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Effects of whole-body cryotherapy and static stretching are maintained 4 weeks after treatment in most patients with chronic fatigue syndrome

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ABSTRACT

In the previous study, whole-body cryotherapy (WBC)+static stretching (SS) has been shown to reduce the severity of some symptoms in Chronic Fatigue Syndrome (CFS) noted just after the therapy. Here we consider the effects of treatment and explore the sustainability of symptom improvements at four weeks (one-month) follow-up. Twenty-two CFS patients were assessed one month after WBC + SS programme. Parameters related to fatigue (Chalder Fatigue Questionnaire (CFQ), Fatigue Impact Scale (FIS), Fatigue Severity Scale (FSS)), cognitive function (Trial Making test part A and B (TMT A and TMT B and its difference (TMT B-A)), Coding) hemodynamic, aortic stiffness (aortic systolic blood pressure (sBP aortic)) and autonomic nervous system functioning were measured. TMT A, TMT B, TMT B-A and Coding improved at one month after the WBC + SS programme. WBC + SS had a significant effect on the increase in sympathetic nervous system activity in rest. WBC + SS had a significant, positive chronotropic effect on the cardiac muscle. Peripheral and aortic systolic blood pressure decreased one month after WBC + SS in comparison to before. Effects of WBC + SS on reduction of fatigue, indicators of aortic stiffness and symptoms severity related to autonomic nervous system disturbance and improvement in cognitive function were maintained at one month. However, improvement in all three fatigue scales (CFQ, FIS and FSS) was noted in 17 of 22 patients. In addition, ten patients were treated initially but they were not assessed at 4 weeks, and are thus not included in the 22 patients who were examined on follow-up. The overall effects of WBC + SS noted at one month post-treatment should be interpreted with caution.

1. Introduction

Results of studies on the clinical course of Chronic Fatigue Syndrome (CFS) have shown that symptom severity tends to fluctuate over time [1,8,12,17,19,41,52]. For instance, Parslow et al. [43] examined 21 adolescents and their parents. Every patient examined in this sample perceived fluctuations in symptom severity, with symptoms worsening after a bout of increased activity. Some CFS patients report having "good" days followed by "bad" days [9,18]. With surveys suggesting that symptom fluctuation does not occur just in day-to-day timeline, but also it seems to fluctuate throughout longer time frames. The majority of the patients report that they "periodically get better and get worse", but that symptoms "never disappear completely" [51].

Previous studies have examined the effects of non-pharmacological treatments in patients suffering from CFS [11,16,32]. Nevertheless, the therapeutic effectiveness of non-pharmacological treatments,

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including aerobic physical exercise programmes on CFS is controversial [39,54]. What seems important is that the long-term (for at least 2 years follow-up) efficacy of physical exercise programs in CFS patients has been contradictory with a lack of significant improvement in fatigue and disability in those receiving some forms of exercise therapy compared to patients allocated to receive standard medical care [39,54].

When using an intention-to-treat protocol, four percent of patients who received graded exercise therapy could be deemed to have "recovered" [54]. In addition, long-term changes were not statistically significant [54].

Whole-body cryotherapy (WBC) is based on repeated sessions of very brief exposure to an extremely low temperature delivered in a chamber. The temperature in the chamber might range from −100 °C to −130 °C and exposure time lasts up to 3 min [35]. WBC leads to improvement in some domains of cognitive function in a randomized clinical trial on patients with mild cognitive impairment (MCI) [49]. Previous studies have explored the molecular mechanisms that might relate to the clinical efficacy of WBC [5]. At least some of the beneficial actions of WBC might be related to its modulatory role on the autonomic nervous system [31] as well as anti-inflammatory effects [36]. In a previous study, we noted that whole-body cryotherapy and static stretching (WBC + SS) decreased fatigue, symptoms of autonomic function disturbance, improved speed of processing visual information and set-shifting immediately on finishing a therapy program in CFS patients [31]. In the current study, we have explored the sustainable impact (one month after) of WBC + SS on the same sample of CFS patients, as in our previous study [31]. We set out to do this, in order to understand the sustained impact of the intervention in the short-term follow-up (four weeks after finishing WBC + SS program).

2. Materials and methods

2.1. Patients

Sample size calculation was made using GLIMMPSE 3.0.0 on-line available calculator for General Linear Mixed Model Power and Sample Size (available at: https://glimmpse.samplesizeshop.org/#) as described previously [31]. To obtain 32 CFS patients, 250 patients were initially assessed for eligibility. One hundred and eighty subjects were excluded as they did not meet the Fukuda criteria (n = 180), had an underlying psychiatric illness (n = 30), had another diagnosis or fatigue was not the primary complaint (n = 8) [31]. We selected 32 patients with chronic fatigue syndrome from 250 individuals who identified themselves as fatigued. The inclusion criteria for the study were as follows: 1) age between 25 and 65 years, men and women, 2) fatigue more than 6 months, due to unknown causes, 3) at least four additional symptoms: malaise after exertion, impaired memory and/or concentration, headache, unrefreshing sleep, tender lymph nodes (cervical or axillary), sore throat, muscle or joint pain. The exclusion criteria were: present illness that might trigger chronic fatigue (e.g. cardiovascular disease, autoimmune disease or psychosocial causes). Patients could participate in this study if they had been referred by a general practitioner, neurology and psychiatry; pre-test health state assessment included: basic psychiatric and neurological, clinical examination. Physicians experienced in CFS diagnosis confirmed the inclusion and exclusion criteria and checked whether an extensive physical examination and laboratory research tests had been performed to exclude any secondary chronic disorder that might explain primary symptoms. Thus, 32 CFS participated in the WBC + SS intervention (Fig. 1). The study was conducted according to the guidelines of the Declaration of Helsinki and

