Effects of a hydrotherapy programme on symbolic and complexity dynamics of heart rate variability and aerobic capacity in fibromyalgia patients

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ABSTRACT

Objective. *To evaluate the effects of a hydrotherapy programme on aerobic capacity and linear and non-linear dynamics of heart rate variability (HRV) in women with fibromyalgia syndrome (FMS).*

Methods. *20 women with FMS and 20 healthy controls (HC) took part in the study. The FMS group was evaluated at baseline and after a 16-week hydrotherapy programme. All participants underwent cardiopulmonary exercise testing on a cycle ergometer and RR intervals recording in supine and standing positions. The HRV was analysed by linear and non-linear methods. The current level of pain, the tender points, the pressure pain threshold and the impact of FMS on quality of life were assessed.*

Results. *The FMS patients presented higher cardiac sympathetic modulation, lower vagal modulation and lower complexity of HRV in supine position than the HC. Only the HC decreased the complexity indices of HRV during orthostatic stimulus. After a 16-week hydrotherapy programme, the FMS patients increased aerobic capacity, decreased cardiac sympathetic modulation and increased vagal modulation and complexity dynamics of HRV in supine. The FMS patients also improved their cardiac autonomic adjustments to the orthostatic stimulus. Associations between improvements in non-linear dynamics of HRV and improvements in pain and in the impact of FMS on quality of life were found.*

Conclusion. *A 16-week hydrotherapy programme proved to be effective in ameliorating symptoms, aerobic functional capacity and cardiac autonomic control in FMS patients. Improvements in the non-linear dynamics of HRV were related to improvements in pain and in the impact of FMS on quality of life.*

Introduction

Fibromyalgia syndrome (FMS) is a multifactorial disease, characterised by chronic widespread pain, stiffness and exaggerated tenderness and sensitivity at 18 specific tender points (1, 2). Although its aetiology and pathophysiology are still unknown, advancements have been made (2, 3) and some studies have suggested that a dysfunction in the autonomic nervous system (ANS) may play an important role and could explain the symptomatology, and the physical and psychological aspects in patients with FMS (4).

Indeed some abnormalities in the ANS have been reported, such as an overall increase in cardiovascular sympathetic activity at rest, blunted sympathetic vascular modulation, impaired cardiac vagal withdrawal during orthostatic stimulus, reduced baroreflex sensitivity and its impaired adjustment during orthostatic challenge, as well as a reduced orthostatic tolerance (5, 6).

Cardiac autonomic control in FMS has been widely studied through heart rate variability (HRV) (5-8), however those studies have predominantly used linear methods of analysis. In addition, its association with clinical features of FMS is still not fully understood. On the other hand, it is known that heart rate (HR) modulation exhibits non-linear dynamics that are difficult to capture using linear methods (9). Therefore, non-linear analyses of HR dynamics have been proposed in order to provide complementary information about the underlying HR regulation mechanisms and to predict a pathological situation and/or a global depression of the organism (10-13).

Another important factor that must be noted, is that FMS patients present a reduced level of aerobic functional capacity (14), which might be due to the symptoms, especially pain and fatigue, limiting their ability to perform physical activity. Therefore, considering that there is still no effective drug treatment for the syndrome itself, non-pharmacological therapies are an important part of the treatment (15, 16). Among several methods and techniques of treatment available, hydrotherapy has been one of the most recommended and perceived as effective by patients (17-19). Latorre *et al*. (20) reported that a physical training programme combining exercises in water and on land reduces pain and disease impact, and improves functional capacity in women with FMS. In addition, a pool-based exercise performed during 12 weeks was effective in increasing the HRV indices at rest related to the cardiac vagal modulation in patients with rheumatoid arthritis (21).

To our knowledge, no studies have assessed the effects of hydrotherapy on cardiac autonomic control in FMS patients and its relationship with symptoms. Considering the important role attributed to the ANS in the FMS, is relevant to elucidate whether the improvement in the cardiac autonomic control is associated with the improvement in the symptoms, since many results are still inconclusive about possible adaptive mechanisms that explain the improvement of clinical features in FMS patients. Thus, this study aimed to test the hypothesis that women with FMS present changes in non-linear dynamics of HRV and that a 16-week hydrotherapy programme could be effective in promoting an improvement in the symptoms, in aerobic functional capacity and in cardiac autonomic control, this amelioration being associated with the clinical improvement.

