cardiovascular variables. After this period, signals were recorded for 15 min at REST. Then, the subject was asked to change posture, and signals were acquired for an additional 15 min during STAND. The STAND session always followed REST. Throughout the procedure, subjects were instructed to breathe spontaneously and were not allowed to talk. HP was determined on the ECG as the temporal distance between two consecutive R-wave peaks. SAP was detected as the maximum arterial pressure signal within the HP. The delineations of the R-wave and SAP peak were carefully checked to avoid erroneous detections or missed beats. If isolated ectopic beats affected HP and SAP, these measures were linearly interpolated using the closest values unaffected by ectopies. Since we were interested in short-term cardiovascular control mechanisms, analyses were carried out over HP and SAP sequences of 256 consecutive values (30a). Sequences were selected in a random position within REST and STAND periods. Attention was paid to avoid the selection of the first three minutes of STAND. Over the same periods, RF was extracted from the respiratory signal.

Time and frequency domain indexes. We computed both time and frequency domain indexes over HP and SAP series. The time domain analysis comprised the computation of HP and SAP mean and variance, indicated respectively as μ_{HP}, μ_{SAP}, σ^2_{HP}, and σ^2_{SAP} in the following (expressed in ms, mmHg, ms^2, and $mmHg^2$, respectively). Frequency domain analysis was carried out via a univariate parametric power spectral approach fitting the series according to an autoregressive model (36). The coefficients of the autoregressive model were estimated via traditional least squares method solved recursively. The model order was optimized according to the Akaike figure of merit in a range from 8 to 14. The parametric power spectral density was factorized into components, each of them characterized by a central frequency. Since we were interested in estimating RSA, we classified spectral components of HP series whose central frequency dropped in the HF band. The final spectral marker was defined as the sum of the powers of all HF spectral components detected in the HP series. The HF power of HP series was expressed in absolute units (i.e., ms^2) and labeled as HFa_{HP}. This index was taken as a marker of the parasympathetic modulation directed to the heart (40, 30a). The spectral components of HP series with central frequency in the LF band (30a) were calculated as well. The sum of the spectral powers of the LF components was labeled as LFa_{HP} and expressed in absolute units (i.e., ms^2). LFa_{HP} and HFa_{HP} allowed the computation of LFa_{HP}/HFa_{HP} index defined as the ratio of LFa_{HP} to HFa_{HP} power. According to Pagani et al. (36), the LFa_{HP}/HFa_{HP} index was utilized to typify the balance between vagal and sympathetic controls directed to the sinus node. Parametric spectral analysis was performed over SAP series as well. In this case, we classified the spectral components whose central frequency dropped in the LF band (30a). The final spectral marker of the SAP series was defined as the sum of the powers of all LF spectral components of the SAP series. The LF power of SAP series was expressed in absolute units (i.e., $mmHg^2$) and labeled as LFa_{SAP}. This index was taken as a marker of the sympathetic modulation directed to the vessels (36).

Complexity analysis. Assigned a pattern of length L formed by current HP and past L-1 HP values, the complexity of HP series was computed as the conditional entropy quantifying the new information carried by current HP that could not be derived from the past L-1 HP values averaged over all possible L-1-dimensional patterns (46). The more irregular and unpredictable the HP series (i.e., the smaller ability of past HPs to indicate future HP behaviors), the larger the conditional entropy. Among the possible techniques for the practical computation of conditional entropy (46), we followed the approach set in Porta et al. (42) to estimate it and deal with the challenge posed by the limited length of the HP series. The approach exploited a uniform quantization procedure to perform coarse graining of the HP series and decide the similarity of patterns utilized to forecast future HP values. In addition, the selected approach adopted a correction strategy of the bias leading to the progressive reduction of the amount of information while increasing L regardless of the HP time course. The optimal value of CE, obtained as the best compromise between the better ability of longer patterns to predict future behaviors and their more limited reliability when short series are considered, was taken as complexity index (CI). CI was computed over HP series and denoted as CI_{HP}. The larger the CI_{HP}, the higher the complexity of the HP series (42, 47). It was observed that CI_{HP} was smaller in healthy subjects during experimental conditions, leading to the sympathetic activation and vagal withdrawal (45–47).

Cardiac baroreflex assessment. We applied the sequence technique to characterize cardiac baroreflex from spontaneous HP and SAP variability series (7, 37), as implemented in Porta et al. (41, 43). More specifically, we defined as the HP-SAP pattern of baroreflex origin an HP-SAP joint scheme featuring three consecutive and contemporaneous HP and SAP increases or decreases. Therefore, a HP-SAP pattern of baroreflex origin is characterized by same-sign HP and SAP ramps with a delay between them equal to 0 beats. All the detected HP-SAP patterns of baroreflex origin were retained in this analysis regardless of the magnitude of total or partial SAP and HP variations and the strength of the linear association between HP and SAP values (41). The baroreflex sensitivity (BRS) was computed as the mean of the slopes of the regression lines of HP on SAP calculated over all HP-SAP patterns of baroreflex origin. BRS was positive by definition (expressed in ms/mmHg). The percentage of HP-SAP patterns of baroreflex origin with respect to the total amount of HP-SAP schemes (SEQ%) was assessed as well and taken as a measure of the degree of involvement of cardiac baroreflex control. By definition, SEQ% ranged between 0 and 100.