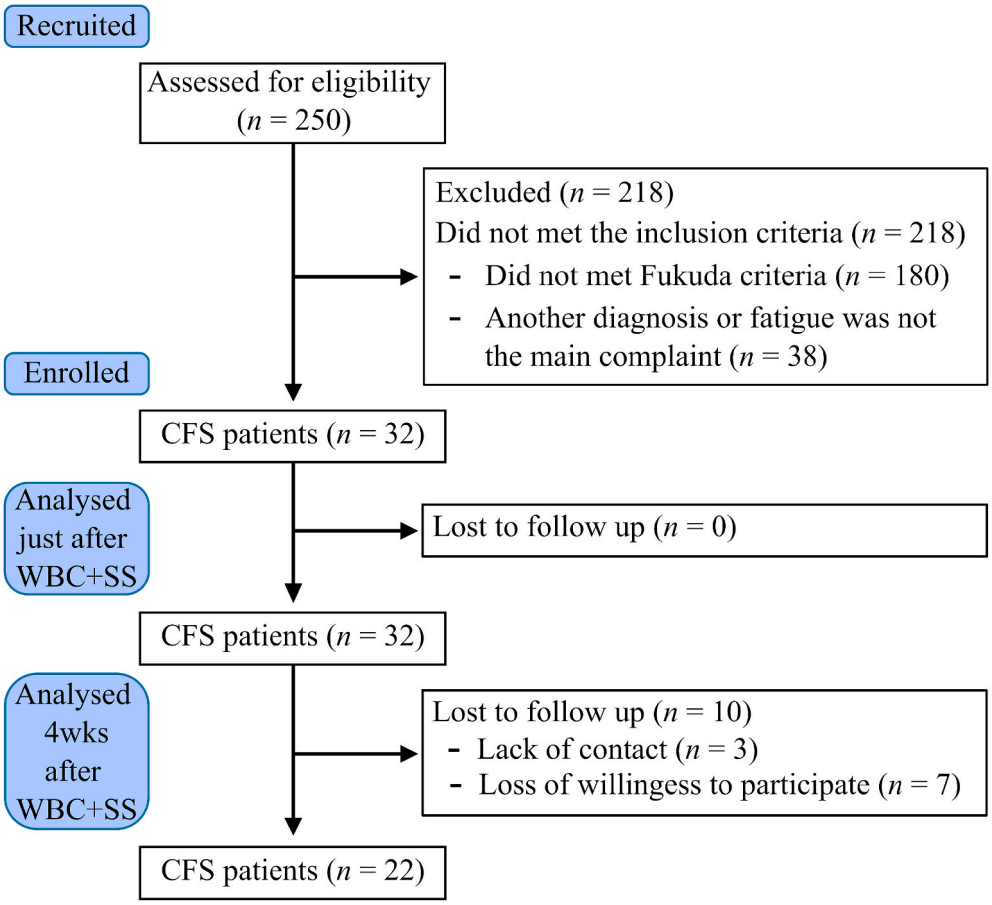

Fig. 1. Flow chart of the study.

approved by Ethics Committee, Ludwik Rydygier Memorial Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń (KB 660/2017). Informed, written consent was obtained from all subjects involved in the study.

2.2. Intervention – whole body-cryotherapy with a static stretching exercise (WBC + SS)

The applied intervention has been described previously [31]. In brief, patients entering the cryochamber were outfitted in swimsuits, face masks to cover their mouths and noses, cotton socks, slippers, and gloves, as well as ear protection and wooden shoes. Before entering the chamber, all jewellery, glasses, and contact lenses were taken off. During the WBC exposure, patients walked round the chamber without touching each other. The temperature in the antechamber was −60 °C, whereas in the proper chamber, it reached −120 °C. Therapy programs consisted of 10 sessions in cryogenic chamber over a period of 12 days (from Monday to Friday, one session per day). Exposure time in a proper chamber ranged from 0.5 to 2.5 min. Exposure time increased from day to day, starting from 30 s during the first session, to 60 s during the fourth session, 90 s on the sixth session, eventually reaching 120 and then 150 s on the last session [31].

Immediately after leaving the cryogenic chamber participants' kinesiotherapy sessions were applied. A single session consisted of breathing exercises and passive stretching exercises of the muscles of the major joints (including the ankle, knee, hip, wrist, elbow, shoulder, thoracolumbar spine, and cervical spine) [2,31]. Each stretch was hold for 20 s, pain induction was avoided. Patients were advised to hold to the point of slight discomfort. Then, 10 s passive rests in a neutral position were applied [60]. All sessions were carried out under the supervision of experienced physiotherapists.

2.3. Measures

The clinical examination was performed in the chronobiology laboratory (temperature 22 °C, humidity 60%, windowless and sound-insulated room) at approximately the same time of day. Patients were assessed before, just after, and one month after WBC + SS.

2.3.1. Fatigue measurements

Chalder Fatigue Questionnaire (CFQ), Fatigue Severity Scale (FSS) and Fatigue Impact Scale (FIS) were applied to assess the severity of fatigue.

The Chalder Fatigue Questionnaire includes 11 items divided into two subgroups– physical ((CFS physical) items 1–7) and mental fatigue ((CFQ mental) items 8–11). The total score ranged from 0 to 33 and a higher score indicates greater fatigue severity [25]. Also, the higher the score in CFQ physical and CFQ mental, the higher the severity of fatigue in physical and mental dimensions, respectively.

The Fatigue Severity Scale (FSS) is composed of nine items that assess the severity of fatigue symptoms on a scale of 1–7 [29]. Therefore, the total score might range from 0 to 63 points and a higher score indicates greater fatigue severity.

The Fatigue Impact Scale (FIS) is composed of forty items. Each item assesses the impact of fatigue on various aspects of life on a 5-point Likert scale (0–4). Therefore, the total score might range from 0 to 160 points, and a higher score indicates a greater impact [20]. Binary categories of improvement (yes or no) were created according to the presence of improvements in CFQ, FIS and FSS (results of the fatigue scale higher at baseline vs. one month after the WBC + SS) vs. no presence of improvement (results of the fatigue scale higher at one month after the WBC + SS vs. at baseline).

2.3.2. Assessment of cognitive function

Cognitive function assessment in the current research has been described in detail previously [31]. In brief, the executive functioning domain: working memory, visuospatial skills and task switching was assessed using Trail Making Test (TMT) [34]. To measure visual search and motor speed skills, Trial Making Test part A (TMT A) was used [34]. To assess cognitive flexibility and executive control Trial Making Test part B (TMT B) was used [3,14,28,46].

Attention, working memory, motor speed, scanning, associative learning and executive functions were measured using Coding [26,34].

2.3.3. Objective assessment of autonomic and cardiovascular function

Heart rate (HR), systolic, diastolic and mean blood pressures (sBP, dBP, mBP), stroke and cardiac indexes (SI, CI), total peripheral resistance index (TPRI), index of contractility (IC), left ventricular ejection time (LVET), left ventricular work index (LVWI), total fluid content (TFC) were calculated as hemodynamic and left ventricular function parameters. In addition, low frequency (LF) and high frequency (HF) of heart rate variability (HRV) and blood pressure variability (BPV) and ratio of LF to HF (LF/HF) served as indicators of autonomic nervous system function. Ratios of LF-dBP/HF-RRI (LF/HF) and LF and HF calculated based on RRI (LF/HF-RRI) were treated as indicators of sympathovagal balance [7]. All parameters were measured in beat-to-beat manner at rest, after giving a proper time for parameters to stabilize using Task Force Monitor (TASK FORCE® MONITOR 3040i SET, CNSystems, Medizintechnik, Graz, Austria). Power spectral analysis for HRV and BPV were calculated using built-in algorithm [7,21,22]. SBP, dBP and mBP were automatically measured in a continuous beat-to-beat manner using vascular unloading technique. The heart rate (HR) was calculated as time distance between 2 R-peaks in the electrocardiogram (ECG) (sampling frequency: 1000 Hz). Impedance cardiography (ICG) technique was applied to derivate TFC, SI, TPRI, IC, LVET (the time interval from the opening to the closing of the aortic valve [mechanical systole]). CI was calculated using ICG to calculate SV and ECG to calculate HR. Then, cardiac output was divided by a body surface area of a patient. LVWI was calculated from CI and mPB. Pulmonary Artery Occlusion Pressure is fixed at 7 mmHg. All objective parameters describing autonomic and cardiovascular function were calculated using in-built algorithms.