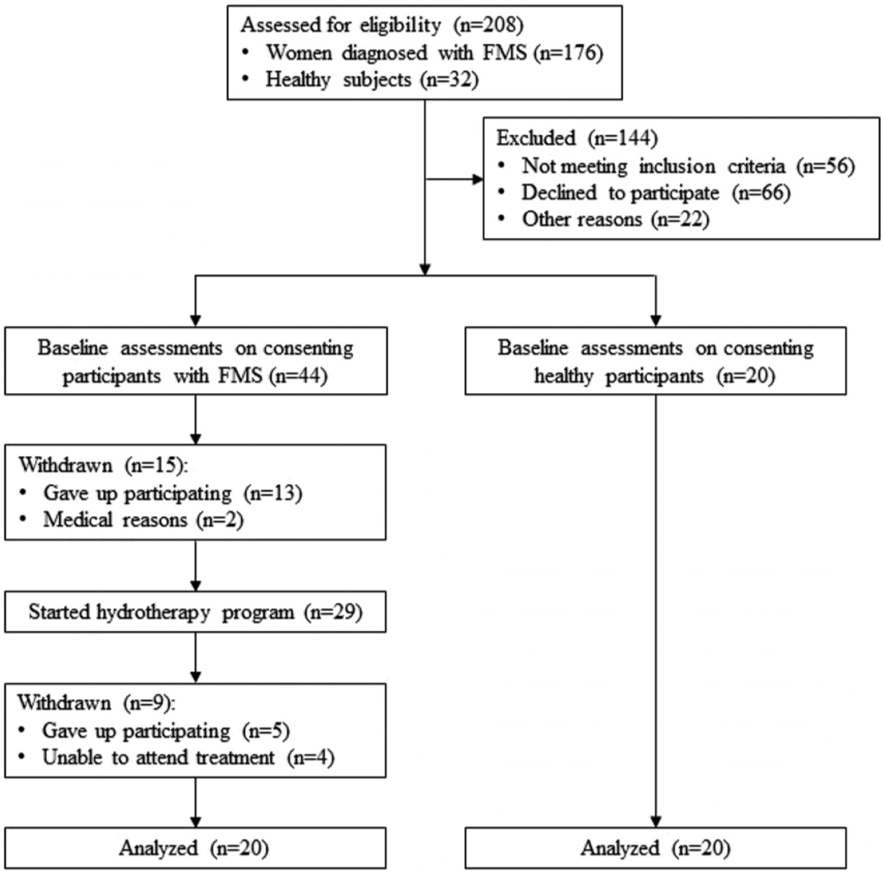


Fig. 1. Flow chart of participants through the study. FMS: fibromyalgia syndrome.

Methods

Participants

176 women with a clinical diagnosis of FMS were recruited from the local community through advertisements in university buildings, orthopedic and rheumatology clinics, or from our database of FMS patients. Of these, 29 were eligible and agreed to participate, and 20 participants completed the study. Figure 1 shows a flow chart demonstrating the flow of participants in the study. The diagnosis was made by a board certified rheumatologist according to criteria established by the American College of Rheumatology (22). The healthy control group (HC) comprised 20 healthy women matched with the FMS group according to age and body mass index. The HC participants were recruited from the local community and through personal contacts of the researchers.

To satisfy the inclusion criteria, it was a requirement that subjects had never had a history of cardiovascular, respiratory or metabolic disease of any kind (*e.g.* hypertension, diabetes, cardiac arrhythmias, thyroid disorders, etc.) or inflammation as a cause of pain, nor any neurological disorder, or cognitive deficits that would prevent understanding or the conduct of the evaluations, nor could they be smokers, or be engaged in regular physical activity or be continuously using drugs or alcohol. None of the participants had taken any psychotropic or other medications known to alter autonomic activity for at least 4 weeks before the study, including antihypertensives, tranquilisers or antidepressant drugs. The study was carried out in accordance with the Declaration of Helsinki, was approved by the Ethics in Research Committee of the institution (protocol number 112.508), and is registered at ClinicalTrials.gov under the number NTC01839305. All subjects signed an informed consent form prior to participation in the study.

All participants underwent an initial interview, in which the study selection criteria were assessed, and those eligible for the study were invited to participate. The participants of the FMS group were evaluated at baseline and after a 16-week hydrotherapy programme (Post16).

Experimental procedure

All experiments were carried out in the morning (8 to 12 a.m.) in order to minimise circadian changes. Room temperature was maintained at 22°C and relative air humidity at between 40%

and 60%. Participants were acquainted with the experimental protocol and were instructed to abstain from stimulants (*e.g.* coffee, tea, soft drinks) and alcoholic beverages during the 24 h preceding the examination, and to have a light meal at least two hours before the test. To avoid any residual fatigue, subjects were asked to refrain from strenuous physical activity at least two days before the tests.

Recording R-R intervals

The subjects rested for about 20 min for the HR to return to control conditions. Then, RR intervals were collected for a period of 15 min in supine position and 15 min during active standing with spontaneous breathing. The participants' breaths per minute were recorded during the entire collection period by the evaluator, through visual inspection of thoracoabdominal movements. The participants who presented a respiratory rate below 9 rpm (0.15 Hz) would be excluded due to the fact that breathing influences the frequency bands of spectral analysis (23). The participants were requested not to talk or move in order to avoid alterations and artefacts in the HR and RR intervals collection.

The data were collected using a HR monitor and a transmitter belt (Polar Advanced RS800CX, Polar Electro Co.Ltda. Kempele, Finland) placed on the thoracic region at the fifth intercostal space. The HR monitor instantly detects the ventricular depolarisation, corresponding to wave R of the electrocardiogram with a 1000 Hz sampling frequency and one-millisecond time resolution (24). After transferring the data to the computer, the R-R interval series (RR) were analysed and any interference was observed.

HRV analyses

HRV was analysed using linear and non-linear methods. The region of 256 consecutive beats with the greatest stability in the RR time series was selected for analysis for all participants (23).

Linear analysis

Spectral analysis was carried out by applying an autoregressive model in the previously selected RR section. The spectral components were obtained in low frequency (LF, 0.04–0.15 Hz) and high frequency bands (HF, 0.15–0.4 Hz) in absolute units (ms^2). Normalised units were computed by dividing the absolute potency of LF or HF components by the total potency component minus the very low frequency component (0.003–0.04 Hz) and multiplying this ratio by 100. Considering that the LF band is modulated by the sympathetic and parasympathetic ANS (with sympathetic predominance), and the HF band is correlated with vagal control, the LF/HF ratio was calculated to evaluate sympathovagal balance (23).

