Pain Physician 2018; 21:E13-E24 • ISSN 2150-1149

Randomized Trial

Cerebral Blood Flow and Heart Rate Variability in Chronic Fatigue Syndrome: A Randomized Cross-Over Study

Anneleen Malfliet, PT, MSc[1,2,12], Roselien Pas, PT, MSc[1,2], Raf Brouns, MD, PhD[4,5], Joris De Win, MD[6], Samar M. Hatem, MD, PhD[2,7-9], Mira Meeus, PT, PhD[1,10,11], Kelly Ickmans, PT, PhD[1-3], Robbert-Jan van Hooff, MD, PhD[4,5], and Jo Nijs, PT, PhD[1-3]

From: [1]Pain in Motion International Research Group, www.paininmotion.be; [2]Department of Physiotherapy, Human Physiology and Anatomy (KIMA), Vrije Universiteit Brussel, Belgium; [3]Department of Physical Medicine and Physiotherapy, University Hospital Brussels, Belgium; [4]Department of Neurology, Universitair Ziekenhuis Brussel, Belgium; [5]Center for Neurosciences (C4N), Vrije Universiteit Brussel, Belgium; [6]Department of Physical Medicine and Rehabilitation, University Hospital Gent, Ghent University, Belgium; [7]Clinic of Physical Medicine and Rehabilitation, Brugmann University Hospital, Belgium; [8]Physical Medicine and Rehabilitation, Vrije Universiteit Brussel, Belgium; [9]Systems & Cognition division (COSY), Institute of Neuroscience, Université catholique de Louvain, Belgium; [10]Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Belgium; [11]Department of Rehabilitation Sciences and Physiotherapy, University of Antwerp, Belgium; [12]FWO - Research Foundation Flanders, Brussels, Belgium

Address Correspondence:
Anneleen Malfliet, PT, MSc
Vrije Universiteit Brussel
Brussels Health Campus, Building F-Kine,
Laarbeeklaan 103,
BE-1090 Brussels, Belgium
E-mail:
anneleen.malfliet@vub.ac.be

Disclaimer: The study was funded by ME Research United Kingdom, a national charity funding biomedical research into Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. The funder did not have any influence in the study design, the collection or analysis of the data, or the conception of this manuscript.

Manuscript received:03-10-2017
Revised manuscript received:
05-19-2017
Accepted for publication:
06-27-2017

Free full manuscript:
www.painphysicianjournal.com

Background: Pain, fatigue, and concentration difficulties are typical features of chronic fatigue syndrome (CFS). The exact underlying mechanisms of these symptoms are still unknown, but available evidence suggests an important role for impaired pain modulation. As evidence also suggests that pain modulation is related to cardiovascular mechanisms, it seems logical to investigate whether cerebral blood flow (CBF) and heart rate variability (HRV) are altered in these patients.

Objectives: We aimed to investigate the role of the cardiovascular system in pain modulation and symptoms of CFS; the response of CBF and HRV to physical stress and their relation to the change in temporal summation (TS) of pressure pain and self-reported symptoms was evaluated.

Study Design: A controlled, randomized cross-over trial.

Setting: University Hospital Brussels.

Methods: Twenty CFS patients and 20 sedentary healthy controls were included in this study. In both of the groups, the change in TS of pressure pain, CBF (using transcranial Doppler), and HRV (using finger plethysmography) was examined during physical and emotional stress (to control for potential bias), as well as their association mutually and with self-reported symptoms of pain, fatigue, and concentrations difficulties.

Results: There was no significant interaction or group (F-values ranging from .100 to 1.862, *P*-values ranging from .754 to .181) effect in CBF or HRV parameters. HRV and CBF did change during physical exercise, but the changes did not differ between patients and controls. While pain scores during TS at the trapezius site reduced in the control group after the physical exercise protocol ($P = .037$), they did not change in the CFS group ($P = .108$), suggesting impaired pain modulation. There were no significant correlations between CBF, HRV, TS, and self-reported symptoms (all *P*-values of correlation analyses > .01).

Limitations: Although effect sizes were medium to large, the study sample was relatively low. Also, the mild nature of the exercise bout is discussable. Nonetheless, this mild exercise was able to provoke endogenous pain modulation in the control group, which endorsed a proper execution of the cycling exercise. Moreover, mild exercises are more applicable to daily physical activities in CFS patients than vigorous exercises.

Conclusion: These results seem to refute the previously suggested alterations of CBF/HRV in CFS patients. These cardiovascular parameters appear not to explain pain before, during, and following exercise.

Key words: Chronic pain, physical exercise, emotional stress, pain modulation, cardiovascular systems, temporal summation, pain pressure thresholds, transcranial Doppler, plethysmography

Pain Physician 2018; 21:E13-E24

Chronic fatigue syndrome (CFS) is a disabling condition, characterized by unexplained, severe fatigue that is not lessened by rest, which leads to functional impairment (1). Besides fatigue, other symptoms include multi-joint pain, headaches, unrefreshing sleep, impaired concentration, and post-exertional malaise (1).