2.3.4. Arterial stiffness measurement

To analyse indicators of aortic stiffness in a non-invasive manner, Arteriograph (TensioMed Software v.1.9.9.2; TensioMed, Budapest, Hungary) was used. Pulse wave velocity (PWV aortic) augmentation index (Aix aortic) and aortic sBP (sBP aortic) [6,48].

2.3.5. Body composition analysis

Multi-frequency bioelectrical impedance analyzer (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.) was used to assess body composition [53]. First, body mass in kilograms and weight were measured. BMI was calculated as follows: (weight [kg]/height^2 [m^2]). Based on the in-built algorithms available in bioelectrical impedance analyser weight of fat-free mass and bone mass in kilograms and visceral fat level in units were estimated.

2.3.6. Statistical analysis

All data are presented as mean ± SD. The assumption on normal distribution of the variables was examined with the Shapiro-Wilk test and by visual inspection of histograms. The assumption of homoscedasticity was examined using Levene's test. If both assumptions were met then, Independent *t*-test was applied to compare the subgroup which was re-examined on follow-up vs not examined. Otherwise, the Mann-Whitney *U* test was applied. The assumption of sphericity was examined using Mauchly's Test of Sphericity. Data on effects of WBC + SS (before vs after vs follow-up) on was submitted to analysis of variance (ANOVA) with repeated measures and post hoc analysis using paired *t*-test and FDR correction of p-value was applied, if all of the three assumptions were met. Effects size (omega squared (ω_p^2) and confidence interval (CI) [−95%; 95%] for effect size) were calculated. In the case of

violation of the assumption of sphericity, then correction was applied. In the case of violation of normality assumption, then Friedman rank sum test was used and post hoc analysis using Durbin-Conover with FDR adjustment of p-values. Effect size (Kendall's coefficient of concordance (*WKendall)*) and CI [−95%; 95%] for effect size) were calculated. Violin graphs and effect size calculation for both tests were done using R with a ggstatsplot package (ver. 0.6.5) [13,44].

3. Results

3.1. Comparison of patients who participated in follow-up vs not

32 patients (25 females) underwent WBC + SS. The disease duration was 3.7 ± 2.9 years.

All patients were current non-smokers. Twenty-two patients (seventeen females) participated in the follow-up phase of the study, while ten patients (eight females) were not examined on the follow-up. There was no statistically significant difference at the baseline between patients in symptoms duration (3.3 ± 2.4 years in patients who were examined at the follow-up vs 4.5 ± 3.7 years in patients who did not show up on the follow-up examination).

Table 1
Baseline comparison of patients examined on follow-up vs not. Abbreviations explanation is available in the appendix. P-values less than 0.05 are considered significant.

Parameter [units]	Examined on follow-up (n = 22)	Not examined on follow-up (n = 10)	p-value
Age [years]	37.2 ± 8.1	35.7 ± 9.5	0.65
BMI [kg/m^2]	25.6 ± 3.1	23.0 ± 4.1	0.16
Body fat [%]	29.1 ± 7.5	24.6 ± 6.9	0.11
Fat-free mass [kg]	53.1 ± 7.7	48.8 ± 10.3	0.20
Visceral fat level [units]	5.1 ± 2.3	3.7 ± 2.5	0.15
Bone mass [kg]	2.7 ± 0.4	2.5 ± 0.5	0.19
TMT A [seconds]	23.1 ± 6.6	22.78 ± 5.3	0.89
TMT B [seconds	51.0 ± 13.2	48.22 ± 16.3	0.74
TMT B-A [seconds	27.9 ± 11.3	25.44 ± 12.2	0.79
Coding I min symbols to go [symbols to go]	53.9 ± 10.4	50.22 ± 6.7	0.12
Coding II min symbols to go [symbols to go]	14.3 ± 11.7	8.78 ± 10.6	0.19
Coding time of completion [seconds]	111.5 ± 1.3	116 ± 3.8	0.07
CFQ [points]	20.7 ± 3.7	25 ± 4.9	0.01
CFQ physical [points]	13.7 ± 3	16.1 ± 3.1	0.05
CFQ mental [points]	7.0 ± 1.7	8.9 ± 2.3	0.01
FIS [points]	51.1 ± 24.4	61.9 ± 23.3	0.25
FSS [points]	44.2 ± 9.6	48 ± 5.5	0.39
PWVaortic [m/s]	8.3 ± 1.9	8.8 ± 2.6	0.82
Aix aortic [%]	27.7 ± 12.3	31.07 ± 13	0.26
SBPaortic [mmHg]	138.3 ± 19.5	127.59 ± 10.3	0.11
HR [n/1]	68.9 ± 8.6	72.3 ± 9.1	0.32
sBP [mmHg]	118.1 ± 8.9	113.41 ± 11.1	0.21
dBP [mmHg]	79.6 ± 9.1	78.4 ± 9.8	0.73
mBP [mmHg]	96.5 ± 8.5	94.25 ± 10.1	0.52
SI [ml/m^2]	53.2 ± 13.8	56.43 ± 9.9	0.51
CI [l/min/m^2]	3.7 ± 1	4.03 ± 0.6	0.28
TPRI [dyne*s*m2/cm5]	2243.1 ± 735.1	1873.44 ± 404.5	0.15
IC [1000/s]	63.4 ± 23.6	71.85 ± 15.2	0.31
LVWI [mmHg*l/[min*m^2]]	4.7 ± 1.2	5.02 ± 0.8	0.40
LVET [ms]	317.6 ± 12	315.05 ± 12.4	0.59
TFC [1/Ohm]	31.6 ± 4.7	33.43 ± 5.5	0.35
LFnu-RRI [%]	53.8 ± 18.2	56.24 ± 14	0.71
HFnu-RRI [%]	46.2 ± 18.2	43.76 ± 14	0.71
LF/HF-RRI [n/1]	2.1 ± 3.2	1.65 ± 1.1	0.70
LF/HF [n/1]	1.8 ± 2.3	1.23 ± 0.7	0.61
LFnu-dBP [%]	53.7 ± 14.5	44.72 ± 16.5	0.13
HFnu-dBP [%]	14.1 ± 10.4	11.61 ± 8.7	0.38
LF/HF-dBP [n/1]	6.0 ± 4.3	6.74 ± 5.2	0.89
LFnu-sBP [%]	43.8 ± 13.4	36.2 ± 17.4	0.19
HFnu-sBP [%]	15.2 ± 12.1	15.5 ± 4	0.40
LF/HF-sBP [n/1]	4.4 ± 2.7	2.5 ± 1.4	0.049

As shown in Table 1, patients examined in the follow-up phase had significantly less fatigue measured by an overall score of CFQ and physical and mental components and apparently higher LF/HF-sBP. There were no statistically significant differences in the baseline comparison in the rest of the measured parameters in patients who were examined at the follow-up vs in patients who did not show up on the follow-up examination, $p > 0.05$.

All of the following results are provided for within-group comparison of patients that completed measurement just after WBC + SS and at one-month follow-up (22 patients).

