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Neurohumoral and Endothelial Responses to Heated Water-Based Exercise in Resistant Hypertensive Patients

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Background: The neurohumoral and endothelial responses to the blood pressure (BP) lowering effects of heated water-based exercise (HEx) in resistant hypertension (HT) patients remain undefined.

Methods and Results: We investigated these in 44 true resistant HT patients (age 53.3±0.9 years, mean±SEM). They were randomized and allocated to 2 groups, 28 to a HEx training protocol, which consisted of callisthenic exercises and walking in a heated pool for 1 h, three times weekly for 12 weeks and 16 patients to a control group maintaining their habitual activities. Measurements made before and after 12 weeks of HEx included clinic and 24-h BP, plasma levels of nitric oxide, endothelin-1, aldosterone, renin, norepinephrine and epinephrine, as well as peak $\dot{V}O_2$, and endothelial function (reactive hyperemia). After 12 weeks of HEx patients showed a significant decrease in clinic and 24-h systolic and diastolic BPs. Concomitantly, nitric oxide increased significantly (from 25±8 to 75±24 μmol/L, P<0.01), while endothelin-1 (from 41±5 to 26±3 pg/mL), renin (from 35±4 to 3.4±1 ng/mL/h), and norepinephrine (from 720±54 to 306±35 pg/mL) decreased significantly (P<0.01). Plasma aldosterone also tended to decrease, although not significantly (from 101±9 to 76±4 pg/mL, P=NS). Peak $\dot{V}O_2$ increased significantly after HEx (P<0.01), while endothelial function was unchanged. No significant change was detected in the control group.

Conclusions: The BP-lowering effects of HEx in resistant HT patients were accompanied by a significant reduction in the marked neurohumoral activation characterizing this clinical condition.

Key Words: Endothelial dysfunction; Heated water-based exercise; Neurohumoral activation; Resistant hypertension

We recently showed that heated water-based exercise (HEx) training exerts blood pressure (BP) lowering effects in patients with true resistant hypertension (HT) by significantly reducing office as well as ambulatory BP values recorded during a 24-h period.[1] Whether and to what extent these BP-lowering effects are associated with, and presumably triggered by, modifications in the behavior of the neurohumoral systems that participate in the homeostatic control of BP and are activated in resistant HT, such as the sympathetic nervous system and the renin-angiotensin-aldosterone axis,[2–5] is largely unknown. In addition, no information is available on whether and to what extent HEx training is capable of exerting favorable effects on the endothelial dysfunction that characterizes resistant HT patients.[5,6]

The present study was designed to provide information on the neurohumoral mechanisms of the BP-lowering effects of HEx training in resistant HT patients by assessing the sympathetic and renin-angiotensin as well as the endothelial responses to this intervention in patients with documented true resistant HT.

Methods

Population

Between August 2011 and August 2013 155 patients with a diagnosis of resistant HT were screneed for the HEx trial in the HT outpatient clinic of the University Hospital of São Paulo, Brasil. We excluded 111 patients, with the remaining 44 patients included in the present study. They were sedentary, aged between 40 and 65 years, diagnosed as affected by resistant HT for more than 5 years, and who in the previous 6 months had unchanged and regular use of at least 3 optimally dosed antihypertensive drugs, including a diuretic, and displayed office systolic BP >140 mmHg, and/or diastolic BP >90 mmHg.[7] We used medical records to exclude patients with a history of secondary HT [e.g., chronic kidney disease (creatinine

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>2.0 mg/dL), severe obesity (body mass index ≥40 kg/m^2) and obstructive sleep apnea], and those with documented evidence of coronary artery disease. We also excluded patients with pseudo-resistance,[7] such as poor adherence to antihypertensive drugs, smoking and diabetes mellitus, and patients with any chronic illness that could limit their capacity to exercise, or if they were participating in regular physical activity those patients who were unable to complete the cardiopulmonary exercise test.