Available evidence suggests that these typical symptoms are associated with central sensitization mechanisms (2), which is characterized by enhanced functioning of pain pathways and exercise induced hyperalgesia (EIH) (3-5). The latter is thought to result from impaired descending inhibiting pathways (central pain modulation), leading to greater pain sensitivity and severity, which is reflected in the tendency of pain thresholds to decrease (instead of increase) in response to physical exercise in CFS patients (6). Currently, the underlying pathophysiology remains unclear.

In pain-free people, evidence indicates that experimental pain leads to a decrease in cerebral blood flow (CBF) (7), causing symptoms such as fatigue, dizziness, memory loss, and headaches. These symptoms, related to a decrease in CBF, are also known to be distinctive symptoms of CFS, making CBF an element of interest for investigation within the CFS population (8). However, up to now, this was rarely the subject of research. Available evidence on CBF in CFS patients suggests an abnormal CBF response in different positions (9), but CBF has not been investigated in relation to physical exercise. Yet, as physical exercise is known to evoke typical symptoms of CFS (due to problems in central pain modulation) (10,11) and as pain and fatigue appear to be related to changes in CBF, it could be possible that inadequate CBF responses to physical exercise are a key factor in unravelling the underlying mechanisms of CFS.

Another cardiovascular parameter is heart rate variability (HRV). HRV is associated to CBF, is defined as the variation over-time of the period between consecutive heartbeats, and represents the well-functioning of the autonomic nervous system by giving an impression on the balance between the sympathetic and parasympathetic nervous system (12). A balance between these 2 systems is required to adaptively respond to external stressors (13), making HRV a worthwhile parameter to investigate as a possible explaining factor in the inability of CFS patients to respond adequately to physical exercise. In other non-CFS chronic pain populations, disruptions in HRV have been shown (13), and similar to CBF, CFS patients show an abnormal HRV response in certain positions or states (e.g., while asleep) (14-16). However, possible HRV alterations during physical activity have not been investigated yet.

Based on the suggested association between cardiovascular and pain modulatory systems (17) and the observation that pain and fatigue (2 core symptoms of CFS) are related with symptoms of autonomic dysfunction (18), alterations in CBF/HRV may explain exercise intolerance in CFS patients. Therefore, this study investigates CBF and HRV in relation to pain modulation during a mild physical exercise in CFS patients. Additionally, the potential association of CBF and HRV parameters mutually as with self-reported symptoms will be considered.

Methods

Study Protocol

The study protocol was performed according to the Declaration of Helsinki and was approved by the local ethical committee (Universitair Ziekenhuis Brussel/Vrije Universiteit Brussel). Before participation, all of the patients read and signed the informed consent.

This study was designed as a controlled, randomized cross-over trial (see Fig. 1). The patients were randomized (computer-generated randomization list) according to the type of stressor that was presented first (i.e., emotional stress test or physical exercise). The patients were asked to sit down in a comfortable position for 10 minutes, after which the CBF and HRV recording was initiated. Both cardiovascular parameters were recorded throughout the whole protocol. First, pain pressure thresholds (PPTs) and temporal summation (TS) were measured, after which patients underwent a physical and emotional stressor in a randomized order. In between both stressors, as well as after finalization of the last stressor, TS was measured again. All outcome measures and the protocol of the used stressors are described below.

Participants

Female CFS patients and healthy, pain-free, sedentary women (age span: 18 – 65 years) were included. Only women were included in the present study to account for possible gender bias and because of the higher prevalence of CFS in women compared to men (19). CFS was diagnosed by an experienced physician, according to the Centre of Disease Control criteria, before study participation. Patients were asked not to change or start new treatment (pharmacological or other) 4 weeks prior to and during study participation