3.1.1. Effects of WBC + SS after 1-month follow-up

3.1.1.1. Fatigue. Fatigue measured by the three scales used decreased after WBC + SS and at the one-month follow-up scores were still significantly lower than before WBC + SS (effect of WBC + SS on CFQ score: Chi-Square = 28.5, $p < 0.0001$, *WKendall*=0.44 [0.44; 1]). CFQ score after WBC + SS was 7.5 ± 5.3 and 7.3 ± 6.5 one month after WBC + SS, which was significantly lower than before WBC + SS 20.7 ± 3.7 (both p-values <0.0001) (Fig. 2A). Similarly, FIS score decreased (Chi-Square = 14.3, $p = 0.001$, *WKendall*=0.61 [0.61; 1]) from 52.2 ± 23.6 before WBC + SS to 38.8 ± 21 points after WBC + SS, $p = 0.02$, and at the one month follow-up FIS score was 28.0 ± 25.2, which was lower than before WBC + SS ($p = 0.0002$) (Fig. 2B). In addition, FSS score decreased (Chi-Square = 9.6, $p = 0.008$, *WKendall*=0.52 [0.52; 1]) from 44.8 ± 9.8 before WBC + SS to 38.6 ± 9.3 after WBC + SS, $p = 0.02$, and at the one month follow-up it was 36.5 ± 9, which was lower than before WBC + SS ($p = 0.007$) (Fig. 2C). However, high variance in response from individual patients was observed in the changes of fatigue scales scores in response to WBC + SS (Fig. 1A).

Table 2 presents results from a binary classification (yes or no) according to the presence of improvement (result of the fatigue scale higher at baseline vs at one month after the WBC + SS) vs no presence of improvement (result of the fatigue scale higher at one month after the WBC + SS vs at baseline. Seventeen CFS patients out of twenty-two have noted improvement (i.e. decrease) in all three fatigue scales at one month after WBC + SS comparing to baseline (Table 2).

3.1.1.2. Cognitive function. Both TMT A, TMT B, TMT B-A, Coding I min, Coding II min improved in response to WBC + SS programme (TMT A: Chi-Square = 27.2, $p < 0.0001$, WKendall = 0.7 [0.7; 1], TMT B: Chi-Square = 20.7, $p < 0.0001$, WKendall = 0.66 [0.66; 1], TMT B-A: Chi-Square = 9.5, $p = 0.009$, WKendall = 0.63 [0.63; 1], Coding I min: Chi-Square = 31.4, $p < 0.0001$, WKendall = 0.82 [0.82; 1], Coding II min: Chi-Square = 29.8, $p < 0.0001$, WKendall = 0.84 [0.8; 1]).

Symbols left in Coding test after I minute decreased in response to WBC + SS from 53.9 ± 10.4 symbols left before to 48.6 ± 5.6 after WBC + SS ($p = 0.00002$). At the one-month follow-up symbols patients had 45.0 ± 6.6 left in Coding test after I minute in coding, which was lower than in comparison to before WBC + SS ($p < 0.0001$) and in comparison to just after WBC + SS ($p < 0.0001$) (Fig. 3A). Similar pattern of changes was observed in Coding score after II minutes (Fig. 3B).

TMT A score decreased from 23.6 ± 6.3 s before WBC + SS to 18.4 ± 4.6 s after WBC + SS, $p < 0.0001$, and after one month after WBC + SS, TMT A score was lower than before WBC + SS (16.4 ± 5.0 s) ($p < 0.0001$) and in comparison to just after WBC + SS ($p = 0.002$) (Fig. 3C). WBC + SS influence on decrease of TMT B score just after WBC + SS in comparison to before ($p = 0.001$) and at the one-month follow-up in comparison both to before WBC + SS ($p < 0.0001$) and after WBC + SS ($p = 0.02$) (Fig. 3D). Moreover, TMT B-A decreased at the one month follow-up comparing to before ($p = 0.005$) (Fig. 3E).

3.1.1.3. Cardiac, autonomic and self-reported measures. WBC + SS had a significant, positive chronotropic effect on the cardiac muscle. Significant effect of WBC + SS on HR was noted ($F = 6.9$, $p = 0.004$, $\omega_p^2 = 0.07$