Statistical analysis. One-way analysis of variance, or Kruskal-Wallis one-way analysis of variance on ranks when appropriate, was applied to test the presence of significant differences among the continuous variables reported in Table 1. χ^2 test was applied to aerobic functional classification markers. Two-way repeated measures analysis of variance (Holm-Sidak test for multiple comparisons) was applied to variability indexes to detect the effect of training given the same experimental condition (i.e., REST or STAND) and the response to postural challenge given the training status (i.e., PRE or POST). Paired t test, or Wilcoxon signed rank test if appropriate, was applied to test the difference of absolute POST-PRE variation markers computed during STAND versus those calculated at REST. No formal statistical analysis was carried out between different groups (i.e., SHAM, MIP60, and CIP), and comparison between different groups was qualitative and based on the observation of significances detected within the group by the previously mentioned two-way repeated measures approach. Continuous data are expressed as means ± SD and categorical data as number with the percentage in parentheses. Statistical analysis was carried out using a commercial statistical program (Sigmaplot, v.14.0, Systat Software, Chicago, IL). $P < 0.05$ was always considered statistically significant.

RESULTS

Baseline characteristics and effect of IMT. The flowchart of the study is shown in Fig. 1. A total of 100 recreational male cyclists were screened for eligibility. Only 50 individuals met the eligibility criteria and were randomized into the 3 groups undergoing SHAM ($n = 17$), MIP60 ($n = 18$), and CIP ($n = 15$) training. Eight, eight, and three cyclists were excluded mainly because they did not conclude the training in the SHAM, MIP60, and CIP groups, respectively. Therefore, 9, 10, and 12 subjects concluded the SHAM, MIP60, and CIP training and could undergo the POST session of recording. Unfor-

Table 1. *Baseline characteristics of the SHAM, MIP60, and CIP groups*

Parameter	SHAM (n = 9)	MIP60 (n = 10)	CIP (n = 11)	P
Age, yr	29.56 ± 5.05	32.10 ± 7.05	29.27 ± 6.59	0.551
Height, m	1.79 ± 0.04	1.77 ± 0.06	1.77 ± 0.05	0.663
Weight, kg	74.91 ± 10.91	75.04 ± 7.56	76.05 ± 10	0.957
Body mass index, kg/m^2	23.51 ± 3.91	24.01 ± 1.81	24.22 ± 2.77	0.863
Peak $\dot{V}O_2$, mL·min^{-1}·kg^{-1}	42.03 ± 8.49	47.97 ± 9.06	51.17 ± 11.69	0.141
MIP, cmH_2O	148.22 ± 11.13	158 ± 24.87	146.18 ± 14.81	0.302
MEP, cmH_2O	166.44 ± 31.02	171 ± 29.14	186 ± 37.51	0.416
RF, apm	15.8 ± 3.7	18.3 ± 4.5	17 ± 3.6	0.433
		Aerobic functional classification according to AHA		
Very weak				
Weak	1 (11)			
Regular	2 (22)	2 (20)	2 (18)	>0.05
Good	4 (45)	5 (50)	4 (36)	>0.05
Excellent	2 (22)	3 (30)	5 (46)	>0.05

Continuous data are expressed as means ± SD and categorical data as number (percentage). AHA, American Heart Association; CIP, IMT at critical inspiratory pressure; IMT, inspiratory muscle training; MEP, maximal expiratory pressure; MIP, maximal inspiratory pressure; MIP60, IMT at 60% of MIP; P, type I error probability; RF, spontaneous respiratory frequency expressed in acts per minute (apm); SHAM, sham IMT; V_{O_2}, oxygen consumption.

tunately, in 1 subject belonging to the CIP group, the signals were of poor quality, thus allowing the analysis of the recordings of 9, 10, and 11 subjects in the SHAM, MIP60, and CIP groups, respectively. Baseline characteristics of the SHAM, MIP60, and CIP groups are summarized in Table 1. The SHAM, MIP60, and CIP groups were homogeneous in terms of age, height, weight, body mass index, peak VO_2, MIP, MEP, and aerobic functional classification according to the American Heart Association. According to the individual values of MIP and MEP, the subjects did not show any sign of respiratory weakness or inspiratory muscle fatigue either in PRE or POST or intermediate sessions.

We characterized the effect of IMT on peak V_{O_2}, MEP, and MIP in terms of variations of POST markers with respect to PRE ones. Variations of peak V_{O_2} were similar in SHAM, MIP60, and CIP groups, being respectively 7.75 ± 2.58, 7.76 ± 2.45, and 6.22 ± 1.88 mL·min^{-1}·kg^{-1}. Variations of MEP were not significantly different across the types of IMT, being respectively 12.00 ± 35.46, 26.00 ± 45.95, and 24.78 ± 30.16 cmH_2O in SHAM, MIP60, and CIP groups. The effect of training was evident over MIP in MIP60 and CIP groups (i.e., 44.60 ± 17.42 and 59.45 ± 23.29 cmH_2O) compared with SHAM (i.e., 23.22 ± 14.17 cmH_2O) with the variation of MIP after CIP training significantly larger than those after SHAM training.

Time domain HP variability markers. The grouped vertical bar graphs shown in Fig. 2 report μ_{HP} computed over SHAM (Fig. 2*A*), MIP60 (Fig. 2*B*), and CIP (Fig. 2*C*) groups in PRE (black bars) and POST (white bars) sessions as a function of the experimental condition (i.e., REST and STAND). All the groups responded to the orthostatic challenge by decreasing μ_{HP}, and this result was observed in both PRE and POST sessions. Training increased μ_{HP}, and this effect was visible at REST in SHAM and MIP60 groups. Remarkably, this outcome disappeared during STAND. Conversely, in the CIP group, the effect of training on μ_{HP} was not evident at REST and,