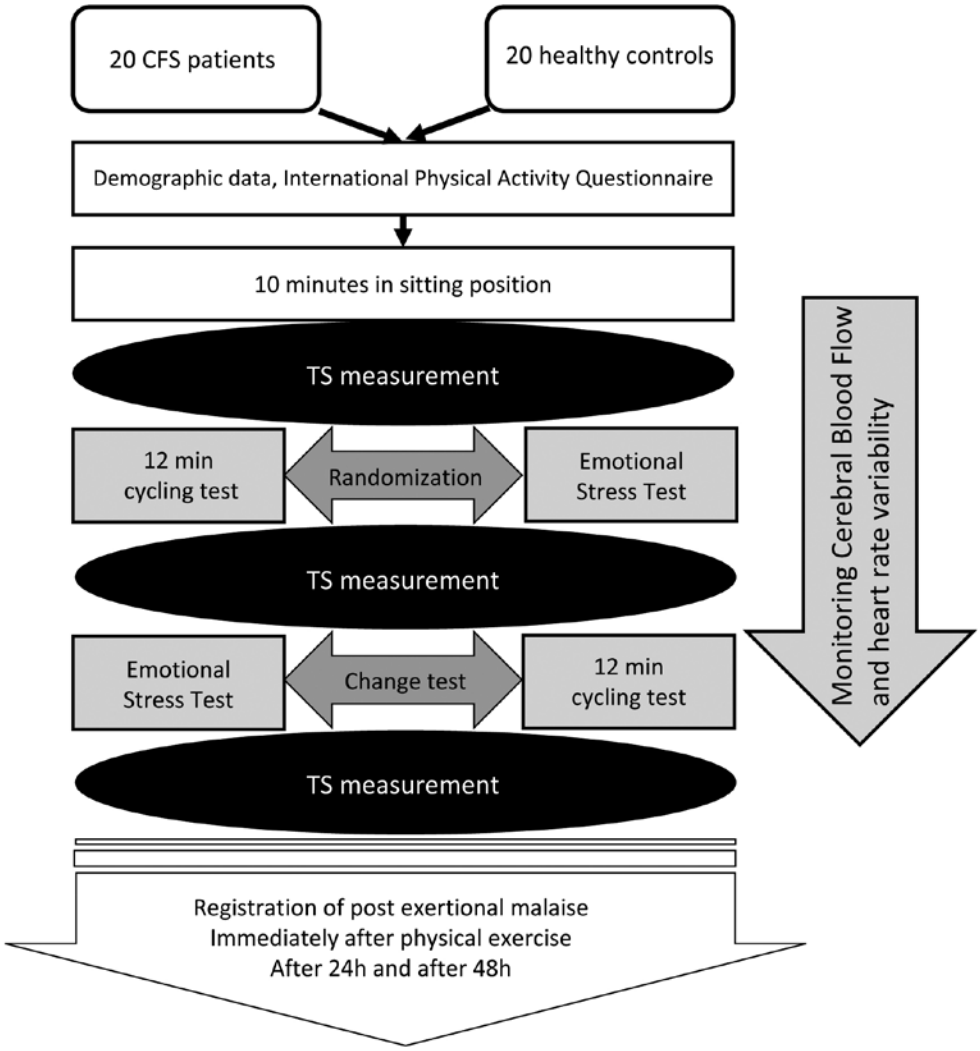

Fig. 1. *Flow chart of the study protocol.*
TS= temporal summation of pressure pain

in order to obtain a steady state; they could continue usual medication. Healthy controls were identified as not having a pain-related medical condition as well as sedentary, to control for physical fitness. All patients were asked to refrain from physical exercise, analgesics, caffeine, alcohol, and nicotine on the day of testing.

Exclusion criteria for both groups were: (1) pregnancy or delivery in the past year, (2) current use of opioid medication, (3) BMI > 30, (4) inability to perform a maximal exercise test, or (5) diabetes mellitus.

Outcomes

Demographic Data

The collected demographic data included age, height, body weight, BMI, and physical activity. To evaluate usual physical activity, the Dutch short version of the International Physical Activity Questionnaire (IPAQ-sf) was used. This 7-item questionnaire asks participants to write down the amount of days, hours, and minutes spent at sitting, walking, moderate activity (e.g., lifting light weights, cycling at moderate speed, etc.), and

vigorous activity (e.g., lifting heavy weights, aerobics, cycling fast, etc.) during the last 7 days. A higher score represents higher physical activity (20) . The IPAQ-sf has a good test-retest reliability and a moderate criterion validity in healthy adults (20).

HRV and CBF

The Nexfin (BMEYE, Amsterdam, The Netherlands) was used to obtain data on HRV in resting state and during the physical and emotional stress test. This non-invasive device uses finger plethysmography measurement and has been validated previously (21). HRV assessment comprises analysis of the intervals between consecutive systolic peaks (P wave to P wave or normal-to-normal intervals). A major advantage of this assessment is its reproducibility, but the recording duration varies significantly between studies. HRV assessment can include time domain analysis, frequency domain analysis, nonlinear analysis, and time-frequency analysis. Recordings were visualized and processed using HRV Analysis Software (HRVAS). For each condition an artefact-free segment with a duration of 5 minutes was chosen for the analysis, in order to correct for noise or extrabeats, etc (22).

CBF was continuously monitored using transcranial Doppler echography (SONARA™, Viasys Healthcare Inc., Conshohocken, PA). Insonating both middle cerebral arteries mean velocity (MV), pulsatility index (PI), resistance index (RI), and heart rate were continuously registered. The average of the last recorded 5 minutes of each condition were processed and used in the analysis (46). Both PI and RI are associated with vascular resistance, referring to the resistance that must be overcome to create blood flow. Transcranial Doppler shows good validity in measuring CBF and is representative of CBF velocity (23). CBF changes as measured in the middle cerebral arteries are supposed to represent changes in brain perfusion as the diameter of such great arteries is invariable. The perfusion territory of these arteries covers lateral parts of the cortex, including the primary somatosensory cortex, the inferior parietal lobe, the insular cortex, and parts of the prefrontal cortex among others. Two 2MHz probes were held in fixed position over the left and right temporal bone window. The angles and the insonation depth of the transcranial Doppler transducer were modified until the correct flow was found.

PPTs and TS of Pressure Pain

TS of pressure pain was used to evaluate the excitability of the dorsal horn neurons and the functionality of pain modulation during physical exercise. When pain modulation is working adequately, one would expect a reduction in pain scores related to the TS protocol.