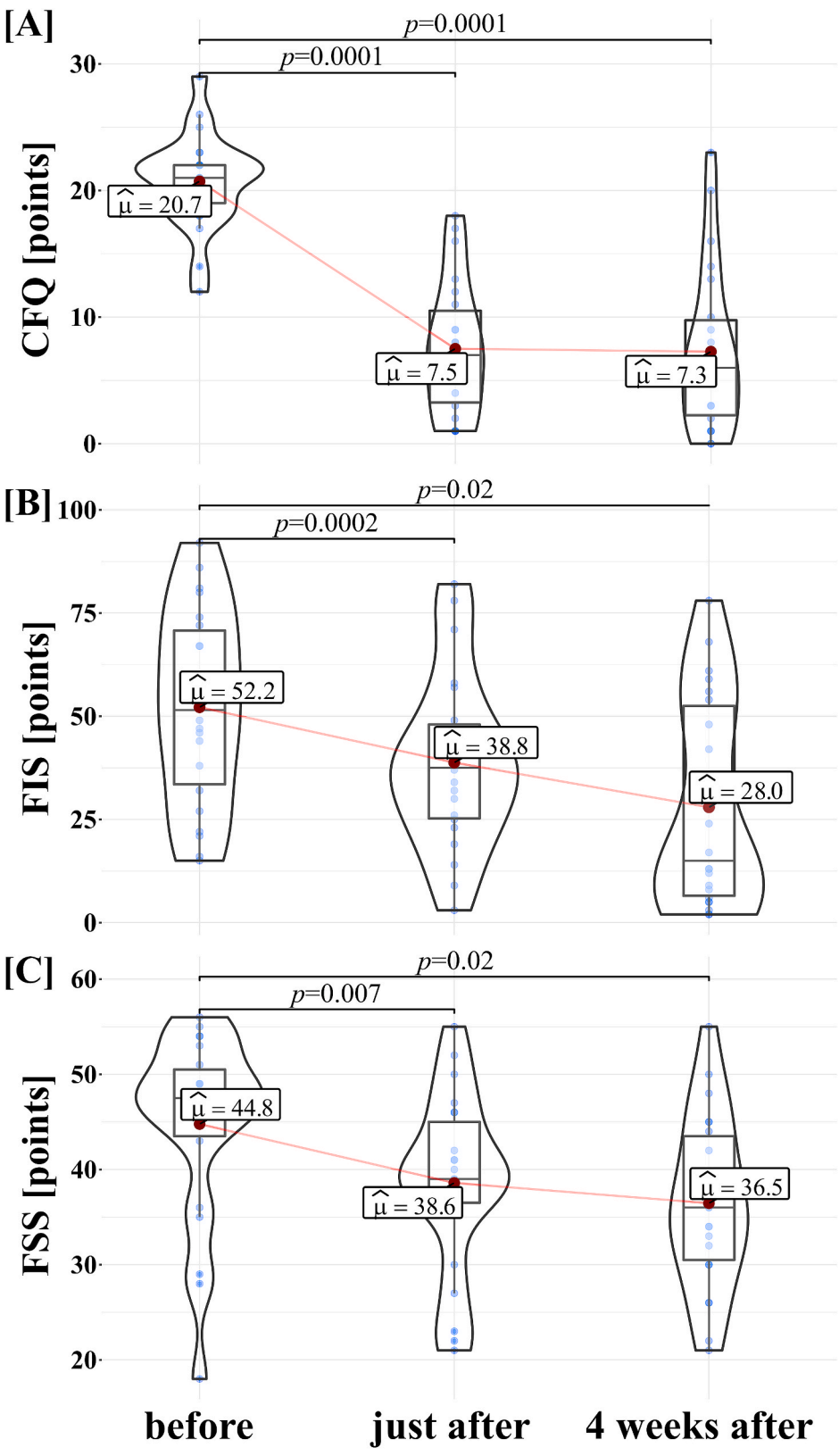

Fig. 2. CFS group mean values ± SD before-, after, and follow-up WBC + SS intervention for fatigue scale results. [A] CFQ Total (Chalder Fatigue Scale), [B] FIS (Fatigue Impact Scale) [C] FSS (Fatigue Impact Scale). Red dots connected by the red line indicate the mean value, horizontal black line inside the box denotes median value. Blue dots denote scores of individual patients. The shape of the violin graph indicates the distribution of results. P-values from post-hoc testing are provided. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[0; 0.23] (Fig. 4A)). Post-hoc analysis indicates that HR was higher at the one-month follow-up than before WBC + SS 74.9 ± 7.7 bpm vs 68.8 ± 8.6 before, p = 0.001. In addition, a significant effect of WBC + SS was noted on LVET (F = 7.6, p = 0.002, ω_p^2 = 0.09 [0; 0.27]. Post-hoc analysis revealed that LVET was significantly lower at the one-month follow-up than before WBC + SS (309.4 ± 10.5 ms vs 318.7 ± 11.0, p = 0.005) (Fig. 4B). Significant effect of WBC + SS on LVWI was noted (F = 3.8, p = 0.03, ω_p^2 = 0.02 [0; 0.13]). LVWI decreased after WBC + SS in comparison to before 4.3 ± 1.1 vs 4.8 ± 1.2 [mmHg*l/[min*m^2]], p = 0.03 (Fig. 4C). No significant changes at the one-month follow-up were

Table 2
Binary categories of improvement (yes or no) were created according to the presence of improvements in CFQ, FIS and FSS (results of the fatigue scale higher at baseline vs. one month after the WBC + SS) vs. no presence of improvement (results of the fatigue scale higher at one month after the WBC + SS vs. at baseline). 10 of 32 original participants did not participate in the fourth week assessments.

Improvement in CFQ score	Counts	% of Total
no	1	5%
yes	21	95%
Improvement in FIS score		
no	2	9%
yes	20	91%
Improvement in FSS score		
no	5	23%
yes	17	77%
Improvement in FIS and FSS		
no	4	18%
yes	18	82%
Improvement in CFQ, FIS and FSS		
no	5	23%
yes	17	77%

noted in LVWI with a tendency to return to baseline values.

Both sBP ($F = 4.8$, $p = 0.01$, $\omega_p^2 = 0.03$ [0; 0.17]) (Fig. 4D), and mBP ($F = 5.2$, $p = 0.01$, $\omega_p^2 = 0.03$ [0; 0.16]) (data not shown) were reduced in response to WBC + SS. Systolic blood pressure decreased one month after WBC + SS in comparison to before (117.4 ± 9.2 before in comparison to 112.0 ± 11.8 mmHg one month after WBC + SS ($p = 0.007$) (Fig. 4D).

WBC + SS had a significant effect on increase in sympathetic nervous system activity in rest in LFnu-RRI ($F = 8.0$, $p = 0.001$, $\omega_p^2 = 0.07$ [0; 0.23]), in comparison to parasympathetic part (HFnu-RRI ($F = 8.0$, $p = 0.001$, $\omega_p^2 = 0.07$ [0; 0.23]). WBC + SS increased LFnu-RRI both in comparison baseline vs to just after WBC + SS 53.9 ± 18.3 vs 57.0 ± 18.3% ($p = 0.001$) and baseline vs at the one-month follow-up (53.9 ± 18.3 vs 65.8 ± 16.9% ($p = 0.02$) (Fig. 4E), with similar changes in HFnu-RRI (Fig. 4F). Moreover, indicators of sympathovagal balance increase in response to WBC + SS (LF/HF-RRI, Chi-Square = 9.9, $p = 0.007$, *WKendall*=0.77 [0.77; 1]; LF/HF, Chi-Square = 13.4, $p = 0.001$, *WKendall*=0.78 [0.78; 1]). In addition, LF/HF-RRI increased one month after WBC + SS in comparison to before (3.2 ± 3.0 vs 2.1 ± 3.2 before, $p = 0.008$) and in comparison to just after WBC + SS ($p = 0.01$) (Fig. 4G). Similar pattern of effects of WBC + SS was observed in the case of LF/HF (Fig. 4H).

3.1.1.4. Arterial stiffness assessment. Aortic systolic blood pressure decreased both just after and after 1-month follow-up after WBC + SS, when compared to before WBC + SS (Chi-Square = 9.3, $p = 0.01$, *WKendall*=0.66 [0.66; 1]). Aortic systolic blood pressure was on mean 138.6 ± 19.9 mmHg before vs 129.4 ± 14.3 mmHg just after ($p = 0.02$) and 138.6 ± 19.9 mmHg before vs 127.5 ± 14.3 mmHg after one month ($p = 0.009$) (Fig. 5). However, the PVW aortic did not show significant changes after 1 month follow-up WBC + SS intervention, $p > 0.05$.

4. Discussion

In a previous study, we have shown that a program composed of whole-body cryotherapy and static stretching led to a decrease in fatigue and autonomic nervous symptoms. In addition, the speed of processing visual information and set-shifting also improved in response to the program. All these effects were measured immediately on therapy completion [31]. In the current study, we have shown that the effect of this program on fatigue-related symptoms reduction was maintained for 17 of 22 patients assessed at one month. However, ten CFS patients from thirty-two patients that underwent therapy, did not participate in the follow-up phase. This subgroup was characterized by higher fatigue at the baseline indicated by one of the three applied fatigue scales and higher LF/HF-sBP. In a study by Maclachlan et al., higher LF/HF-sBP in CFS patients was interpreted as an indicator of greater sympathetic activity [37]. Taking into account baseline differences between patients who showed up on the one-month follow-up vs not, and the relatively small sample size (22 patients) who were examined one month after the therapy, the suitability of effects of WBC + SS should be interpreted with caution.

4.1. Tolerability of cold exposure in CFS patients

Cold intolerance seems to be commonly noted by patients with CFS [47,55] and is mentioned as a neuroendocrine symptom in the Canadian Consensus Criteria [10]. Nevertheless, no medium-term complications or side effects of WBC + SS were noted in the current study. In the current study, the tolerability of WBC + SS by patients was high. Patients examined in the follow-up phase had significantly less fatigue measured by overall score of CFQ and physical and mental components and apparently higher LF/HF-sBP. There were no statistically significant differences in the baseline comparison in the rest of measured parameters in patients who were examined at the follow-up vs in patients who did not show up on the follow-up examination. Cutell et al. observed that higher body fat percentage might be related to a greater decrease in skin temperature noted 35 min after WBC exposure [15]. Presumably, because of the between-sex differences (i.e. tendency for females to have a higher body fat percentage in comparison to males) sex differences in immediate reaction to WBC might be observed [15]. In the current study, baseline comparison showed no significant differences in body composition between patients who appeared on follow-up examination vs not. Data on immediate WBC exposure was not measured in this study. In addition, no data on patients' views on the WBC + SS benefits was obtained, however, some patients articulated that they would like to repeat the therapy as soon as possible indicating good tolerability of the therapy for them.