Study Design

The study followed a randomized controlled, parallel group design and was carried out in a single hospital center in Brazil. The patients fitting the study inclusion criteria were invited to participate, read a detailed description of the protocol and gave written informed consent. All patients underwent initial evaluations (see below) and were then allocated to either the intervention group (n=28), or control group (n=16) with a 2:1 randomization using a drawing of lots (envelopes in a bag). The active group underwent a 12-week exercise training, while the control group was instructed to maintain their habitual activities without any type of exercise training for 12 weeks. All patients were instructed to keep unchanged (doses and medications) their antihypertensive drug treatment during the entire study period and at the end of the 12-week period all measurements were repeated. The HEx trial is registered at ClinicalTrials.gov (NCT01863082). The local Ethics Committee of the University Hospital approved all procedures.

Exercise Test and BP Evaluation

Evaluations performed in the HEx trial have been previously described in detail.[1] Briefly, a cardiopulmonary exercise test was performed to exclude patients with coronary artery disease and to assess the training effect. Before the test perfomance all patients followed the strict behavioral criteria previously described. The test was carried out on a programmable treadmill (Series 2000, Marquette Electronics, Milwaukee, WI, USA) in a temperature-controlled room (21–23°C) between 08:00 and 11:00 AM, with a standard 12-lead continuous ECG monitor (Max 1, Marquette Electronics). BP monitoring was performed with the patient at rest, during effort and recovery. Minute ventilation, oxygen uptake, carbon dioxide output, and other cardiopulmonary variables were acquired breath-by-breath by a computerized system (Vmax 229 model, SensorMedics, Yorba Linda, CA, USA). The respiratory exchange ratios were recorded as the 1-minute averaged samples, obtained during each stage of the Balke protocol.[8] A peak respiratory exchange ratio ≥1.05 and symptoms of maximum effort characterized a satisfactory test. The highest $\dot{V}O_2$ uptake level was considered the peak value (peak $\dot{V}O_2$).

BP and heart rate measurements included both office and ambulatory values. Office BP measurements were done according to recommended guidelines,[9] while heart rate was assessed at the level of the radial artery by the palpatory method. The BP value used was the average of 3 readings performed within a 2-min period. Ambulatory BP monitoring was performed before patients began the program and 72 h after the last session in the heated pool. Both measurements started at the same time of the day (between 01:00 and 02:00 PM) using a Spacelabs model 90207 monitor (Spacelabs Medical Inc., Redmond, WA, USA), set to obtain automated BP and heart rate oscillometric readings every 20 min over the 24 h.[10] During the monitoring period, patients were asked to follow their normal activities. The monitor was programmed to measure BP every 15 min during the daytime and every 20 min during the nighttime period. Ambulatory BP data were accepted only if at least 85% of the measurements were successfully taken.

Assessment of Endothelial Function

All patients underwent evaluation of endothelial function before and at the end of the 12-week study period. For this evaluation a non-invasive method that checks the peripheral arterial tone (PAT) signal using equipment called the Endo-PAT 2000 was used.[11] This method measures arterial tone changes in peripheral arterial beds. The PAT signal is measured from the fingertip by recording finger arterial pulsatile volume changes. Results of the 15-min test are automatically calculated and an index is generated. Endo-PAT quantifies the endothelium-mediated changes in vascular tone, elicited by a 5-min occlusion of the brachial artery (using a standard BP cuff). With the patient lying on the examination table, in a quiet environment with controlled temperature (21–23°C), a standard inflatable BP cuff is placed around the non-dominant arm. Finger biosensors containing plethysmographic probes, which are connected to the recording device, are placed over the index finger of each hand to monitor the function of the blood vessels. A reading of the fingers' blood flow rate begins and measures PAT: after having monitored BP while the patient rested for 5 min, the cuff on the non-dominant arm is inflated until occlusion of the brachial artery and 5 min later, the BP cuff is deflated, releasing blood through the brachial artery to the fingers. When the cuff is released, the surge of blood causes an endothelium-dependent flow-mediated dilatation.[11] The dilatation, manifested as reactive hyperemia, is captured by Endo-PAT as an increase in the PAT signal amplitude. The finger sensors monitor the reactive hyperemia, and then automatically convert the changes in PAT into a graph, which is used to assess the blood vessels and their hyperemic response before, during, and after occlusion of the brachial artery. A post-occlusion to pre-occlusion ratio is calculated by the software, providing the reactive hyperemia index (RHI), (normal endothelial function: RHI >1.67, endothelial dysfunction: RHI <1.67).[11]