First the PPTs, which are defined as the minimal pressure inducing pain, were determined using a digital Fisher algometer (FPX™, Wagner Instruments, Greenwich, CT) applied on both the trapezius (between the processus spinosus C7 and the acromio-clavicular joint) and quadriceps (halfway between the spina iliaca anterior superior and the patella). These 2 test sites were assessed in randomized order and the PPT was measured twice per test site, after which the mean was calculated and used in the analysis. To obtain the PPT, pressure was increased at a constant rate of 1 kg/s until the patient indicated an unpleasant, but not yet painful feeling. The procedure for determining the PPT and the TS of pressure pain is described in detail elsewhere by Cathcart et al (24). To assess TS, 10 stimuli were given with the algometer at the PPT intensity with an interstimulus interval of one second. Patients were asked to rate the first, fifth, and tenth stimulus on a scale from 0 to 10 (10 being highly unpleasant), and TS was obtained by extracting the first score from the last. This procedure has been found reliable in healthy patients and has been repeatedly used for studying patients with chronic pain, including CFS (25).

Self-Reported Symptoms

The patients were asked to rate pain severity, fatigue severity, and concentration difficulties (main CFS symptoms) on a scale from one to 10 before the start of the experimental protocol, as well as immediately after, 24 hours, and 48 hours after finishing the protocol.

Stressors

Physical Stressor

All patients performed a mild cycling exercise on a Monark ergometer with a large flywheel (Monark Exercise AB, Sweden). The seat was adjusted to the level of the greater trochanter. All of the patients performed a submaximal exercise test of 12 minutes cycling at 55–65 cycles per minute, against a constant resistance of 50 W.

Emotional Stressor

The International Affective Picture System (IAPS) (26) was used as the emotional stressor, to control for potential bias. The protocol is well-described by Lang et al (26) and was used as such in this study. The IAPS provides a standardized set of photographic slides

that vary in emotional evocation. The pictures include mutilated faces, burn victims, accidents, etc. and were presented for 6 seconds followed by a white screen. The mean inter-picture interval is 12 seconds. The patients were asked to watch closely and to let the pictures 'sink in.' Afterwards, they were asked to give the picture a score ranging from 1 to 9, with 9 being highly unpleasant. This system is a well-validated research-based inventory (26).

Statistical Analysis

Data were analyzed using SPSS Version 22.0 (IBM Corporation, Armonk, NY). Comparability of the groups at baseline was assessed using the independent t-test or Mann-Whitney U-test, depending on the normality of the data. Possible alterations in CBF, HRV, and TS during physical and emotional stress were assessed using a repeated-measure ANCOVA (group x stressor), with significance set at $P < .05$. Physical activity (IPAQ-sf) was added as a covariate. The assumption of homogeneity and sphericity was checked by Levene's and Mauchly's test, respectively. Greenhouse-Geisser corrections were used when sphericity was violated. Post-hoc analysis was performed using Bonferroni. In the CFS group, correlation analysis (Pearson or Spearman respectively) was performed to examine the association between changes in CBF, HRV, self-reported post-exertional malaise, and EIH (change in TS due to physical exercise). To correct for multiple comparisons, correlations were only deemed significant below the 0.01 level (2-tailed).

Results

Subject Characteristics and Comparability

Twenty female CFS patients and 20 female sedentary pain-free controls were included. The patients and controls were comparable for demographic variables. The groups differed at baseline for the PPT measured at the trapezius site ($P = .002$), and self-reported symptoms of pain, fatigue, and concentration difficulties were significantly higher in CFS patients at each point of measurement ($P < .002$). On average, CFS patients reported a pain score of 6 out of 10 at baseline, which relates to severe pain; details can be found in Table 1.

HRV Response in CFS

No significant group or interaction effect was seen for HRV parameters. The main effect of condition was significant in several HRV parameters, including time-domain standard deviation of all NN intervals (SDNN) (Df = 1.364; F = 10.511; $P = .001$) and triangular interpolation of the NN interval histogram (TINN) (Df = 1.450; F = 8.053; $P = .004$), frequency-domain low frequency/high frequency (LF/HF) ratio (Df = 1510; F = 4.495; $P = .027$) and nonlinear alpha-1 (Df = 1.449; F = 40.806; $P < .001$), and time-frequency LF/HF ratio (Df = 1.352; F = 6.026; $P = .013$). Details on the ANCOVA results and Bonferroni post-hoc analysis can be found in Table 2.

CBF Response in CFS

Similarly, for CBF, no main group effects or interaction effects were found. For all parameters, the main effect of condition was significant (F-values ranging from 4.524 to 184.849; *P*-values ranging from .014 to < .001) (Table 3). Bonferroni post-hoc tests revealed that all CBF parameters showed a significant increase from baseline to physical exercise (*P*-values ranging from .005 to < .001), while CBF parameters did not significantly differ between baseline and the emotional stressor (*P*-values ranging from .291 to 1.000), except for heart rate ($P < .001$).

TS of Pressure Pain in CFS

For the changes in TS, a significant interaction effect was found for both the quadriceps (Df = 2; F = 3.213; $P = .046$) and trapezius (Df = 2; F = 5.07; $P = .009$) (Table 4). Post-hoc analysis of the interaction effect only showed a significant decrease in TS (trapezius site) between baseline and both physical ($P = .037$) and emotional stress ($P = .005$) in the control group (see Fig. 2 and data in supplementary material S1).