4.2. Symptoms severity fluctuation in CFS patients

In the current study, a high heterogeneity of response on WBC + SS was noted. Overall, seventeen CFS patients of twenty-two (77%) have noted improvement (i.e. decrease) in all three fatigue scales one month after WBC + SS compared to baseline. In our previous study, we applied cluster analysis on the cohort of CFS patients and four groups with distinct autonomic profiles were identified [50]. Arguably this indicates initial evidence on the existence of distinct profiles in patients with CFS. If this were validated in further studies, then a difference in response to a particular therapy might be observed in relation to a patient profile. In line with that, in our previous study, we created a model that might predict the likelihood of completion of aerobic physical activity programmes in CFS patients based on baseline values obtained before taking part in the intervention [30]. Further studies should also examine predictors of WBC + SS completion and effectiveness to better tailor treatment to a particular patient.

A potential confounding factor of the above results is the tendency to fluctuation in symptoms of CFS patients. Therefore, changes in parameters observed in the current study might be not influenced effects of WBC + SS sustained for one month after the therapy only. Nevertheless, the individual experience in terms of how they describe it and the degree and impact varies. In a study in which CFS patients were monitored up to 3 years, the majority of patients initially diagnosed as CFS underwent partial or total remission or sustained total remission (56.9% and 10% of patients, respectively) [41]. However, patients suffering from CFS were recruited in the study using a random-digit-dialling survey [41], therefore there might be some limitations in extrapolating results from this

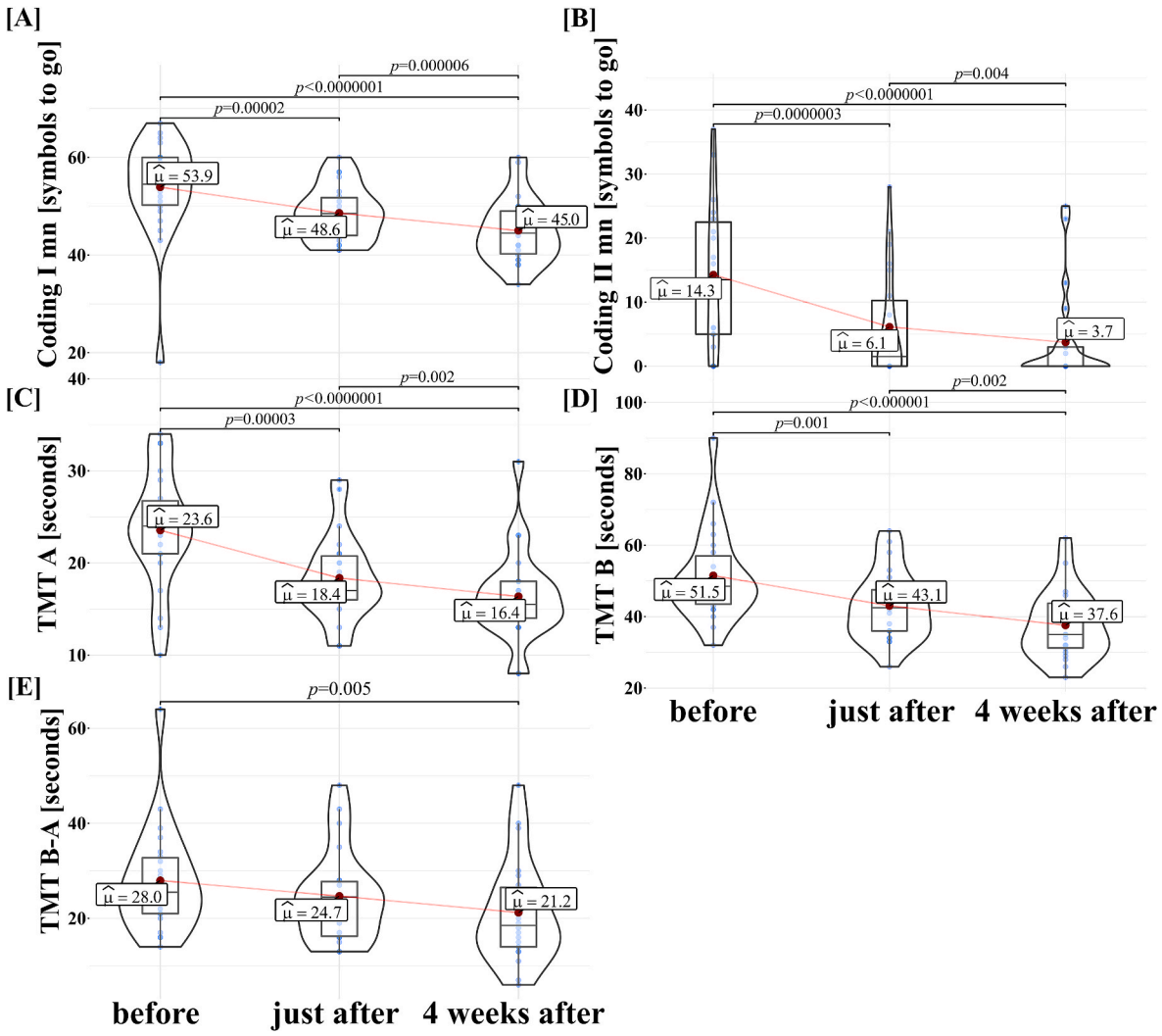

Fig. 3. Effects of WBC + SS on cognitive function in CFS. [A] Coding I minute – number of symbols left after I minute of execution of Coding test; [B] Coding II minute – number of symbols left after II minutes of execution of Coding test [C] TMT A – time of execution of Trial Making Test part A; [D] TMT B - time of execution of TMT B Trial Making test part B; [E] TMT B-A - Difference in time of execution between Trail Making Test par B and A. Red dots connected by the red line indicate the mean value, horizontal black line inside the box denotes the median value. Blue dots denote scores of individual patients. The shape of the violin graph indicates the distribution of results. P-values from post-hoc testing are provided. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

study to a general population. In a study on 150 subjects fitting Fukuda 1994 CFS, 4% experienced decrease of symptoms severity over time and 50% perceived symptoms fluctuation. In a survey based on 551 subjects 1.1% experienced symptoms severity decrease and more than a half of patients described symptoms course as "fluctuating/remitting/relapsing" and 27% as "worsening" [12]. Proportions of CFS patients that experience health state improvement over time range from 7% [19] to 5% [8,52]. Higher recovery rates were reported in individual studies, however, such results seem to be not accurate because of differences in definitions of recovery [1,17]. Comparing the clinical course (over a year) in patients with Long COVID (post-acute sequelae of COVID-19) to CFS patients it was observed that symptom severity decreased in Long COVID patients which was not the case in CFS patients [42]. A longitudinal study on Australian ME/CFS cohort over five months showed symptoms such as muscle pain and weakness, orthostatic intolerance and intolerance to extreme temperatures tended to fluctuate over time [4]. Overall, it seems that fluctuation of symptom severity is a common feature in patients suffering from CFS [4,27,38]. Patients might report some days as "better" or "worse" even in a relatively short period of time, as 10 days [18], which presumably indicates an existence of daily fluctuation of symptoms. Unfortunately, due to the lack of a control group in the current study composed of patients with CFS undergoing no intervention or sham-intervention, it is impossible to say if the noted decrease in symptoms severity in the current study is related to the intervention itself or more to this natural fluctuation of symptoms severity in CFS.