Neurohumoral Measurements

Blood samples were collected before and after the 12-week study, with patients fasting for 12 h and abstaining from alcohol, chocolate, coffee, cola, tea or any other beverage that contains caffeine for 24 h preceding the sample collection. Patients remained at rest for at least 30 min prior to collection; the samples were taken into polyethylene tubes with anticoagulant (EDTA) with an intravenous cannula inserted into the antecubital vein at least 20 min before blood sampling. After collection, samples were centrifuged to obtain the plasma, and then stored in a freezer at −80°C until subjected to assay for norepinephrine, epinephrine, plasma renin activity, aldosterone, nitric oxide (NO) and endothelin-1.[12–16]

Exercise Training Protocol

The exercise sessions took place in the early afternoon (from 1:30 to 2:30 PM) and were performed in a controlled temperature (32°C) swimming pool; patients were immersed

Table 1. Baseline Characteristics of the Study Patients With Resistant HT

	Total group (n=44)	Training group (n=28)	Control group (n=16)
Sex (F/M)	21/23	14/14	7/9
Age (years)	53.3±0.9	54.4±1.2	52.4±1.5
BMI (kg/m^2)	29.4±0.7	29.1±0.9	30.1±1.1
Ethnicity (W/B)	15/29	9/19	6/10
No. of anti-HT drugs	4 (3–6)	4 (3–6)	4 (3–6)
Diuretics	100%	100%	100%
CCBs	78%	75%	81%
ACEIs	62%	62%	62%
ARBs	27%	28%	25%
β-blockers	63%	64%	62%
Vasodilators	42%	36%	50%
Aldosterone antagonists	37%	30%	43%
Central sympatholytics	28%	29%	25%
Oral antihyperglycemics	29%	34%	25%
ASA	13%	22%	6%

Data are shown as mean±standard errors or as percent. ACEIs, angiotensin-converting enzyme inhibitors; anti-HT, antihypertensive drug; ARBs, angiotensin receptor blockers; ASA, acetylsalicylic acid; BMI, body mass index; CCBs, calcium channel blockers; HT, hypertension; SBP and DBP, systolic and diastolic blood pressures; W/B, white/black.

in warm water up to the xiphoid process, and the sessions were performed three times each week for 12 weeks. All subjects were instructed not to add any other leisure-time exercise during the study period. All patients were either unused to or had no previous experience with swimming. The exercise sessions consisted of a 60-min schedule: 5 min of warming up, 20 min of callisthenic exercises against water resistance (upper and lower limbs), 30 min of walking in the pool at a pace that was between "fairly light, somewhat hard" (between 11 and 13 on the Borg Scale), and 5 min of cooling down and stretching.[17] The control group was requested to maintain daily activities without exercise training during the entire 12-week period.

Statistical Analysis

Descriptive statistics were used to analyze patients' characteristics. Data are presented as mean±standard error unless otherwise specified. The Shapiro-Wilk test was applied to ensure a Gaussian distribution of the data. Unpaired t-test was used to compare baseline characteristics between the training and control groups. Repeated measures analysis of variance (ANOVA) were performed with time (pre and post) and intervention (HEx and control) as repeated factors. Bonferroni post-hoc analysis was performed to identify significant differences between values. The Mann-Whitney U test was also used for non-parametric analyses. The relationships between clinical and ambulatory BP changes and the concomitant changes in plasma norepinephrine, epinephrine, renin, aldosterone, NO and endothelin-1 were investigated by linear regression analysis. Pearson product moment correlation coefficients (r) were calculated. The level of statistical significance was set at $P<0.05$ and data analyses were performed by using SPSS 20.0 for Mac (SPSS Inc., Chicago, IL, USA). All data evaluations and analyses were made by investigators unware of the experimental study design.