Associations between Changes in CBF, HRV, and TS

No significant correlations were observed between changes in CBF, HRV, or TS within or across conditions (all $P > .01$).

Self-Reported Symptoms and the Associations with CBF and HRV

No significant correlations were observed between self-reported concentration difficulties, fatigue, and pain, with CBF or HRV parameters at baseline, during physical exercise, or the change in these parameters (from baseline to physical exercise) (all $P > .01$).

Discussion

The aim of this study was to investigate CBF and HRV in CFS patients - with regard to EIH and self-reported symptoms - at rest and during physical and emo-

Table 1. *Demographic and clinical characteristics.*

Variable		CFS Group (95% CI)	Control Group (95% CI)	Mean Difference (95% CI)	*P*-value
Age[a] (yrs)		41.10 ± 8.90 (36.89;44.94)	39.85 ± 14.20 (33.32;46.20)	-1.25 (-8.84;6.34)	0.741
Height[a] (m)		1.67 ± 0.07 (1.63;1.70)	1.68 ± 0.06 (1.66;1.71)	0.02 (-0.03;0.06)	0.427
Weight[a] (kg)		68.94 ± 11.02 (64.36;73.70)	67.53 ± 8.54 (63.80;71.40)	-1.41 (-7.72;4.90)	0.654
BMI[a] (kg/m^2)		24.82 ± 3.46 (23.39;26.39)	23.86 ± 2.93 (22.61;25.17)	-0.97 (-3.02;1.09)	0.347
PPT Quadriceps[a] (kgf)		4.34 ± 2.20 (3.52;5.39)	5.44 ± 1.41 (4.85;6.05)	1.10 (-0.09;2.28)	0.068
PPT Trapezius[b] (kgf)		1.79 (1.57) (1.73;2.63)	3.04 (1.36) (2.84;3.77)	1.14 (0.44;1.84)	0.002
PA Total[b]		612.25 (539.30) (478.55;1113.61)	1063.50 (1926.00) (1210.24;3112.51)	1264.78 (229.69;2299.86)	0.068
Post-Exertional Malaise[b] Pain severity (1–10)	Base	6.00 (3.75) (3.50;6.00)	1.00 (1.00) (1.00;1.50)	-3.45 (-4.66;-2.24)	0.002
	0h	6.00 (5.00) (3.50;7.50)	1.00 (0.80) (1.00;1.00)	-3.70 (-5.09;-2.31)	< 0.001
	24h	6.00 (4.60) (4.50;7.25)	1.00(0.00) (--)	-4.83 (-5.98;-3.64)	< 0.001
	48h	6.00 (5.00) (3.00;7.00)	1.00 (0.00) (--)	-4.40 (-5.51;-3.29)	< 0.001
Post-exertional Malaise[b] Fatigue severity (1–10)	Base	8.00 (4.00) (6.00;9.50)	2.00 (1.00) (1.00;2.00)	-5.55 (-6.77;-4.33)	0.001
	0h	7.50 (2.10) (7.00;8.00)	1.50 (1.80) (1.00;2.00)	-5.13 (-6.16;-4.09)	< 0.001
	24h	8.00 (1.90) (6.75;8.00)	1.00 (0.00) (1.00;1.00)	-5.85 (-6.71;-4.99)	< 0.001
	48h	8.00 (1.00) (7.00;8.00)	1.00 (0.00) (--)	-6.30 (-7.08;-5.52)	< 0.001
Post-exertional Malaise[b] Concentration difficulties (1–10)	Base	7.00 (4.50) (6.00;9.00)	1.00 (1.00) (1.00;2.00)	-5.15 (-6.51;-3.79)	0.001
	0h	6.00 (5.50) (4.50;8.00)	1.00 (1.00) (1.00;2.00)	-4.60 (-5.83;-3.37)	< 0.001
	24h	6.00 (3.00) 5.00;8.00)	1.00 (0.00) (--)	-4.93 (-6.01;-3.84)	< 0.001
	48h	6.50 (6.00) (5.00;7.00)	1.00 (0.00) (--)	-4.95 (-5.81;-4.09)	< 0.001

Abbreviations: CFS = chronic fatigue syndrome; BMI = body mass index; PPT = pain pressure threshold; PA = physical activity; 0h = symptoms of post-exertional malaise reported directly after the experimental protocol; 24h = symptoms of post-exertional malaise reported 24 hours after the experimental protocol, 48h = symptoms of post-exertional malaise reported 48 hours after the experimental protocol

[a]Values are represented as Mean ± Standard Deviation and comparability of groups was evaluated using the independent t-test.

[b]Values are represented as median with interquartile range, and comparability of groups was evaluated using the Mann-Whitney U-test.

tional stress. Overall, results seem to refute a role for altered CBF and HRV in CFS patients and do not support the hypothesis that EIH and self-reported symptoms are associated with altered CBF or HRV.