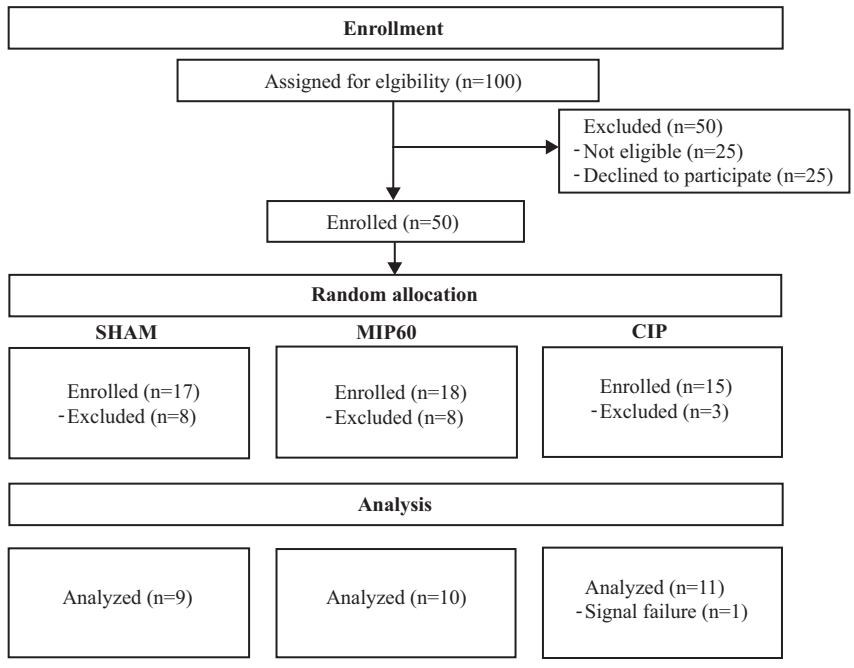

Fig. 1. Flowchart of the study.

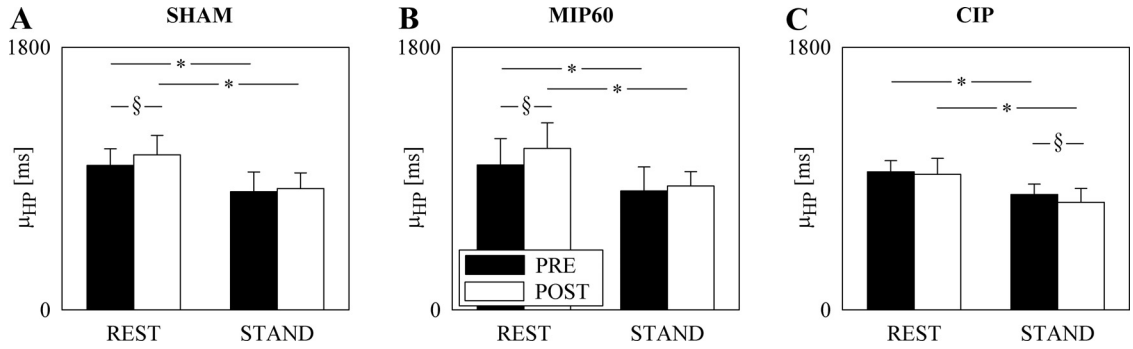

Fig. 2. The grouped vertical bar graphs show heart period mean (μ_{HP}) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). Values are reported as mean plus standard deviation. *Statistically significant difference vs. REST within the same training condition (i.e., PRE or POST) with $P < 0.05$. §Statistically significant difference vs. PRE within the experimental condition (i.e., REST or STAND) with $P < 0.05$.

remarkably, during STAND, μ_{HP} was lower in POST compared with PRE.

Figure 3 has the same structure as Fig. 2, but it shows σ^2_{HP}. This parameter did not change with either training status (i.e., PRE and POST) or experimental condition (i.e., REST and STAND). This conclusion held regardless of the type of training, namely SHAM (Fig. 3*A*), MIP60 (Fig. 3*B*), and CIP (Fig. 3*C*).

Frequency domain HP variability markers. Figure 4 has the same structure as Fig. 2, but it shows the HFa_{HP} power. In the SHAM group, the effect of training and modification of posture were not visible (Fig. 4*A*). In the CIP group, the effect of the postural challenge was evident in both PRE and POST sessions and led to a decrease of the HFa_{HP} power in response to STAND (Fig. 4*C*). The MIP60 group (Fig. 4*B*) exhibited a higher HFa_{HP} power after training at REST, whereas no significant difference between PRE and POST sessions was detected during STAND. Moreover, in the same group, the expected decrease of the HFa_{HP} power in response to STAND was visible only in the POST session.

Figure 5 has the same structure as Fig. 2, but it shows the LFa_{HP}/HFa_{HP} marker. The effect of the orthostatic stimulus was visible in both PRE and POST sessions in the MIP60 and CIP groups and only in POST session in the SHAM group. Conversely, when assigned the experimental condition (i.e., REST or STAND), the LFa_{HP}/HFa_{HP} marker remained unvaried during POST compared with PRE, and this finding held irrespective of the group.

HP variability complexity markers. Figure 6 has the same structure as Fig. 2, but it shows CI_{HP}. The expected decrease of complexity during STAND was evident in both PRE and POST sessions solely in the CIP group (Fig. 6*C*). In both SHAM (Fig. 6*A*) and MIP60 (Fig. 6*B*) groups, CI_{HP} decreased in response to STAND only in the PRE session, whereas STAND did not affect CI_{HP} during POST. Remarkably, in the MIP60 group during STAND, the CI_{HP} was higher during POST compared with PRE, whereas in both SHAM and CIP groups during STAND, the complexity of HP variability was similar both before and after training. At REST, the effect of training was not visible, and this finding was observed irrespective of the group.

Modifications of RF with IMT. Figure 7 has the same structure as Fig. 2, but it shows the RF. In the SHAM (Fig. 7*A*) and CIP (Fig. 7*C*) groups, the RF remained unvaried irrespective of the experimental condition and training status. The MIP60 training (Fig. 7*B*) led to a decrease of the RF at REST, whereas no effect of training was visible during STAND. In the same group, modification of posture did not change the RF regardless of training status.