Results

As shown in **Table 1** the resistant HT patients recruited in

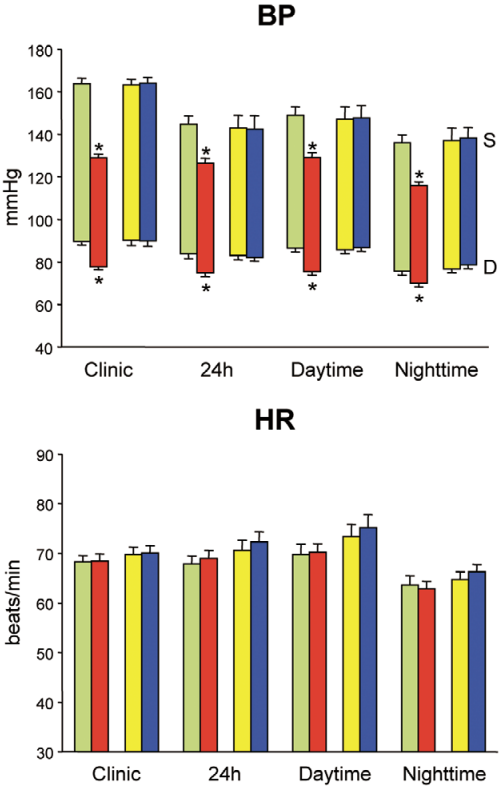

Figure 1. Effects of 12 weeks' heated water-based exercise training (Hex) or observation on clinic, 24-hour (24 h), daytime and nighttime systolic (S) and diastolic (D) blood pressure (BP) and corresponding heart rate (HR) values in the resistant hypertensive patients enrolled in the study. Green and yellow bars refer to baseline values before Hex or observation without intervention, respectively. Red and blue bars refer to the values recorded following the 12 weeks of Hex or obeservation without intervention, respectively. Data are shown as mean±standard error. *P<0.01 refers to the statistical significance between values recorded before and after Hex.

Table 2. Cardiopulmonary Exercise Test Results in the Study Patients With Resistant HT

	Training group (n=28)		Control group (n=16)	
	Pre	Post	Pre	Post
HR (beats/min)				
Rest	67.8±3.2	64.5±3.1	70.5±3.7	72.1±3.8**
Peak	139±4.7	145.1±4.5	136.7±6.2	136.2±6.1
Recovery	116.8±4.8	112.1±4.2	116.3±5.9	116.1±4.3
Recovery (Delta)	22.2±14.7	33±17.7*	20.4±14.2	20.5±13.2**
SBP (mmHg)				
Rest	162.2±23.2	135.5±11*	157.6±17.6	157.8±16.6**
Peak	197.7±26.4	175±20.7*	193.7±17.3	194.1±17.2**
Recovery	183±23.2	160.6±17*	180.9±17.4	181.4±13.6**
DBP (mmHg)				
Rest	83.8±2.5	76.7±2.1*	86.4±2.5	87.1±2.0**
Peak	95.5±2.9	86.8±3.3	100.9±4.6	101.5±4.4
$\dot{V}O_2$ (mL/kg/min)				
Peak	23.9±4.6	29.8±3.9*	25.1±4.8	20.7±4.0**
RER	1.2±0.1	1.2±0.1	1.1±0.1	1.1±0.1

Data are shown as mean±standard error. *Difference within the group (P<0.05), **difference between the groups (P<0.05). HR, heart rate; HR recovery Delta, difference between the peak HR and the recovery HR; RER, respiratory exchange ratio; $\dot{V}O_2$, oxygen consumption. Other abbreviations as in Table 1.