HRV and CBF Response in CFS

The present results do not provide evidence for altered HRV in CFS patients at rest or during physical and emotional stress. Both groups displayed a similar and normal reduction of HRV during physical exercise,

Table 2. *Changes in heart variability in response to a physical vs. emotional stressor in patients with CFS vs. healthy controls.*

		CFS Patients Mean ± SD (95% CI)	Controls Mean ± SD (95% CI)	Mean Difference (95%CI)	ANCOVA				Bonferroni Post-Hoc Analysis		
					Interaction Effect	Physical Activity Effect	Main Effect of Exp. Condition	Main Effect of Group	Base vs. Phys	Base vs. Emo	Phys vs. Emo
Time SDNN	Base	53.21 ± 23.69 (41.13;66.73)	52.20 ± 24.96 (39.86;66.99)	-0.76 (-21.91;20.40)	NS	Df = 1.364 F = 0.483 $P = 0.550$ $\eta_p^2 = 0.021$	Df = 1.364 F = 10.511 $P = 0.001$ $\eta_p^2 = 0.323$	Df = 1 F = 0.979 $P = 0.333$ $\eta_p^2 = 0.043$	$P < 0.001$	$P = 0.025$	$P < 0.001$
	Phys	24.32 ± 11.63 (18.42;31.25)	21.48 ± 8.27 (17.17;25.17)	-3.48 (-12.22;5.19)							
	Emo	47.44 ± 20.13 (37.15;58.05)	41.90 ± 19.54 (31.56;52.77)	-6.13 (-23.26;11.01)							
Time RMSSD	Base	35.79 ± 18.64 (26.32;46.56)	34.08 ± 20.90 (23.07;46.19)	-2.78 (-19.71;14.14)	NS	Df = 1.173 F = 0.472 $P = 0.528$ $\eta_p^2 = 0.021$	Df = 1.173 F = 2.509 $P = 0.121$ $\eta_p^2 = 0.102$	Df = 1 F = 0.813 $P = 0.377$ $\eta_p^2 = 0.036$	N/A	N/A	NA
	Phys	20.46 ± 10.94 (14.93;26.98)	21.49 ± 7.99 (17.22;25.75)	1.52 (-6.76;9.79)							
	Emo	30.45 ± 12.75 (23.48;37.52)	28.03 ± 20.39 (17.54;39.72)	-3.25 (-17.87;11.21)							
Time TINN	Base	187.86 ± 118.47 (132.92;254.88)	162.84 ± 74.57 (124.96;207.81)	-21.47 (-107.59;64.66)	NS	DF = 1.450 F = 0.408 $P = 0.603$ $\eta_p^2 = 0.018$	Df = 1.450 F = 8.053 $P = 0.004$ $\eta_p^2 = 0.268$	Df = 1 F = 0.881 $P = 0.358$ $\eta_p^2 = 0.038$	$P < 0.001$	$P = 0.399$	$P < 0.001$
	Phys	63.27 ± 34.90 (44.49;82.71)	68.43 ± 39.97 (45.72;89.75)	4.06 (-28.23;36.35)							
	Emo	166.76 ± 84.16 (123.88;212.29)	145.16 ± 88.17 (96.82;192.83)	-25.11 (-99.23;49.01)							
Freq AR LFHF	Base	2.55 ± 2.04 (1.66;3.77)	2.86 ± 1.36 (2.18;3.65)	0.79 (-0.27;1.86)	NS	Df = 1.510 F = 1.080 $P = 0.335$ $\eta_p^2 = 0.047$	Df = 1.510 F = 4.495 $P = 0.027$ $\eta_p^2 = 0.170$	Df = 1 F = 0.176 $P = 0.679$ $\eta_p^2 = 0.008$	$P = 0.262$	$P = 0.527$	$P = 0.097$
	Phys	2.21 ± 1.57 (1.46;3.00)	1.72 ± 1.57 (1.03;2.65)	-.031 (-1.61;0.99)							
	Emo	2.90 ± 1.65 (2.10;3.86)	3.33 ± 2.02 (2.27;4.49)	0.69 (-0.78;2.17)							
Poincare SD1	Base	25.33 ± 13.21 (19.13;33.50)	24.13 ± 14.80 (16.65;32.98)	-1.97 (-13.96;10.02)	NS	Df = 1.172 F = .473 $P = 0527$ $\eta_p^2 = 0.021$	Df = 1.172 F = 2.503 $P = 0.122$ $\eta_p^2 = 0.102$	Df = 1 F = 0.806 $P = 0.379$ $\eta_p^2 = 0.035$	N/A	N/A	N/A
	Phys	14.48 ± 7.75 (10.13;18.43)	15.22 ± 5.66 (12.15;18.42)	1.08 (-4.78;6.95)							
	Emo	21.56 ± 9.04 (17659;26.99)	19.84 ± 14.45 12.91;28.64)	-2.30 (-12.55;7.95)							
Nonlinear Sampen	Base	2.38 ± 0.66 (2.14;2.79)	2.32 ± 0.65 (1.96;2.66)	-0.14 (-0.68;0.40)	NS	Df = 1.585 F = 0.274 $P = 0.710$ $\eta_p^2 = 0.012$	Df = 1.585 F = 1.311 $P = 0.277$ $\eta_p^2 = 0.056$	Df = 1 F = 1.329 $P = 0.261$ $\eta_p^2 = 0.057$	N/A	N/A	N/A
	Phys	2.03 ± 0.40 (1.80;2.24)	2.05 ± 0.38 (1.83;2.26)	0.03 (-0.31;0.37)							
	Emo	2.35 ± 0.47 (2.08;2.60)	2.12 ± 0.37 (1.92;2.33)	-0.21 (-0.57;0.16)							
Nonlinear Alpha 1	Base	1.17 ± 0.28 (0.99;1.28)	1.26 ± 0.17 (1.16;1.35)	0.12 (-0.07;0.31)	NS	Df = 1.449 F = 1.586 $P = 0.222$ $\eta_p^2 = 0.067$	Df = 1.449 F = 40.806 $P < 0.001$ $\eta_p^2 = 0.650$	Df = 1 F = 0.100 $P = 0.754$ $\eta_p^2 = 0.005$	$P < 0.001$	$P = 0.148$	$P < 0.001$
	Phys	0.76 ± 0.19 (0.68;0.88)	0.72 ± 0.21 (0.60;0.83)	-0.06 (-0.23;0.10)							
	Emo	1.28 ± 0.19 (1.16;1.35)	1.30 ± 0.20 (1.19;1.41)	0.05 (-0.12;0.20)							
Time Freq AR rLFHF	Base	4.44 ± 5.05 (2.03;7.45)	5.36 ± 4.55 (3.27;8.05)	0.93 (-3.14; 4.99)	NS	Df = 1.352 F = 0.688 $P = 0.456$ $\eta_p^2 = 0.032$	Df = 1.352 F = 6.026 $P = 0.013$ $\eta_p^2 = 0.223$	Df = 1 F = 0.118 $P = 0.734$ $\eta_p^2 = 0.006$	$P = 0.015$	$P = 0.218$	$P = 0.043$
	Phys	1.84 ± 1.22 (1.24;2.58)	1.65 ± 1.12 (1.16;2.39)	-0.19 (-1.18;0.80)							
	Emo	2.73 ± 1.55 (1.99;3.63)	3.32 ± 2.04 (2.23;4.46)	0.59 (-0.95;2.12)							