Non-linear analysis

The non-linear methods used in the present study comprised the symbolic analysis (25), Shannon entropy (SE) (25) and corrected conditional entropy (26).

In symbolic analysis, the RR time series selected for analysis is uniformly distributed in six levels, where each beat receives a symbol (from 0 to 5). After that, patterns (sequences of three symbols) are built based on the sequence of symbols and grouped in the following four families: patterns without variation (0V), patterns with one variation (1V), patterns with two like variations (2LV), and patterns with two unlike variations (2UV). Since previous studies (11, 25, 27) have found that the 0V% index represents sympathetic cardiac autonomic modulation, the 1V% and the 2LV% indices represent both parasympathetic and sympathetic cardiac autonomic modulation, and 2UV% index represents parasympathetic cardiac modulation, the percentage of each family's appearance was calculated.

SE and corrected conditional entropy reflect the complexity of the RR time series (25, 26). SE is a measure of the patterns' distribution complexity (sequences of three symbols) obtained in the RR time series. The presence of peaks (relevant to patterns more frequently detected) or valleys (relevant to missing or less frequent patterns) in the patterns' distribution determines the reduction of SE. On the other hand, the maximum SE is obtained when the patterns are identically distributed (25).

Corrected conditional entropy provides measures of the complexity of the dynamic relation between patterns that follow each other. In the present study we used the complexity index (CI) provided by the corrected conditional entropy. This index value will be zero if the patterns' time series is completely regular (predictable). However, maximum values will be obtained when there is no relation between patterns that follow each other, that is, when the patterns' time series is completely random. Thus, the larger the index, the greater the complexity, and the lesser the regularity (26).

Cardiopulmonary exercise testing

The cardiopulmonary exercise testing (CPET) was performed on a cycle ergometer with electromagnetic braking (Quinton Corival 400, Seattle, WA, USA). The participants were instructed not to perform an isometric contraction while holding onto the handlebar of the cycle ergometer, and to maintain the pedaling rate at 60 rpm. The CPET consisted of a continuous ramp type protocol, comprising 1 min pretesting in the sitting, resting position on the cycle ergometer followed by a 4 min warm-up period at 4 W and power output increments until physical exhaustion, which corresponded either to the point at which the participant was unable to maintain 60 rpm or to the manifestation of a limiting symptom (*i.e.* pain, dizziness) or respiratory fatigue. Power output increments were determined for each subject according to the formula proposed by Wasserman *et al.* (28): Power output increase (W) = [(height - age) x 14] – [150 + (6 x body mass)]/100.

During the CPET ECG and HR were recorded beat-to-beat (Welch Allyn CardioPerfect Workstation, Skaneateles Falls, NY). Ventilatory and metabolic variables such as oxygen uptake (VO_2) and carbon dioxide output (VCO_2) were obtained breath-by-breath during CPET by means of an expired gas measurement system (CPX/D, Medical Graphics, St Paul, MN) that was calibrated before each test. After the ventilatory and metabolic measurements were obtained, aerobic capacity was evaluated by considering power output (W), absolute VO_2 (mL.kg.min^{-1}) and HR (bpm) data obtained at the peak of the exercise test and at the ventilatory anaerobic threshold (VAT). Three properly trained observers evaluated the VAT using a graphic visual method to estimate the disproportional increase in ventilatory and metabolic variables during the incremental dynamic exercise. The criterion adopted was a loss of parallelism between carbon output VCO_2 and VO_2 (29, 30).

Table I. Demographic, haemodynamic and respiratory variables at rest. Data are expressed as mean ± SD.

	HC (n = 20)	FMS Group (n = 20)
Age (years)	46 ± 7	48 ± 7
Body mass index (Kg/m^2)	24.7 ± 3.0	26.1 ± 2.5
Education (years)	12 ± 3	11 ± 3
Disease duration (years)	-	8 ± 4.6
Beck Inventory Depression	6.2 ± 5.5	16.8 ± 9.4*
Beck Inventory Anxiety	4.5 ± 3.9	16.6 ± 13.1*
Heart rate (bpm)	67 ± 7	72 ± 2
Systolic blood pressure (mmHg)	119 ± 8	120 ± 9
Diastolic blood pressure (mmHg)	71 ± 7	75 ± 6
Respiration (cycles/min)	16 ± 2	16 ± 4

Clinical features
In order to study some clinical features at baseline and Post16, we assessed the current level of pain, the tender points, the pressure pain threshold (PPT) and the impact of fibromyalgia on quality of life. These assessments were conducted by a researcher experienced in the use of these tools, but blind to the experimental protocol.
The current level of pain was assessed by a visual analogue scale (VAS) ranging from 0 (no pain) to 100 mm (worst pain possible). The PPT was determined, using a digital algometer (OE-220: Tissue Hardness Meter & Algometer, Ito Co., Japan), at the 18 tender points described by Wolfe *et al*. (22). The tender points were counted when the PPT reported was less than a pressure of 4 kg (22). The average of the PPT measured at the 18 TP was considered for analysis.
The impact of fibromyalgia on quality of life was assessed by the Fibromyalgia Impact Questionnaire (FIQ). The score of this questionnaire ranges from 0 to 100, and the higher the score, the greater the impact of FMS on the subject's quality of life.