Analysis of SAP variability and cardiac baroreflex. Table 2 reports time and frequency domain markers of SAP variability (i.e., μ_{SAP}, σ^2_{SAP}, and LFa_{SAP}) as well as the characterization of cardiac baroreflex carried out via BRS and SEQ% indexes. None of the variables changed during POST compared with PRE, and this conclusion held regardless of the group (i.e., SHAM, MIP60, and CIP). Only the effect of the orthostatic

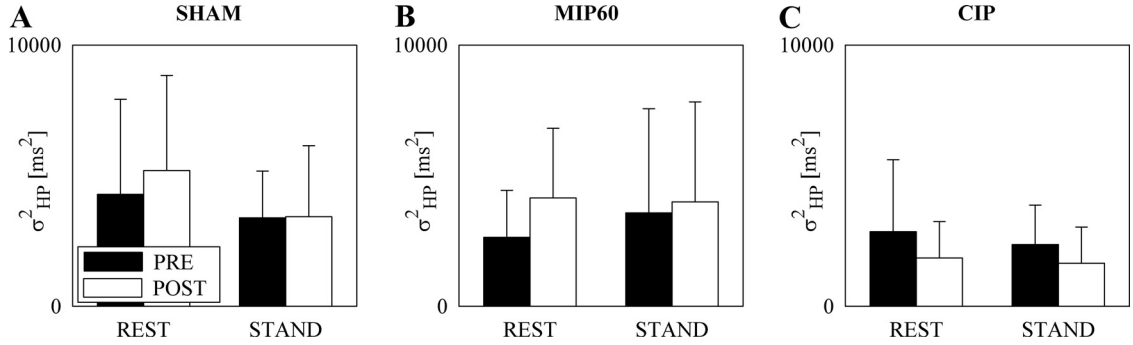

Fig. 3. The grouped vertical bar graphs show heart period variance (σ^2_{HP}) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely, SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). The values are reported as mean plus standard deviation.

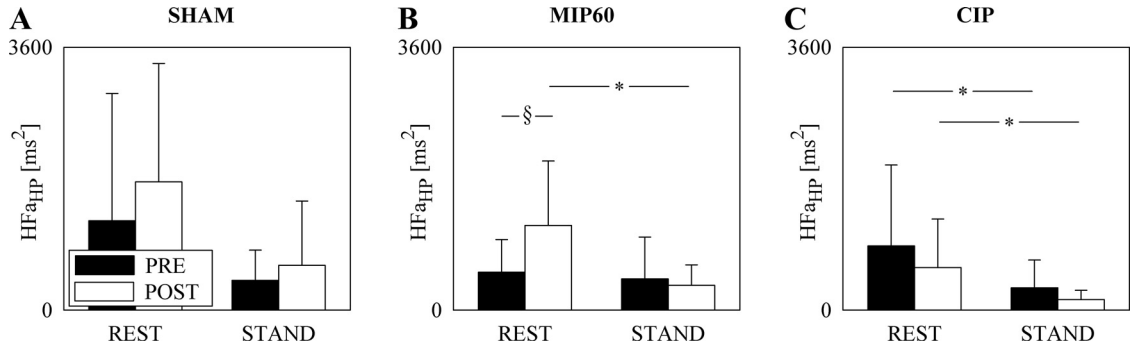

Fig. 4. The grouped vertical bar graphs show high frequency power of heart period series expressed in absolute units (HFa_{HP}) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). The values are reported as mean plus standard deviation. *Statistically significant difference vs. REST within the same training condition (i.e., PRE or POST) with $P < 0.05$. §Statistically significant difference vs. PRE within the experimental condition (i.e., REST or STAND) with $P < 0.05$.

challenge was visible. More specifically, during STAND compared with PRE in the SHAM group, the LFa_{SAP} power increased during both PRE and POST sessions and BRS decreased only in PRE; in the MIP60 group during STAND, σ^2_{SAP}, LFa_{SAP}, and SEQ% rose, whereas BRS declined regardless of the training status, and in the CIP group, LFa_{SAP} power and SEQ% increased, whereas BRS decreased during both PRE and POST sessions. Training status and experimental condition did not affect μ_{SAP}, and this conclusion held regardless of the group.

POST-PRE variations of cardiovascular variability markers. The absolute POST-PRE variation (Δ) of all variability indexes is reported in Table 3. A significant response to STAND was detected in the case of $\Delta\mu_{HP}$, ΔHFa_{HP}, and ΔCI_{HP} in the MIP60 group and in the case of $\Delta\mu_{HP}$ in the CIP group. These STAND-REST differences were mainly the consequence of the bradycardic effect of MIP60 during REST, the tachycardic effect of CIP during STAND, the remarkable increase of HFa_{HP} at REST induced by MIP60 training, and the relevant rise of CI_{HP} during STAND induced by MIP60 training. None of the remaining variation markers were varied in response to STAND, and this result held regardless of IMT type.

DISCUSSION

Although previous studies have reported positive effects of moderate-intensity IMT on athletes' performance (25, 30), to our knowledge, this is the first study that assesses the chronic influences of IMT on cardiovascular control in athletes and how these effects depend on the IMT intensity. The main findings of this study can be summarized as follows: *1*) the MIP60 training induced a bradycardia and a more important RSA at REST as well as a greater cardiac control complexity during STAND; *2*) the effect of SHAM training was more limited because it was able to exclusively evoke a bradycardia at REST; *3*) CIP training did not produce any bradycardia at REST, and, conversely, a tachycardia appeared during STAND; *4*) different from MIP60 training, RSA and cardiac autonomic control complexity remained unvaried in the CIP group; and *5*) regardless of the exercise intensity, IMT did not affect markers of vascular regulation derived from SAP variability and cardiac baroreflex control analyses.