Abbreviations: CFS = chronic fatigue syndrome; η_p^2 = partial eta squared (effect size); Base = baseline value; Phys = value measured during physical stressor; Emo = value measured during emotional stressor; SDNN = standard deviation of all NN intervals; rMSSD = the root-mean-square of differences of adjacent NN intervals; TINN = triangular interpolation of NN interval; rLFHF = ratio low frequency/high frequency; NS = not significant; N/A = not applicable

Table 3. *Changes in CBF in response to physical vs. emotional stressor in patients with CFS vs. healthy controls.*

		CFS patients Mean ± SD (95% CI)	Controls Mean ± SD (95% CI)	Mean Difference (95% CI)	ANCOVA				Bonferroni Post-Hoc Analysis		
					Interaction Effect	Physical Activity Effect	Main Effect of Exp. Condition	Main Effect of Group	Base vs. Phys	Base vs. Emo	Phys vs. Emo
MV R	Base	54.95 ± 10.69 (50.32;60.93)	59.12 ± 14.05 (52.47;65.03)	3.52 (-5.23;12.27)	NS	Df=2 F = 0.375 P = 0.689 η_P^2 = 0.010	Df = 2 F = 5.915 P = 0.004 η_P^2 = 0.138	Df = 1 F = 0.889 P = 0.352 η_P^2 = 0.023	P < 0.001	P = 1.000	P = 0.003
	Phys	58.38 ± 13.01 (52.65;65.04)	64.03 ± 14.85 (57.36;71.05)	5.53 (-4.17;15.22)							
	Emo	55.94 ± 11.27 (50.18;61.06)	59.94 ± 13.03 54.11;66.78)	4.74 (-4.12;13.60)							
MV L	Base	56.17 ± 11.22 (51.01;61.52)	59.06 ± 12.90 (53.35;64.63)	2.89 (-5.30;11.08)	NS	Df = 2 F = 0.199 P = 0.820 η_P^2 = 0.006	Df = 2 F = 4.524 P = 0.014 η_P^2 = 0.121	Df = 1 F = 0.384 P = 0.540 η_P^2 = 0.011	P = 0.005	P = 1.000	P = 0.002
	Phys	59.17 ± 12.90 (53.32;65.46)	62.87 ± 12.73 (57.19;68.41)	3.70 (-4.98;12.38)							
	Emo	55.88 ± 11.85 (50.50;61.49)	58.72 ± 12.47 (53.27;64.56)	2.84 (-5.40;11.08)							
PI R	Base	0.87 ± 0.18 (0.81;0.98)	0.96 ± 0.18 (0.89;1.06)	0.07 (-0.05;0.20)	NS	Df = 2 F = 0.444 P = 0.643 η_P^2 = 0.012	Df = 2 F = 64.355 P < 0.001 η_P^2 = 0.635	Df = 1 F = 1.862 P = 0.181 η_P^2 = 0.048	P < 0.001	P = 0.291	P < 0.001
	Phys	1.18 ± 0.27 (1.07;1.33)	1.23 ± 0.18 (1.17;1.31)	0.04 (-0.11;0.19)							
	Emo	0.84 ± 0.16 (0.79;0.93)	0.90 ± 0.16 (0.84;0.99)	0.05 (-0.06;0.16)							
PI L	Base	0.90 ± 0.22 (0.82;1.01)	0.94 ± 0.22 (0.85;1.03)	0.03 (-0.12;0.18)	NS	Df = 1.648 F = 0.596 P = 0.523 η_P^2 = 0.018	Df = 1.648 F = 38.229 P < 0.001 η_P^2 = 0.537	Df = 1 F = 0.087 P = 0.770 η_P^2 = 0.003	P < 0.001	P = 1.000	P < 0.001
	Phys	1.28 ± 0.34 (1.14;1.46)	1.26 ± 0.19 (1.19;1.34)	-0.02 (-0.21;0.16)							
	Emo	0.93 ± 0.26 (0.83;1.07)	0.87 ± 0.11 (0.82;0.92)	-0.06 (-0.20;0.07)							