Hydrotherapy treatment programme
The hydrotherapy programme comprised 32 sessions of 45 min of pool exercise twice a week for 16 weeks in a warm pool (30°C ± 2°C), in a closed room at a gym club. The sessions were conducted in groups of up to 5 subjects and were supervised by a physical therapist. The hydrotherapy programme comprised the following phases: 1) warm-up (5 min) 2) aerobic and resistance exercises (30 min), 3) stretching (5 min) and 4) relaxation (5 min). The exercise was planned to permit individual progress, aiming to improve overall function but respecting the patients' pain and fatigue limits. The rating perceived exertion (RPE) measured on the Borg CR-10 scale (31), ranged from 1 ("very weak") to 2 ("weak") during flexibility and stretching exercises, while it was 7 ("strong") to 10 ("very, very strong") during aerobic exercise. The mean value for HR during the programme ranged from 50% to 80% of the HR obtained at the peak of CPET.

Statistical analysis
Shapiro-Wilk's and Levene's test were used to verify the normality and homogeneity, respectively. Two-way repeated measures analysis of variance with Bonferroni adjustment (one factor repetition) was used to examine the differences between HC individuals and FMS patients at baseline in supine and orthostatic conditions. Two-way repeated measures analysis of variance with Bonferroni adjustment (two factor repetition) was used to assess the effects of hydrotherapy on the ability of FMS patients to deal with the orthostatic challenge. Unpaired two-tailed Student's *t* test was used to analyse the differences between controls and FMS group for the variables obtained during CPET at baseline. The association between the changes in the HRV indices and the changes in the clinical features of FMS was assessed by Pearson's correlation coefficient. Within-group effect size was calculated by Cohen d coefficient. An effect size greater than 0.8 was considered large; around 0.5, moderate; and less than 0.2, small (32). For all statistical procedures, significance was set at 5%. Statistical analyses were performed using the SPSS package (v 20; IBM Corporation, Armonk, NY).
The sample size calculation was based on a pilot study, in which an effect size of 0.70 was obtained for the LF/HF ratio. A sample of 12 subjects was suggested with a level of 0.05 and 90% power. To allow for drop outs, this sample was increased to 20 people in each group.

Results

Participant's characteristics
Data regarding the age, demographic and haemodynamic variables of both groups are presented in Table I. No significant differences between groups were observed for age, education, body mass index and haemodynamics (p>0.05). The FMS group showed higher scores for Beck Inventory Depression (p<0.05) and Beck Inventory Anxiety (p<0.05).

Linear and non-linear analyses of HRV
Table II presents the results regarding the indices of linear and non-linear analyses of HRV from the HC and FMS groups. At the baseline, a significant group × posture interaction was observed only for SE (F=5.38; p<0.05) and CI (F=4.75; p<0.05) indices. Pairwise comparisons revealed that, in supine posture, the FMS group presented lower values of SE (p=0.01) and CI (p=0.04) compared to the HC. Regarding the comparisons between supine and standing postures, the HC presented a significant reduction of SE (p=0.002) and CI (p=0.04), which was not observed in the FMS group (p>0.05). A significant main effect of posture was found for μ_{RR} (F=69.86; p<0.0001), HF (F=11.06;

Table II. HRV analyses in healthy controls and in the FMS patients at baseline and Post16. Data are expressed as mean ± SD.

	HC		FMS			
	Baseline		Baseline		Post16	
	SUP	STAND	SUP	STAND	SUP	STAND
Linear analysis						
μ_{RR} (ms)	899.4 ± 22.9	810.1 ± 23.8	805.4 ± 23.2	709.5 ± 23.6	872.5 ± 22.3	813.2 ± 31.0
σ^2_{RR} (ms^2)	1505.5 ± 346.1	1338.9 ± 263.8	475.4 ± 113.2	354.7 ± 103.5	689.0 ± 105.4	551.5 ± 84.4
HF (ms^2)	508.2 ± 141.8	281.8 ± 75.7	89.2 ± 19.1	30.0 ± 6.4	195.4 ± 46.3	66.8 ± 23.4
HFnu (%)	55.4 ± 3.3	36.9 ± 3.4	43.0 ± 3.8	23.6 ± 2.8	59.6 ± 3.4‖¶	24.8 ± 4.3
LF/HF	0.89 ± 0.1	2.22 ± 0.33	1.83 ± 0.31	4.35 ± 0.6	0.82 ± 0.13	4.41 ± 0.75
Non-linear analysis						
Symbolic analysis						
0V (%)	12.7 ± 1.6	28.2 ± 3.2	22.5 ± 2.9	34.8 ± 2.3	8.1 ± 1.2‖¶	32.6 ± 2.4
1V (%)	49.1 ± 1.4	46.8 ± 1.0	48.8 ± 1.3	46.0 ± 0.8	47.8 ± 1.5	47.4 ± 0.8
2LV (%)	14.4 ± 1.2	9.9 ± 1.6	10.1 ± 1.1	6.4 ± 1.0	15.9 ± 1.2‖¶	6.2 ± 0.7
2UV (%)	23.7 ± 2.5	15.1 ± 1.8	18.7 ± 2.0	12.8 ± 1.6	28.3 ± 2.4‖¶	13.7 ± 1.6
SE	3.80 ± 0.10 *¥	3.40 ± 0.11	3.40 ± 0.10	3.40 ± 0.06	3.81 ± 0.06‖¶	3.35 ± 0.05
CI	1.15 ± 0.03 *¥	1.02 ± 0.04	1.01 ± 0.04	0.98 ± 0.03	1.14 ± 0.04‖¶	0.94 0.02