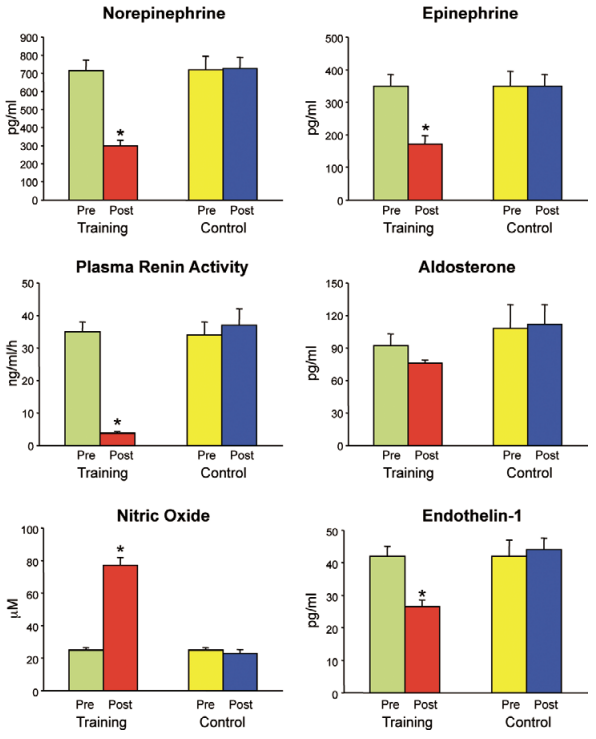

Figure 2. Effects of 12 weeks' heated water-based exercise training (training) or observation (control) on plasma norepinephrine, epinephrine, renin activity, aldosterone, nitric oxide and endothelin-1 values in the resistant hypertensive patients enrolled in the study. Green and yellow bars refer to baseline values before training or observation without intervention, respectively. Red and blue bars refer to the values recorded following the 12 weeks of training or obeservation without intervention, respectively. Data are shown as mean±standard error. *P<0.01 refers to the statistical significance between values recorded before and after the training program.

the present study and allocated according to a randomized schedule to either HEx training or control group had similar ages, body mass index, and preexisting antihypertensive medication use, which was based on an average of 4 drugs per day. All patients included showed optimal (100%) adherence to the training protocol, which was well tolerated and without any adverse events. Throughout the study period all patients followed their antihypertensive medication regimens (drugs and dosages) established at baseline.

Figure 1 shows the clinic and ambulatory BP values, as well as the corresponding heart rate values, in the training and control groups before and after HEx training or observation without intervention, respectively. At baseline there was no significant difference in office BP and heart rate values detected in the 2 groups. This was also the case also for ambulatory BP and heart rate values. HEx training, which significantly increased peak oxygen consumption and decreased peak systolic BP (**Table 2**), induced a significant reduction in clinic, 24-h, daytime, nighttime systolic and diastolic BP values (P<0.01) without affecting the corresponding heart rate values. No significant changes in clinic or ambulatory BP and heart rate were detected in the control group before and after 12 weeks of observation without intervention.

Figure 2 shows the effects of HEx training on the neurohumoral variables assessed in the present study. HEx significantly reduced venous plasma norepinephrine, epinephrine and endothelin-1 levels, and plasma renin activity, while significantly increasing plasma NO values (all P<0.01). Plasma aldosterone levels were also reduced by the intervention; however, the decrease did not achieve the minimal level of statistical significance. In contrast no change in the various variables was detected in the control group before and after 12 weeks of observation without the HEx training program.

Endothelial function as assessed by the Endo-PAT reactive hyperemia procedure is shown in **Table 3**. At baseline, deflation of the cuff positioned at the brachial artery level,

Table 3. Endothelial Function Test Using Endo-PAT in the Study Patients With Resistant HT

	Training group (n=28)		Control group (n=16)	
	Pre	Post	Pre	Post
SBP (mmHg)	163.2±3.5	130.4±2.5*	162.2±4.3	161.1±3.3**
DBP (mmHg)	95.1±1.6	80.6±1.9*	94.1±1.5	93.9±1.4**
RHI	2±0.1	1.8±0.1	2.1±0.2	2.1±0.1

Data are shown as mean±standard error. *Difference within the group (P<0.05), **difference between the groups (P<0.05). RHI, reactive hyperemia index. Other abbreviations as in Table 1.