4.3. Further studies

The sample examined in the current study consisted of patients with moderate to medium symptom severity, based on the results in fatigue measurement scales. All patients were able to participate in therapy without external assistance. Further studies should explore whether the route through which cold therapy is delivered (whole-body cryotherapy,

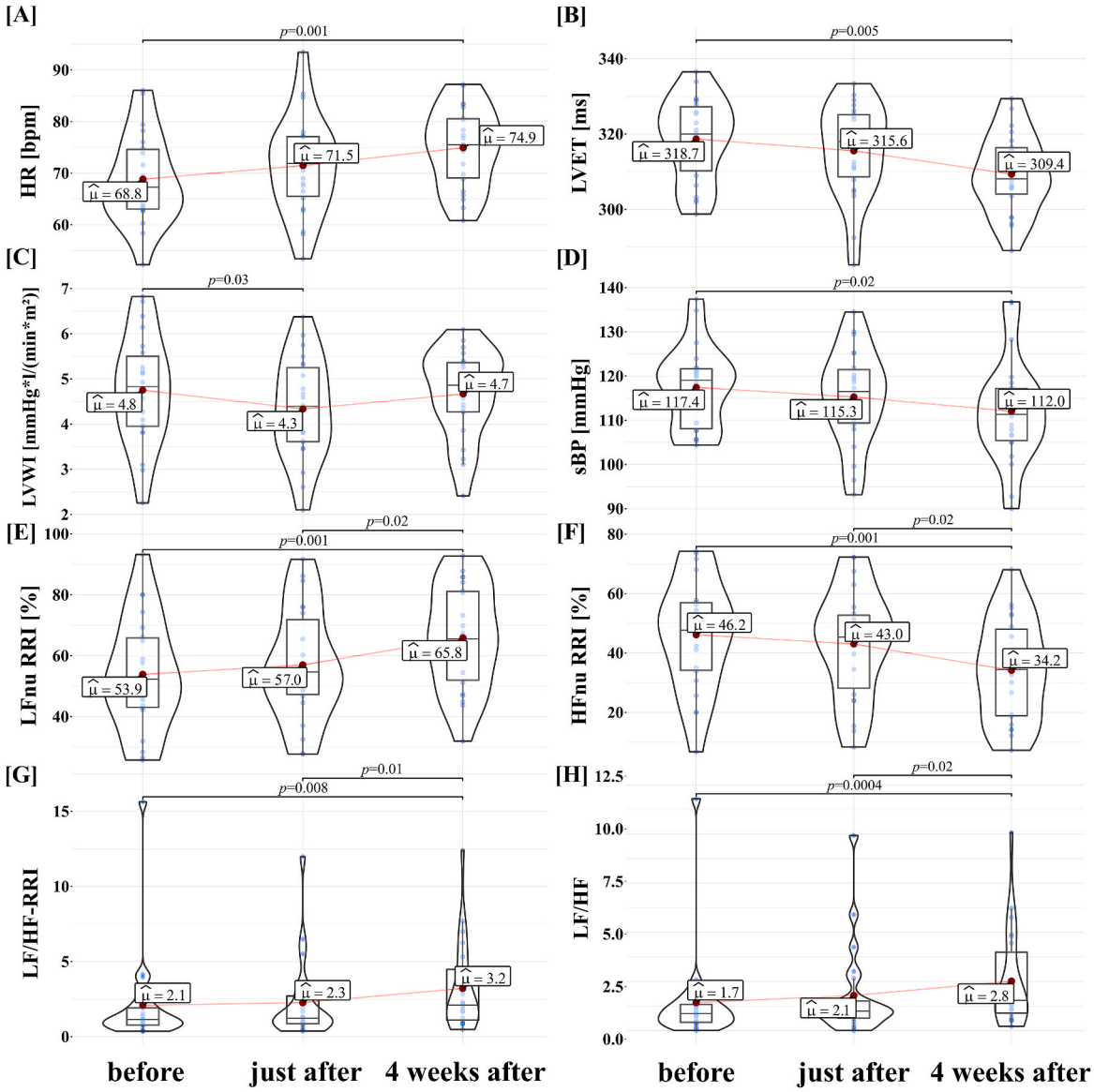

Fig. 4. Effects of WBC + SS on hemodynamic, autonomic and vascular parameters. [A] HR – heart rate measured in beats per minute; [B] LVET – left ventricular ejection time – time for ejection of blood from left ventricle in milliseconds; [C] LVWI – left ventricular work index – an indicator of physical work undertaken by left ventricle; [D] – sBP – systolic blood pressure measured in mmHg; [E] LFnu RRI – low-frequency normalized units of R-R interval in %; [F] HFnu RRI – high-frequency normalized units of R-R interval in %; [E] LF/HF-RRI – low to high frequency R to R interval – indicator of sympathovagal balance; [F] LF/HF – low frequency dBP to high frequency RRI - indicator of sympathovagal balance. Red dots connected by the red line indicate the mean value, horizontal black line inside the box denotes median value. Blue dots denote scores of individual patients. The shape of the violin graph indicates the distribution of results. P-values from post-hoc testing are provided. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

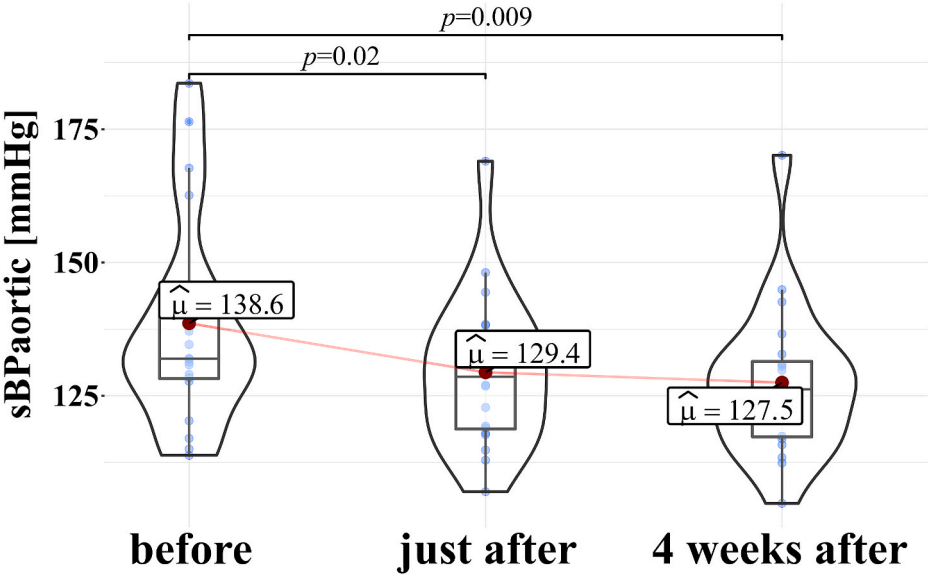

Fig. 5. Effects of WBC + SS on aortic systolic blood pressure. sBPaortic – aortic systolic blood pressure measured in mmHg. Red dots connected by the red line indicate the mean value, horizontal black line inside the box denotes the median value. Blue dots denote scores of individual patients. The shape of the violin graph indicates the distribution of results. P-values from post-hoc testing are provided. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