Cardiac autonomic control in athletes. Controversial results are present in the literature about cardiac autonomic control in athletes based on the analysis of HP variability. Some studies found modifications of the HFa_{HP} power in endurance athletes and interpreted this finding as a consequence of a greater vagal modulation (1, 54). Other studies, although confirming resting bradycardia in athletes, did not identify the influence of training on HP variability at REST and during a postural maneuver compared with sedentary population (32). These controversial findings suggest that resting bradycardia in athletes might be more related to modifications of the autonomic tone (i.e., mean

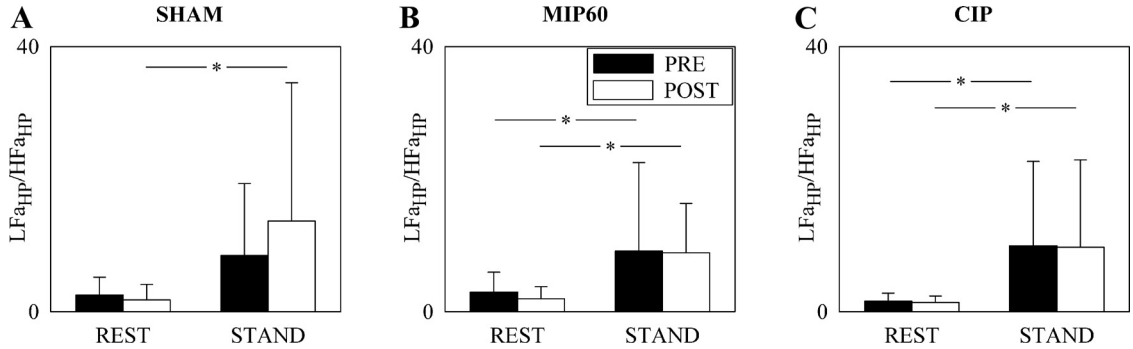

Fig. 5. The grouped vertical bar graphs show the ratio of the low frequency power to the high frequency power of the heart period series expressed in absolute units (LFa_{HP}/HFa_{HP}) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). The values are reported as mean plus standard deviation. *Statistically significant difference vs. REST within the same training condition (i.e., PRE or POST) with $P < 0.05$.

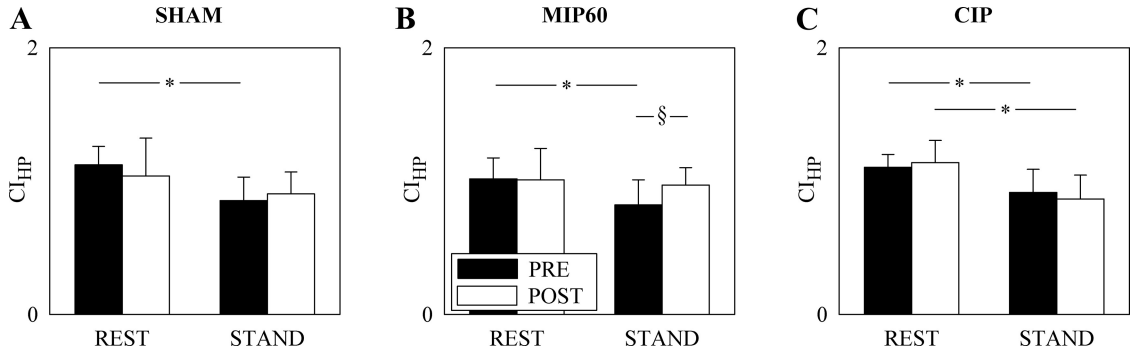

Fig. 6. The grouped vertical bar graphs show complexity index computed over the heart period series (CI_{HP}) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). The values are reported as mean plus standard deviation. *Statistically significant difference vs. REST within the same training condition (i.e., PRE or POST) with $P < 0.05$. §Statistically significant difference vs. PRE within the experimental condition (i.e., REST or STAND) with $P < 0.05$.

value of autonomic activity) (11) than changes of the autonomic modulation (i.e., variability of the firing rate about the mean value) (31, 64). However, even nonautonomic factors might account for resting bradycardia and increased magnitude of HP variability in athletes: indeed, the downregulation of the funny current I_f of pacemaker cells (20) can explain the after-training adaptations of intrinsic heart rate (58), and the influence of the autonomic tone on the velocity of the cardiac pacemaker cell membrane potential to reach the threshold (8) supports the positive association between μ_{HP} and σ^2_{HP} regardless of the magnitude of the autonomic perturbations. These controversial interpretations prompt investigating cardiac autonomic control in athletes, with special emphasis on discussing whether eventual modifications of the HP variability markers are more compatible with autonomic or nonautonomic factors, with modifications of tonic neural activity or its variability about the mean value and the role played by the training intensity. This investigation was carried out through a longitudinal, controlled, randomized blind design assessing the effect of IMT while monitoring modifications of the RF as a possible confounding factor (10, 30a). The computation of markers typical of vascular control, such as the LFa_{SAP} power, and indexes of baroreflex regulation are reported as well to provide a more complete picture of the effect of IMT on the autonomic function.

Effects of SHAM training on cardiovascular variability markers. Although the intensity of SHAM training is minimal (i.e., 6 cmH_2O) and did not produce remarkable MIP modifications, an increase in μ_{HP} was observed at REST. Remarkably, μ_{HP} is the unique HP variability marker that was affected by SHAM training. The finding is incompatible with the simple geometrical relationship linking HP variability indexes to μ_{HP} (8) and/or with modifications of vagal and sympathetic modulations induced by training (31, 36, 40). We suggest that an IMT of minimal intensity could be sufficient to induce a small, but significant, increase of mean vagal activity and/or decrease of mean sympathetic discharge directed to the sinus node (11) and/or modifications of cardiac pacemaker cell activity (20, 58). The limited influence of SHAM on HP variability markers in connection with the absence of modifications of BRS and RF indicates that SHAM training has a very limited impact on cardiovascular control (5, 36, 37, 39, 40, 30a).