Table 4. Correlation Coefficients (r) and P Values (P) of Changes in Clinic and 24 h SBP and DBP (ΔSBP and ΔDBP, Respectively) and Concomitant Changes in Neurohumoral Variables

	ΔSBP (mmHg)	ΔDBP (mmHg)	ΔSBP-24 h (mmHg)	ΔDBP-24 h (mmHg)
ΔNE, pg/mL				
r	0.31	0.28	0.32	0.30
P-value	0.10	0.11	0.09	0.11
ΔE, pg/mL				
r	0.25	0.20	0.27	0.22
P-value	0.14	0.18	0.16	0.13
ΔRA, ng/mL/h				
r	0.32	0.25	0.34	0.31
P-value	0.11	0.13	0.09	0.11
ΔA, pg/mL				
r	0.18	0.21	0.19	0.21
P-value	0.20	0.24	0.19	0.20
ΔNO, μmol/L				
r	−0.33	−0.27	−0.34	−0.31
P-value	0.09	0.14	0.07	0.10
ΔEpi, pg/mL				
r	−0.20	−0.18	−0.22	−0.21
P-value	0.21	0.26	0.22	0.22

A, plasma aldosterone; E, plasma endothelin-1; Epi, plasma epinephrine; NE, plasma norepinephrine; NO, plasma nitric oxide; RA, plasma renin activity. Other abbreviations as in Table 1.

after a 5-min occlusion, triggered a marked increase in blood flow. The magnitude of this hyperemic response, as evaluated by the RHI, was unaffected by the HEx, despite the lower BP values observed following the intervention. No significant change in BP values or in the RHI was observed in the control group after 12 weeks' observation without intervention.

No significant relationship was found between the systolic, diastolic clinic and 24-h ambulatory BP changes induced by HEx and the comcomitant changes in plasma norepinephrine, epinephrine, renin, aldosterone, NO and endothelin-1 (**Table 4**).

Discussion

The present study confirmed in a large population sample of true resistant HT patients the BP-lowering effects of HEx training originally documented for the first time by our group in a previously published study.[1] It adds to this information, however, a number of major novel findings. First, it showed for the first time that the BP-lowering effects of HEx training in resistant HT are accompanied by a marked and significant reduction in the circulating plasma levels of the adrenergic neurotransmitters norepinephrine and epinephrine, which are markedly elevated in this clinical condition.[5,18] Second, it showed, again for the first time, that HEx training triggers in resistant HT patients a significant reduction in the circulating plasma levels of renin (and thus of angiotensin II with its pronounced vasocostrictive properties), which are again remarkably increased in this hypertensive state.[3–5,18] Third, it provided evidence that the BP reduction elicited by HEx training is associated with an increase in the circulating plasma levels of NO and a parallel reduction in plasma concentrations of endothelin-1, a peptide produced by the endothelium with potent vasoconstrictor effects.[5,6] Taken together these findings allow us to conclude that several of the neurohumoral alterations characterizing resistant HT and responsible, at least in part, for the marked systemic vasoconstriction and BP elevation detected in this condition are favorably affected by HEx. This is the case for the sympathetic overactivity documented in resistant HT via indirect and direct techniques.[2,5,18] This is also the case for the renin-angiotensin activation reported in resistant HT patients.[4,5,18] Finally, this is the case for the elevated circulating plasma levels of endothelin-1 and the oxydative stress dysfunction described in patients with resistant HT.[5] These neurohumoral changes may be responsible for the

HEx training-induced BP-lowering effects, by triggering a marked reduction in peripheral vascular resistance and thus a pronounced vasodilatation, which has been documented as a result of the same procedure in patients with congestive heart failure or chronic renal disease.[19,20] Other mechanisms potentially involved in the BP-lowering effects of HEx training include an improvement in sympathomodulatory and BP-lowering properties of the arterial baroceptors, whose function, impaired in resistant HT,[2] can be favorably affected by HEx intervention.