partial-body cryotherapy using cryousauna, etc.) is more or less likely to induce side effects such as post-exertional malaise (PEM) in CFS patients with higher severity of symptoms. PEM could be triggered by cognitive, physical, emotional or social activity and is related to worsening of symptoms [40]. In general, it is recognised that PEM induction by a rehabilitation method could be counterproductive to the clinical efficacy of a program [24]. Further studies should assess more precisely if PEM could be triggered mainly by physical exercise, for instance by some mechanism specific to the response to physical exercise session on systemic and/or molecular level, or by all stressors that induce the response from the autonomic nervous system. Both heat exposure (as in sauna) and cold (as in WBC) might alter autonomic activity [45,56,57]. However, it seems that during a session of sauna exposure an increase in HR is observed [33,59], while a decrease in HR was noted just after WBC exposure [58]. The safety of both response to heat and cold should be examined in patients suffering from CFS in further studies. As the exact dynamics of sympathetic and parasympathetic activity in response to heat and cold exposure might differ, it might be the case that some types of thermal therapy would not trigger PEM. If cold therapy does not induce PEM, then the effects of a more widely-available cold exposure method could be measured, such as cold showers. However, data on such interventions is scarce even in a general population, largely because variables (intensity, area of exposure, duration) are hard to control for. In the current study, static stretching was applied after the WBC exposure. The recent NICE guidelines recommends incorporating movement that helps to maintain joint and muscle flexibility without worsening symptoms of ME/CFS [40]. Clinically, it is important to apply cold therapy in combination with other adjunctive therapies as suggested in the recent NICE guidelines [40]. An active control group composed of ME/CFS patients should also be included. In addition, further longitudinal studies assessing multiple subjective and objective parameters would be needed to assess whether there are factors related to symptoms severity fluctuation. Ideally, some of these parameters might be assessed using continuous measurement of physiological parameters such as blood glucose, blood pressure, heart rate etc. In addition, both subjective and objective measurements of the brain, cardiac, skeletal muscle, level of cytokines, indicators of dysfunction of the immune system, mitochondria, autonomic nervous system, and sleep should be incorporated. In further studies, it might be of importance to apply more recent criteria for ME/CFS diagnosis.

4.4. Study limitations

In the current study, data from just one follow-up (one month after the therapy) was obtained. In addition, no control group was applied, which might underwent WBC or SS alone. CFS diagnosis was made based on Fukuda criteria [23] in which post-exertional malaise presence is not required for CFS diagnosis.

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Declaration of competing interest

The authors declare no conflict of interest.

Appendix

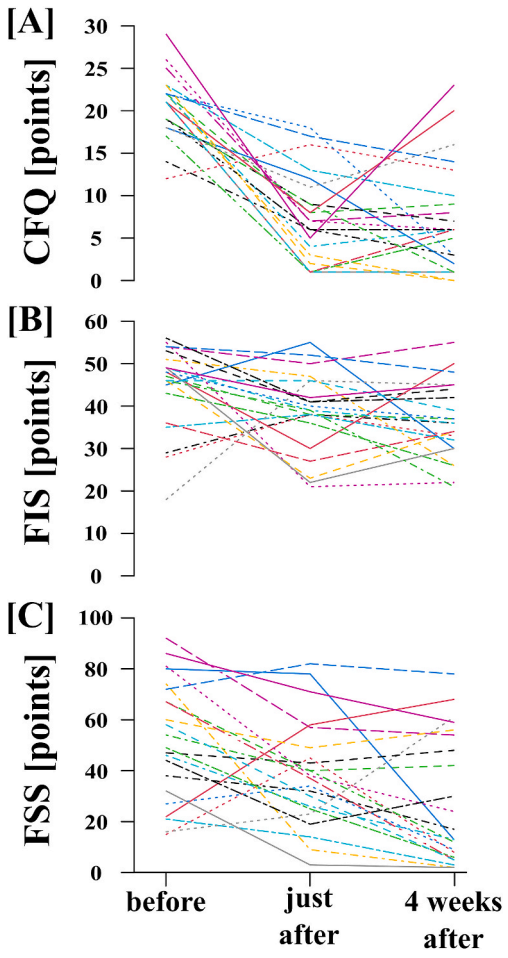

Fig. 1A. Spaghetti plot showing the dynamics before, after, and at one month follow-up after WBC + SS intervention for fatigue scale results. [A] CFQ Total (Chalder Fatigue Scale), [B] FIS (Fatigue Impact Scale) [C] FSS (Fatigue Impact Scale). Individual patient data is shown by an individual line

List of abbreviations

Aix aortic augmentation index
ANOVA analysis of variance
BPV blood pressure variability
CFQ Chalder Fatigue Questionnaire
CFQ MENTAL Chalder Fatigue Questionnaire mental dimension
CFQ PHYSICAL Chalder Fatigue Questionnaire physical dimension
CFS Chronic Fatigue Syndrome
CI cardiac index
CI confidence interval
dBP diastolic blood pressure
FIS Fatigue Impact Scale
FSS Fatigue Severity Scale
HFnu high frequency normalized units
HFnu-dBP high Frequencies normalized units calculated from dBP
HFnu-RRI high Frequencies normalized units calculated from RRI
HFnu-sBP high Frequencies normalized units calculated from sBP
HR Heart rate
HRV heart rate variability
IC index of contractility
LF/HF LF-dBP/HF-RRI ratio
LF/HF-dBP LF/HF ratio, both LF and HF calculated based on dBP
LF/HF-RRI LF/HF ratio, both LF and HF calculated based on RRI
LF/HF-sBP LF/HF ratio, both LF and HF calculated based on sBP
LFnu low frequencies normalized units calculated from RRI

LFnu-dBP low frequencies normalized units calculated from dBP
LFnu-sBP low frequencies normalized units calculated from sBP
LVET left ventricular ejection time
LVWI left ventricular work index
mBP mean blood pressure
mmHg millimetres of mercury
PEM post-exertional malaise
PWV aortic Pulse wave velocity
RRI The interval between R waves in the electrocardiogram
sBP systolic blood pressure
sBP aortic aortic systolic blood pressure
SI stroke index
SS static stretching
TFC total fluid content
TFM Task Force Monitor
TMT A Trial Making test part A
TMT B Trial Making test part B
TMT B-A Difference between Trial Making test part A and B
TPRI total peripheral resistance index
WBC whole-body cryotherapy

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