Effects of MIP60 training on cardiovascular variability markers. We confirmed that even an IMT of moderate intensity tends to increase MIP compared with SHAM while negligibly affecting peak $\dot{V}O_2$ in athletes (30). In addition, this study proves that an IMT of moderate intensity produces a significant bradycardia at REST accompanied by a significant increase of the HFa_{HP} power and by an unvaried LFa_{SAP} and BRS. This finding suggests that MIP60 promotes changes in autonomic

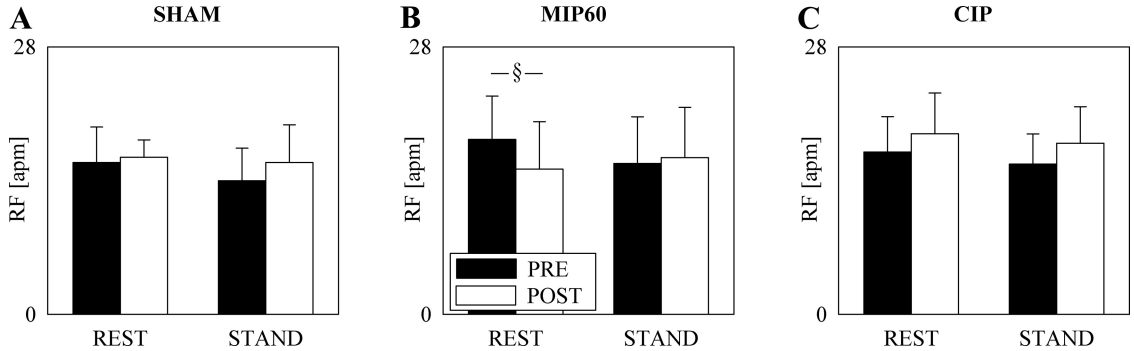

Fig. 7. The grouped vertical bar graphs show respiratory frequency (RF) before (PRE, black bars) and after (POST, white bars) training as a function of the experimental condition (i.e., REST and STAND) in the three considered groups, namely SHAM (*A*), 60% of the maximal inspiratory pressure (MIP60) (*B*), and critical inspiratory pressure (CIP) (*C*). The values are reported as mean plus standard deviation. §Statistically significant difference vs. PRE within the experimental condition (i.e., REST or STAND) with $P < 0.05$.

Table 2. *SAP markers and cardiac baroreflex control indexes in SHAM, MIP60, and CIP groups*

	SHAM		MIP60		CIP	
Index (Experimental Condition)	REST	STAND	REST	STAND	REST	STAND
μ_{SAP}, mmHg						
PRE	111 ± 17	104 ± 16	112 ± 14	116 ± 18	110 ± 9	109 ± 14
POST	98 ± 38	95 ± 39	112 ± 23	114 ± 24	113 ± 16	115 ± 16
σ^2_{SAP}, mmHg^2						
PRE	32 ± 22	52 ± 20	15 ± 5	41 ± 20*	23 ± 13	29 ± 12
POST	36 ± 25	38 ± 30	23 ± 13	46 ± 29*	30 ± 16	39 ± 25
LFa_{SAP}, mmHg^2						
PRE	9.7 ± 11	22 ± 11*	5 ± 5	24.6 ± 21*	5 ± 6	16.4 ± 13*
POST	6.6 ± 7	19.4 ± 18*	5.7 ± 6	26 ± 24*	6.2 ± 9.6	13.7 ± 11*
BRS, ms/mmHg						
PRE	19.7 ± 7.3	9.1 ± 4*	18.6 ± 6.1	9.1 ± 5.5*	20.3 ± 10	9.3 ± 3.4*
POST	20.4 ± 11.7	12.7 ± 6.9	17.9 ± 8.4	9.3 ± 4*	17.7 ± 8	6.5 ± 2.3*
SEQ%						
PRE	7 ± 8.9	11.5 ± 8.2	5.6 ± 5.6	14 ± 6.7*	4.2 ± 3	13.1 ± 7.9*
POST	3.3 ± 3.4	10.2 ± 6.7	4.5 ± 4.9	15.9 ± 10.6*	4.4 ± 2.5	12.7 ± 8.2*

Data are presented as means ± SD. BRS, baroreflex sensitivity computed via sequence method; CIP, IMT at critical inspiratory pressure; IMT, inspiratory muscle training; LF, low frequency; LFa_{SAP}, LF power of the SAP series expressed in absolute units; MIP, maximal inspiratory pressure; MIP60, IMT at 60% of MIP; POST, after training; PRE, before training; REST, at rest in supine position; SAP, systolic arterial pressure; SEQ%, percentage of patterns of baroreflex origin; SHAM, sham IMT; STAND, active standing; μ_{SAP}, SAP mean; σ^2_{SAP}, SAP variance. *$P < 0.05$ vs. REST within the same period of analysis (i.e., PRE or POST) assigned the training group.