Several other results of our study deserve to be discussed. Firstly, in the resistant HT patients recruited in the present study we found that a 12-week HEx training schedule, although capable of significantly improving NO production by the endothelial cells, did not modify the Endo-PAT reactive hyperemic response, which is considered an index of endothelial function. The discrepancy in these results can be explained by the hyperemic response to local ischemia being only in part dependent on NO[21] and that participation in the observed response by functional but also structural vascular factors, such as arteriolar hypertrophy and remodeling, which are common in resistant HT,[22,23] cannot be ruled out.[24] Secondly, our study did not allow us to clarify which mechanisms may concur in determining the neurohumoral and NO effects of the HEx training in the patients enrolled. Several non-mutally exclusive hypotheses can be advanced, however. We can, for example speculate, that, as already mentioned, an improvement in baroreflex control of sympathetic function may occur, which would lead to a decrease in sympathetic activity, inhibition of the renin-angiotensin-aldosterone system, increased NO production and decreased endothelin-1 release, which are all linked, directly or indirectly, to neuroadrenergic function.[25] We can also speculate that an exercise training-dependent improvement in cardiopulmonary receptor control of sympathetic drive and renin release, which is also impaired when the hypertensive state is complicated by left ventricular hypertrophy,[26] as frequently occurs in resistant HT,[27,28] might participate in the phoenomenon. Thirdly, despite the marked decrease in venous plasma norepinephrine and epinephrine values, and thus the marked sympathetic deactivation seen after the 12-week HEx training program, we were unable to find any significant decrease in resting heart rate values, which depend to a consistent extent on sympathetic influences on sinus node activity. We speculate that the dissociation between the behavior of plasma catecholamines and heart rate reflects the fact that the procedure, despite eliciting marked peripheral sympathoinhibitory effects, did not affect cardiac sympathetic neural drive. This explanation, however, does not fit with the well-known evidence that regular physical exercise markedly lowers, throughout a reduction in cardiac sympathetic outflow, heart rate, concomitantly increasing vagal inhibitory influences on sinus node activity.[29] A final consideration is that our study was not designed to determine the relative contribution of exercise training "per se" (i.e., land-based exercise training vs. Hex) to the observed hemodynamic and neurohumoral responses. Although the data obtained in populations recruited in different studies are difficult to be compare with each other, our results show a BP reduction in response to HEx training more pronounced in magnitude than that induced by land-based exercise training in resistant HT patients.[30,31] This appears to be the case also for plasma norepinephrine, epinephrine, NO, endothelin-1 and plasma renin activity changes induced by HEx training when compared with those triggered by land-based exercise training in essential HT patients.[32–34] It can be thus reasonably concluded that the heated water component of the HEx program is an important determinant of both the BP and neurohumoral responses.

Study Limitations

These include the fact that the patients enrolled in the present study were all under multiple antihypertensive drug treatment that for obvious clinical reasons could not be withdrawn before the intervention or in the 12-weeks follow-up. The antihypertensive drugs could indeed have affected the results, in same cases exacerbating the neurohumoral abnormalities described in resistant HT patients. However, the fact that the drug regimens were unchanged in the 12-month follow-up and that the same drug classes were used in the control group of patients, with no evidence of any neurohumoral effects, should rule out the this possibility. A further limitation is that in the assessment of the sympathetic effects of the intervention we used plasma catecholamines as the marker of adrenergic neural function. This marker has been recognized as having a number of methodological and physiological limitations as compared with the gold standard approach to assessing sympathetic neural function; that is, direct microneurographic recording of muscle symapthetic nerve traffic.[35]

Finally, in our study the neurohumoral alterations characterizing resistant HT, although reversed by the HEx training program, failed to show complete normalization, the observed values of the different variables remaining well above those reported in healthy subjects.[36] This result, however, is not peculiar to the HEx training program but effect the other 2 approaches of potential clinical use in the treatment of resistant HT: bilateral renal nerve ablation and carotid baroreceptor stimulation.[18,37,38]

Conflict of Interest

None declared by each author.

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