*$p<0.05$ HC Baseline SUP *vs*. FMS Baseline SUP; ¥$p<0.05$ HC Baseline SUP *vs*. HC Baseline STAND; ‖$p<0.05$ FMS Baseline SUP *vs*. FMS Post16 SUP; ¶$p<0.05$ FMS Post16 SUP *vs*. FMS Post16 STAND. Significant main effects are described in the results. HRV: heart rate variability; STAND: active standing; SUP: supine position; FMS: fibromyalgia syndrome; µ: mean of RR; σ^2: variance of RR; HF: high frequency component of RR variability expressed in absolute units; HFnu: high frequency component of RR variability expressed in normalised units; 0V: patterns with no variation; 1V: patterns with one variation; 2LV: patterns with two like variations; 2UV: patterns with two unlike variations. SE: Shannon entropy; CI: complexity index.

p<0.01), HFnu (F=28.94; *p*<0.0001), LF/HF (F=25.65; *p*<0.0001), 0V% (F=33.48; *p*<0.0001), 2LV% (F=12.16; *p*=0.001) and 2UV% (F=17.64; *p*<0.0001). Regardless of group, lower values of μ_{RR}, HF, HFun, 2LV% and 2UV% were observed compared to the supine posture (*p*<0.05). A significant main effect of group was observed for μ_{RR} (F=9.75; *p*<0.01), σ^2_{RR} (F=10.64; *p*<0.01), HF (F=9.97; *p*<0.01), HFnu (F=15.97; *p*<0.0001), LF/HF (F=19.28; *p*<0.0001), 0V% (F=9.08; *p*=0.01) and 2LV% (F=9.11; *p*=0.01). Regardless of posture, the FMS group presented lower values of μ_{RR}, σ^2_{RR}, HF, HFnu and 2LV%, and higher LF/HF and 0V% (*p*<0.05).

Table II presents also the results regarding the effects of the hydrotherapy programme on the HRV indices of the FMS group. A significant time × posture interaction was observed for HFnu (F=7.35; *p*=0.01), 0V% (F=7.82; *p*=0.01), 2LV% (F=13.68; *p*=0.002), 2UV% (F=10.41; *p*=0.004), SE (F=9.60; *p*=0.006) and CI (F=6.56; *p*=0.02). Pairwise comparisons revealed that after a 16-week hydrotherapy programme, in supine posture, the FMS group increased HFnu (*p*<0.0001; effect size=1.19), 2LV% (*p*<0.0001; effect size=1.34), 2UV% (*p*=0.001; effect size=0.98), SE (*p*=0.002; effect size=1.11) and CI (*p*=0.03; effect size=0.94) and decreased 0V% (*p*<0.0001; effect size =-1.18). After the treatment, during the active standing the FMS group decreased HFnu (*p*=0.001), 2LV% (*p*=0.002), 2UV% (*p*=0.006), SE (*p*<0.0001) and CI (*p*<0.0001) and increased 0V% (*p*=0.002) compared to the supine position, which had not occurred at the baseline assessment. A significant main effect of time was observed for μ_{RR} (F=15.31; *p*=0.001), σ^2_{RR} (F=4.47; *p*=0.04) and HF (F=7.13; *p*=0.02). Thus, regardless of posture, after the treatment the FMS group presented higher values of μ_{RR}, σ^2_{RR} and HF (*p*<0.05).

Changes in the cardiac autonomic indices from supine to standing position in FMS patients were reported in Table III, expressed as delta values (standing values minus supine values). The results show that after the hydrotherapy programme, a significant increase in the delta from supine to standing position was observed for HFnu, 0V%, 2LV%, 2UV%, SE and CI indices (*p*<0.05).

Table III. Power output, cardiorespiratory and ventilatory responses at the peak and ventilatory anaerobic threshold (VAT) of the cardiopulmonary exercise testing. Data are expressed as mean ± SD.

	HC	FMS	
	Baseline	Baseline	Post16
Power Output$_{PEAK}$ (W)	103 ± 5*¥	76 ± 5	92 ± 5¶
Power Output$_{VAT}$ (W)	58 ± 3*	41 ± 5	57 ± 6¶
VO_{2PEAK} (mL.kg.min^{-1})	19.0 ± 1.0*¥	13.2 ± 0.5	15.9 ± 3.3¶
VO_{2VAT} (mL.kg.min^{-1})	12.2 ± 0.5*¥	8.9 ± 0.6	10.7 ± 0.8¶
HR_{PEAK} (bpm)	148 ± 3*	131 ± 4	145 ± 4¶
HR_{VAT} (bpm)	118 ± 4*	108 ± 3	115 ± 4¶

*$p<0.05$ HC Baseline *vs*. FMS Baseline; ¥$p<0.05$ HC Baseline *vs*. FMS Post16; ¶$p<0.05$ FMS Baseline *vs*. FMS Post16 VO_2: oxygen uptake; HR: heart rate.

Variables obtained in CPET

Table IV presents the variables obtained in CPET. Compared to the controls the FMS group presented lower values of Power Output$_{PEAK}$ (*p*<0.05), Power Output$_{VAT}$ (*p*<0.05), VO_{2PEAK} (*p*<0.05), VO_{2VAT} (*p*<0.05), HR_{PEAK} (*p*<0.05) and HR_{VAT} (*p*<0.05). After the hydrothera-

Table IV. Changes in the HRV indices from supine position to active standing in FMS patients. Data are expressed as mean ± SD.