average activity and amplitude of its beat-to-beat fluctuations about the mean, leading to a more dominant action of the vagal branch over the sympathetic one in absence of any modification of cardiac baroreflex regulation (11, 31, 40). Therefore, we conclude that this training practice seems to be valuable when sympathetic predominance needs to be limited (24, 28, 33) or a trend toward sympathetic overactivity need to be counteracted (50). However, it is useless to improve baroreflex control. Some studies linked an improvement of the HP variability magnitude at REST to better results in an intermittent endurance test (38), thus suggesting a positive association between vagal control and physical performances. However, the RF decrease during POST compared with PRE observed at REST might have contributed to the observed after-training increase of the HFa_{HP} power given the low-pass characteristic of the sinus node transfer function (9). Moreover, the effects of nonautonomic mechanisms, such as modifications of cardiac functioning at sinus node pacemaker cell level, could not be dismissed given the possible impact of the training in downregulating cardiac pacemaker cell activity (20, 58) and the consensual significant changes of both μ_{HP} and HFa_{HP} power (8). Additional mechanisms can have played a role in determining the observed modifications of the HFa_{HP} power. The MIP60 training might have potentiated cardiorespiratory coupling (23) by promoting central respiratory network modifications (22, 44, 55) and by improving the action of afferent atrial and pulmonary stretch-activated receptor circuits on central respiratory rhythm generator and central sympathetic drive (12, 53, 60). Moreover, IMT attenuates the human respiratory muscle metaboreflex by increasing respiratory muscle strength and reducing inspiratory muscle fatigue (67). Since the metaboreflex activation induced by voluntary resistive inspiration results in higher muscle sympathetic nerve activity, heart rate, and mean arterial pressure and lower blood flow in the resting limbs (57, 67), the reduced respiratory muscle fatigue after IMT might have contributed to producing some chronic

Table 3. *POST-PRE variations of cardiovascular variability markers in SHAM, MIP60, and CIP groups*

	SHAM		MIP60		CIP	
Variation	REST	STAND	REST	STAND	REST	STAND
$\Delta\mu_{HP}$, ms	72.1 ± 92.7	21.3 ± 101.5	112.6 ± 117.4	34.1 ± 125.8*	−17.8 ± 89.1	−53.4 ± 58.5*
$\Delta\sigma^2_{HP}$, ms^2	901.4 ± 3,124.6	39.2 ± 2,397.3	1,508.1 ± 1,416.2	417.7 ± 3,362.6	−1,003.7 ± 2,108.2	−719.8 ± 1,553.9
ΔHFa_{HP}, ms^2	533.7 ± 1,404	206.4 ± 654	639.9 ± 813.1	−89.3 ± 585*	−297.2 ± 754.8	−162.265 ± 297.6
$\Delta LFa_{HP}/HFa_{HP}$	−0.7 ± 3.0	5.2 ± 24.4	−0.9 ± 1.8	−0.2 ± 15.1	−0.2 ± 1.2	−0.2 ± 17.8
CI_{HP}	−0.08 ± 0.3	0.05 ± 0.1	−0.01 ± 0.1	0.18 ± 0.2*	0.04 ± 0.1	−0.05 ± 0.1
$\Delta\mu_{SAP}$, mmHg	−13.4 ± 49.7	−9.4 ± 39	−0.8 ± 17.6	−2.2 ± 24	3.2 ± 16.8	5.7 ± 22.5
$\Delta\sigma^2_{SAP}$, mmHg^2	3.6 ± 16.3	−13.9 ± 36.7	8 ± 12.4	5.8 ± 31.2	7.1 ± 19.9	10.3 ± 43.2
ΔLFa_{SAP}, mmHg^2	−3.1 ± 7.2	−2.6 ± 17.7	0.7 ± 2.3	1.4 ± 27.6	1.2 ± 4.3	−2.7 ± 7.0
ΔBRS, ms/mmHg	0.7 ± 12.9	3.6 ± 7.7	−0.7 ± 11.5	0.2 ± 3.7	−2.6 ± 15.2	−2.8 ± 3.5
ΔSEQ%	−3.7 ± 9.6	−1.3 ± 12.6	−1.1 ± 5.5	1.9 ± 9.2	0.2 ± 3.8	−0.4 ± 5.7

Data are presented as means ± SD. CI_{HP}, complexity index computed over the HP series; CIP, IMT training at the critical inspiratory pressure; HF, high frequency; HP, heart period; IMT, inspiratory muscle training; LF, low frequency; LFa_{HP}, LF power of the HP series expressed in absolute units; MIP60, IMT at 60% of the maximum inspiratory pressure; REST, at rest in supine position; SAP, systolic arterial pressure; SHAM, sham IMT; STAND, active standing; ΔBRS, variation of the baroreflex sensitivity; ΔHFa_{HP}, variation of the HF power of the HP series expressed in absolute units; $\Delta LFa_{HP}/HFa_{HP}$, variation of the ratio of LFa_{HP} to HFa_{HP}; ΔLFa_{SAP}, variation of the LF power of the SAP series expressed in absolute units; ΔSEQ%, variation of the percentage of patterns of baroreflex origin; $\Delta\mu_{HP}$, variation of the HP mean; $\Delta\mu_{SAP}$, variation of the SAP mean; $\Delta\sigma^2_{HP}$, variation of the HP variance; $\Delta\sigma^2_{SAP}$, variation of the SAP variance. Variations are computed as POST minus PRE index. *$P < 0.05$ vs. REST assigned the training group.

effects on basal autonomic tone, leading to a reduction of sympathetic drive and an improvement of vagal control (15, 30, 57, 67). This mechanism has been advocated to explain the improved basal cardiovascular control and autonomic function observed after IMT in pathological populations (13, 17, 19, 24, 29, 35, 51a). We observe that the invariable value of BRS excludes that the HFa_{HP} increase observed after MIP60 training could be the consequence of the simultaneous improvement of the baroreflex mechanical and neural component (3, 59) resulting from the greater solicitation of stretch-sensitive areas imposed by deeper breathing (6, 16), which leads to bigger changes of venous return and, in turn, more important variations of stroke volume (63), and from an enhancement of vagal reflexes.