	Baseline Δ (STAND – SUP)	Post16 Δ (STAND – SUP)	*p*-value
Linear analysis			
μ (ms)	-95.9 ± 16.9	-56.8 ± 15.0	0.07
$σ^2$ (ms^2)	-120.8 ± 46.4	-136.7 ± 114.6	0.89
HF (ms^2)	-59.2 ± 16.4	-122.1 ± 51.3	0.23
HFnu (%)	-19.4 ± 5.2	-31.8 ± 4.5	0.04
LF/HF	2.52 ± 0.67	3.41 ± 0.76	0.22
Non-linear analysis			
Symbolic analysis			
0V (%)	12.3 ± 3.4	23.3 ± 2.6	0.03
1V (%)	-2.8 ± 1.6	-0.3 ± 1.7	0.37
2LV (%)	-3.7 ± 1.5	-9.2 ± 1.4	0.01
2UV (%)	-5.9 ± 1.9	-13.9 ± 2.0	0.01
SE	-0.08 ± 0.10	-0.44 ± 0.07	0.01
CI	-0.04 ± 0.04	-0.19 ± 0.04	0.03

HRV: heart rate variability; STAND: active standing; SUP: supine position; FMS: fibromyalgia syndrome; μ: mean of RR; $σ^2$: variance of RR; HF: high frequency component of RR variability expressed in absolute units; HFnu: high frequency component of RR variability expressed in normalised units; 0V: patterns with no variation; 1V: patterns with one variation; 2LV: patterns with two like variations; 2UV: patterns with two unlike variations. SE: Shannon entropy; CI: complexity index.

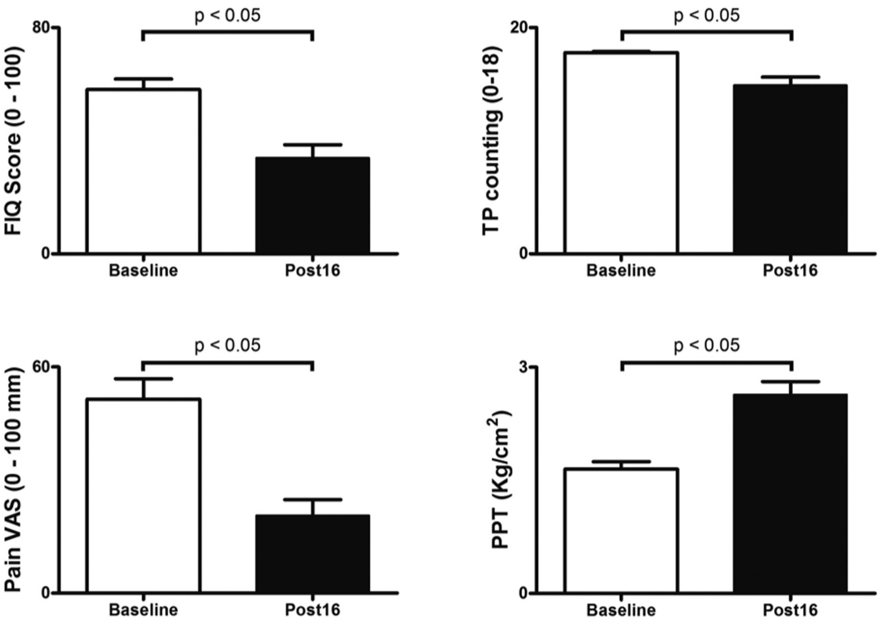


Fig. 2. Clinical features assessed at Baseline and Post16. FIQ: Fibromyalgia Impact Questionnaire; TP: tender points; VAS: visual analogue scale; PPT: pressure pain threshold.

py programme a significant increase was observed for Power $Output_{PEAK}$ (p<0.05; effect size=0.73), Power $Output_{VAT}$ (p<0.05; effect size=0.72), VO_{2PEAK} (p<0.05; effect size=1.21), VO_{2VAT} (p<0.05; effect size=0.73), HR_{PEAK} (p<0.05; effect size=0.83) and HR_{VAT} (p<0.05; effect size=0.48).

Clinical manifestations

A significant reduction in FIQ scores, number of active tender points, current level of pain, and a significant increase in PPT were observed after the treatment period (Fig. 2).

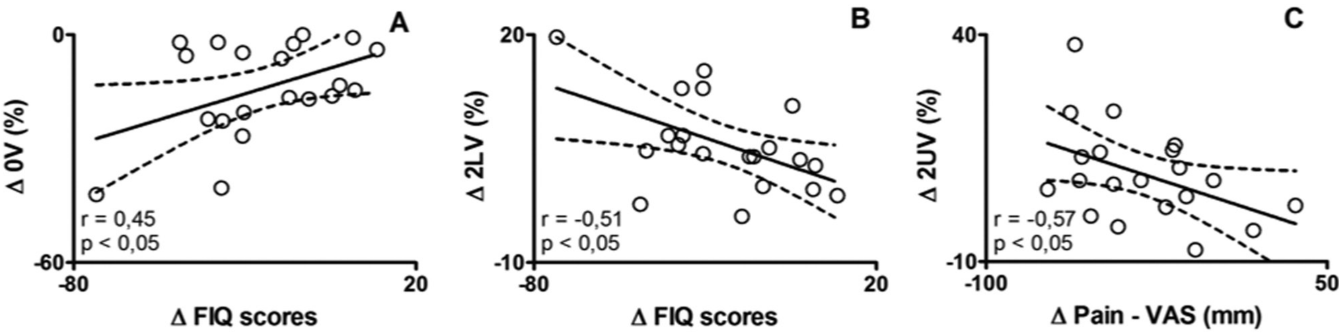
Significant relationships between improvements in the clinical manifestations and improvement in the cardiac autonomic indices assessed in supine position were observed. A positive linear relationship between the reduction in FIQ scores and the reduction in the 0V% pattern was found (ΔFIQ and Δ0V, r=0.45, p<0.05, Fig. 3), as well as a negative linear relationship between decreases in FIQ scores and increases in 2LV pattern (ΔFIQ and Δ2LV, r=-0.51, p<0.05), in SE (ΔFIQ and ΔSE, r=-0.57, p<0.01) and in CI (ΔFIQ and ΔCI, r=–0.55, p<0.01, Fig. 3). A negative linear relationship was also found between decreases in Pain scores and increases in 2UV% pattern (ΔPain and Δ2UV, r=-0.45, p<0.05, Fig. 3).

There were no significant correlations between the amount of improvement in the variables obtained in the CPET and the improvement of clinical manifestations (p>0.05).

Discussion

The main findings of this study are the following: 1) the FMS patients presented alterations in non-linear dynamics of HRV compared to the HC, characterised by higher cardiac sympathetic modulation, lower vagal modulation and lower complexity of HRV in the supine position, and impaired response to orthostatism, identified by the absence of a decrease in complexity indices; 2) after a 16-week hydrotherapy programme, the FMS patients presented decreased cardiac sympathetic modulation and increased vagal modulation and complexity dynamics of HRV in the supine position; their cardiac autonomic adjustments to orthostatic stimulus improved; there was an improvement in their aerobic functional capacity and the symptoms assessed; 3) An association between the improvement in non-linear dynamics of HRV and the improvement in pain and in the impact of FMS on quality of life was found.

The present findings confirm the hypothesis that women with FMS show alteration in the non-linear dynamics of HRV. The symbolic indices used in this study have the potential to detect non-reciprocal changes in sympathetic and parasympathetic modulations (25), differing from spectral analysis (23, 33). However, the results obtained with both methods were similar in the present study, revealing higher cardiac sympathetic and lower cardiac parasympathetic modulations in the supine posture. This is in line with previous studies which reported similar results in patients with FMS (5, 7, 34). On the



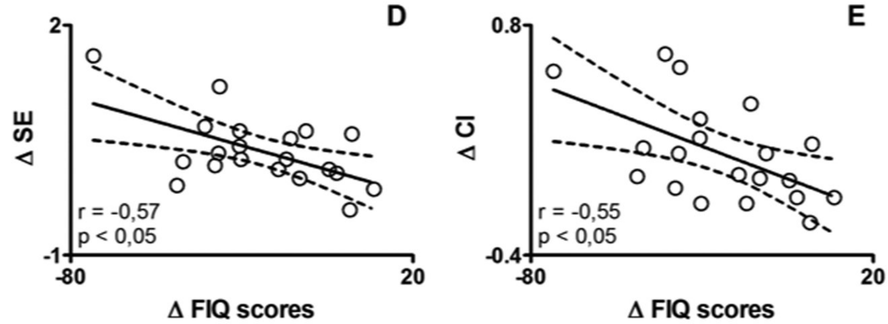


Fig. 3. Correlation between heart rate variability indices and clinical features of fibromyalgia syndrome. **A**, Changes in 0V% (Δ0V) pattern of symbolic analysis in supine position and changes in fibromyalgia impact questionnaire scores (Δ FIQ), **B**, changes in 2LV% (Δ2LV) pattern of symbolic analysis in supine position and ΔFIQ scores, **C**, changes in 2UV% (Δ2UV) pattern of symbolic analysis in supine position and changes in current level of pain assessed by visual analogue scale (Δ Pain VAS), **D**, changes in Shannon Entropy (ΔSE) in supine position and ΔFIQ and **E**, changes in complexity index (ΔCI) in supine position and ΔFIQ. Changes are assessed as Post16 values minus baseline values.

other hand, with regard to SE and CI, in addition to revealing a reduced complexity dynamic of HRV in the supine position, the complexity indices also indicated that patients with FMS have a blunted response to orthostatic stimulus. This confirms that these methods provide different and complementary information to those obtained from spectral and symbolic analyses. Porta *et al.* (12), report that the assessment of cardiovascular regulation complexity could provide important information about the underlying regulatory mechanisms. Therefore, decreased complexity indices might be resulting from depressed organ function, a loss of interaction among subsystems, an overwhelming action of a subsystem over others and an impairment of regulatory mechanisms, which may reflect a pathological situation

After a 16-week hydrotherapy programme, the FMS patients presented a significant improvement in the cardiac autonomic control, characterised by a reduction in cardiac sympathetic autonomic modulation, an increase in vagal modulation and an increase in the complexity dynamics of HRV at rest. Furthermore, the improvement in the cardiac autonomic control in supine position contributed to the improvement in the cardiac autonomic responses to the orthostatic stimulus by preventing a possible ceiling effect, since the hyperactive sympathetic nervous system of FMS patients seems to be unable to further respond to different stressors (35). Therefore, after the treatment, the FMS patients responded with an increase in the cardiac sympathetic modulation and a decrease in the cardiac parasympathetic modulation and complexity of HRV during active standing.