In the MIP60 group, the CI_{HP} increased during STAND in POST session. This rise prevented us from observing the expected decrease of CI_{HP} after the MIP60 training in response to STAND (45, 61). Conversely, this reduction was visible in the PRE session. Since the complexity of the cardiac control decreases during sympathetic activation and vagal withdrawal as a result of the more limited presence of faster oscillations in HP variability (45, 47, 61), the increase of CI_{HP} during STAND in the POST session might indicate that the athlete can cope with postural challenge after MIP60 training with a smaller degree of sympathetic activation and vagal withdrawal compared with the PRE session. This change might be accompanied by an improvement in physical performance: indeed, a previous study found an association between the limited increase of sympathetic modulation during head-up tilt and better work capacity in athletes, whereas the larger magnitude of HFa_{HP} at REST was related to the ability to improve further peak $\dot{V}O_2$ (26) and intermittent endurance performance (38). Remarkably, the increase of CI_{HP} during STAND in the POST session was observed in the presence of no modification of the other HP variability markers, thus suggesting that this result might be primarily ascribed to changes in the dynamics of vagal and sympathetic controls (31, 36, 40, 64), whereas additional factors are unlikely to have played a role (8, 11, 20, 58).

Effects of CIP training on cardiovascular variability markers. As proven in Karsten et al. (30), a high-intensity IMT significantly improves MIP while leaving unmodified MEP and peak $\dot{V}O_2$. Despite significant modifications of MIP compared with SHAM, confirmed in the present study, our data suggest that an IMT of high intensity, such as the CIP training, did not produce any significant modification of HP and SAP variability markers as well as baroreflex indexes both at REST and during STAND. This result suggests that the IMT at the maximum intensity that can be sustained by the subject during a training session without experiencing inspiratory muscle fatigue (48) is useful to increase inspiratory muscle strength but is ineffective to produce modifications of the cardiovascular autonomic modulation and cardiac baroreflex regulation (31, 40, 64). This result might be due to the increase of the intrathoracic pressure associated with the high-intensity respiratory load during CIP sessions. This augmentation might have promoted a reduction of venous return, leading to an acute baroreflex-mediated sympathetic activation and vagal withdrawal to compensate the arterial pressure drop (2). This autonomic response, resulting in a more limited increase of RSA and HP variability markers during CIP sessions compared with lower-intensity IMT (2), might have induced some chronic adaptations of the cardiovascular control that have prevented cardiorespiratory coupling enhancement and central respiratory network modifications. Like MIP60 training, an IMT of high intensity left unmodified cardiac baroreflex control. Indeed, the increase of intrathoracic pressure during CIP training might have exacerbated the inability of IMT in producing an effective solicitation of the mechanical component of the cardiac baroreflex and in triggering adaptations of its neural components (3, 59).

Some effects of the CIP training on μ_{HP} were visible, and these influences took the form of a more tachycardic response to STAND. This finding may indicate the need of a greater sympathetic tone to cope with postural challenge and a more reactive response of sympathetic control to stressors after CIP training. This result cannot be explained as a consequence of some adaptations of cardiac pacemaker current (20, 57) and/or modifications of the basal vagal tone (11) because μ_{HP} was unmodified at REST. Remarkably, the after-training modifications of μ_{HP} during STAND occur in the absence of any change of other HP variability indexes, thus suggesting that μ_{HP} and HP variability markers provide complementary information (31, 40, 64) despite trivial relationships (8). Although chronic effects of high-intensity training remain largely unexplored, this finding is in line with studies that identified possible signs of sympathetic hyperactivity in response to high-intensity physical training (14, 27).

Limitations of the study and future developments. One of the main limitations of the study is the small sample size, which prevented a formal quantitative comparison of autonomic control markers derived from groups performing different types of IMT. A unique type of IMT carried out over a sole group of larger size could be exploited to assess the evolution of the computed indexes as a function of time in the POST session to better understand whether the effect of training was maintained over time. It is well known that the RF decrease is accompanied by the RSA increase (9) and that slowing respiration might produce modifications of baroreflex and chemoreflex controls that, in turn, can affect the magnitude of HP fluctuations (5, 6, 39). Since RF was basically unaffected by IMT (the unique exception is the after training RF decrease at REST in the MIP60 group), the observed cardiac autonomic control changes could not be explained by RF variations. However, this study solely monitored the RF. Future studies should consider additional variables relevant to the respiratory system, such as tidal volume, ratio of inspiratory to expiratory time, and end tidal carbon dioxide, to understand whether their changes can explain the reported modifications of HP variability parameters. Even additional nonrespiratory variables, such as parameters related to cardiac filling and ventricular ejection, should be monitored in future studies to favor the clarification of mechanisms driving the chronic adaptations of the cardiac autonomic control to IMT.

Perspectives and Significance

Our findings support that IMT, when performed at moderate intensity, may promote an improved cardiac vagal control in resting condition as well as a lower autonomic response during orthostatic challenge. This improvement of cardiac autonomic control might have positive consequences for the performance in athletes because it might enlarge the variety of cardiac

control responses to physiological challenges. Moreover, these improvements might be particularly desirable in pathological populations showing signs of sympathetic overactivity and/or postural hypotension as a consequence of a high basal sympathetic drive and in healthy elderly populations to counteract the effect of aging. Conversely, high-intensity IMT might have some undesirable effects in athletes because it reduces the ability to cope with sympathetic stressors like postural challenge, and, as such, it cannot be recommended in either pathological or healthy aged populations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.M.C. conceived and designed research; R.M.d.A., P.R.-S., C.D.d.S., É.D.F.S., and C.A.S. performed experiments; R.M.d.A. and B.C. analyzed data; R.M.d.A., A.P., P.R.-S., B.C., C.D.d.S., É.D.F.S., C.A.S., and A.M.C. interpreted results of experiments; R.M.d.A. and A.P. prepared figures; R.M.d.A. and A.P. drafted manuscript; R.M.d.A., A.P., P.R.-S., B.C., C.D.d.S., É.D.F.S., C.A.S., and A.M.C. edited and revised manuscript; R.M.d.A., A.P., P.R.-S., B.C., C.D.d.S., É.D.F.S., C.A.S., and A.M.C. approved final version of manuscript.

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