To our knowledge, no studies assessed the effects of a hydrotherapy programme on cardiac autonomic control in FMS patients. Nevertheless, some improvements in cardiovascular function were observed regarding pool-based exercises in healthy subjects and in patients with rheumatoid arthritis. Ruoti *et al.* (36) found that water exercises conducted over a 12-week period with an older adult population resulted in increased cardiovascular function during work and decreased resting HR. Janse Rensburg *et al.* (37) applied a pool-based exercise programme to patients with rheumatoid arthritis which is considered to be a primary outcome of HRV indices. The authors found a decrease in resting HR and an improvement in cardiac autonomic control during orthostatic stimulus after the treatment, both of which were attributed to an improvement in cardiac vagal modulation.

It is well established that aerobic training improves cardiac autonomic modulation, decreasing the efferent sympathetic neural flow on the sinoatrial node and promoting an increase in parasympathetic control over the resting heart rate (38, 39). However, several factors might have contributed to the improvement in the cardiac autonomic control after the hydrotherapy programme, such as biochemical, structural, metabolic, hormonal and neural adaptations (39, 40).

The effects of the water properties must also be considered. Xu *et al.* (41) found that balneotherapy (immersion in warm water 34-40ºC) for 21 days leads to a significant improvement in the cardiac function of healthy subjects, as assessed by cardiac pump function and heart contractive function indices. The authors state that the underlying mechanisms need to be further elucidated, including the roles played by the ANS and humoral factors.

One of the main aims of this study was to evaluate whether the improvement in clinical features of fibromyalgia is related to the changes in cardiac autonomic control. Indeed, the results revealed an interesting association between the improvement in non-linear dynamics of HRV and the improvement in pain, and in the impact of FMS on quality of life. These findings indicate that the participants who showed higher decrease in pain and in the impact of FMS on quality of life, were the ones who showed higher decrease in the cardiac sympathetic modulation, higher increase in the cardiac parasympathetic autonomic control (2LV% and 2UV%) and increase in the complexity dynamics (SE and CI) of HRV at rest in supine position. Moreover, it must be highlighted that only the changes in non-linear indices correlated with the changes in FIQ scores, denoting the importance of using these indices in FMS patients. Although it is not possible to infer causality, *i.e.*, whether the improvement in the cardiac autonomic control leads to the improvement of symptoms or vice versa, the present findings are consistent with the model proposed by Martinez-Lavin *et al.* (42) in which the alterations regarding the ANS may explain many common symptoms in patients with FMS. The authors proposed that persistent sympathetic hyperactivity can lead to insomnia, anxiety and chronic pain. Furthermore, this hyperactivity could result in down-regulation and desensitisation of adrenergic receptors, leading to a hyporeactivity of the sympathetic nervous system to stress and thus resulting in fatigue and reduced orthostatic tolerance.

The hydrotherapy programme was also effective in improving the aerobic capacity at the peak and at VAT of the CPET. In keeping with these results, Ruoti *et al.* (36) reported an increase of 15% in the VO_{2peak} after the hydrotherapy programme in patients with FMS. In addition, Assis *et al.* (21) observed anaerobic threshold and VO_{2PEAK} enhancement in 31% and 38%, respectively, of patients subjected to a pool-based exercise programme. The authors attribute the improvements to the predominant aerobic component of the hydrotherapy programme in association with the advantages of the aquatic environment. Performing exercise in water decreases the joint stress in the lower limbs due to the buoyancy effect, and provides resistance to the execution of movements, resulting in high caloric expenditures and heightening cardiovascular activity (36).

It is well documented that aerobic training is effective in improving symptoms and aerobic capacity in patients with FMS (43-45). However, it must be noted that there was not a significant association between clinical improvement and aerobic gain, being in accordance with the results reported by Valim *et al.* (43) and Assis *et al.* (39). This finding reinforce, as proposed by Valim et al (43), that the treatment of patients with FMS should be of low to moderate intensity prioritising adherence rather than aerobic capacity gain.

Despite the interesting results obtained, some limitations must be mentioned. A randomised controlled-trial design could be applied in order to better elucidate the effects of hydrotherapy on important parameters not yet studied in FMS, such as blood pressure variability, baroreflex sensitivity and muscle sympathetic nerve activity. In addition, although the sample size was higher than the minimum suggested by the sample size calculation, it may be considered relatively small. A larger number of subjects would make it possible to take into account possible influences of co-morbidities (*e.g.* hypertension, obesity, thyroid disorders) and especially the impact of concomitant therapies (*e.g.* antidepressants and tranquilisers), since in the present study these subjects were excluded in order to make the sample more homogenous.

On the basis of these observations, the present findings have important clinical implications. The use of HRV should be encouraged in order to assess the cardiac autonomic control in FMS patients, especially using non-linear methods of analysis, since it seems to provide important information related to the symptoms of fibromyalgia. Therefore, the assessment of cardiac autonomic control, which is a non-invasive, simple and low-cost method, might be used as a quantitative index to provide feedback to patients and therapists on the progress and effectiveness of a given therapy.

In conclusion, the women with FMS presented abnormal non-linear dynamics of HRV, suggesting that the integrity of the cardiac autonomic modulation is compromised compared to healthy subjects. A 16-week hydrotherapy program was effective in ameliorating the symptoms, physical capacity and cardiac autonomic control in FMS patients, and improvements in the non-linear dynamics of HRV seem to be related to improvements in the impact of FMS on quality of life and pain.

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