RI R	Base	0.55 ± 0.06 (0.53;0.58)	0.58 ± 0.04 (0.56;0.59)	0.02 (-0.01;0.05)	NS	Df = 2 F = 0.066 P = 0.936 η_P^2 = 0.002	Df = 2 F = 64.657 P < 0.001 η_P^2 = 0.636	Df = 1 F = 0.953 P = 0.335 η_P^2 = 0.025	P < 0.001	P = 0.345	P < 0.001
	Phys	0.65 ± 0.07 (0.62;0.68)	0.66 0.03 (0.65;0.68)	0.01 (-0.02;0.05)							
	Emo	0.55 ± 0.07 (0.53;0.59)	0.56 0.05 (0.54;0.58)	0.00 (-0.03;0.04)							
RI L	Base	0.57 0.07 (0.54;0.61)	0.57 ± 0.05 (0.55;0.59)	-0.00 (-0.05;0.04)	NS	Df = 1.707 F = 0.193 P = 0.791 η_P^2 = 0.006	Df = 1.707 F = 30.523 P < 0.001 η_P^2 = 0.481	Df = 1 F = 0.033 P = 0.857 η_P^2 = 0.001	P < 0.001	P = 0.965	P < 0.001
	Phys	0.67 ± 0.07 (0.64;0.70)	0.67 0.04 (0.66;0.69)	0.00 (-0.03;0.04)							
	Emo	0.56 ± 0.07 (0.53;0.60)	0.56 0.05 (0.54;0.58)	-0.00 (-0.04;0.04)							
HR	Base	76.78 ± 11.39 (72.33;82.80)	79.48 ± 12.70 (74.30;85.89)	2.85 (-5.46;11.17)	NS	Df = 1.239 F = 1.800 P = 0.186 η_P^2 = 0.046	Df = 1.239 F = 184.849 P < 0.001 η_P^2 = 0.833	Df = 1 F < 0.001 P = 0.993 η_P^2 < 0.001	P < 0.001	P < 0.000	P < 0.001
	Phys	125.97 ± 20.85 (117.77;136.43)	122.67 ± 18.70 (112.71;129.59)	-6.01 (-19.53;7.51)							
	Emo	82.54 ± 13.98 (77.04;90.05)	83.22 ± 12.36 (76.86;88.36)	-0.37 (-9.93;8.88)							

Abbreviations: CFS = chronic fatigue syndrome; η_p^2 = partial eta squared (effect size); R = right; L = left; MV = mean cerebral blood flow velocity; PI = pulsatility index; RI = resistance index; HR = heart rate; Base = baseline value; Phys = value measured during physical stressor; Emo = value measured during emotional stressor; NS= not significant

which normalizes (back to baseline values) again during emotional stress. Similar findings have been reported for supine position and upright tilt (14). These results refute our hypothesis of altered HRV as seen in other chronic pain populations (17,18).

Likewise, none of the CBF parameters differed between the CFS patients and healthy controls, and both of the groups showed a similar evolution in CBF parameters over the different experimental conditions. CBF data of the study population was within normal

Table 4. *Changes in TS in response to physical vs. emotional stressor in patients with CFS vs. healthy controls.*

		CFS Patients Mean ± SD (95% CI)	Controls Mean ± SD (95% CI)	Mean Difference (95% CI)	ANCOVA				Bonferroni Post-Hoc Analysis
					Interaction Effect	Physical Activity Effect	Main Effect of Exp. Condition	Main Effect of Group	
M. Quadriceps	Base	2.88 ± 1.87 (2.07;3.71)	1.25 ±0.92 (0.86;1.69)	-1.61 (-2.58;-0.64)	Df = 2 F = 0.106 *P* = 0.046 η_p^2 = 0.078	NS	N/A	N/A	See Fig. 2. Data presented in supplementary material S1.
	Phys	2.95 ± 1.98 (2.05;3.79)	1.23 ±0.98 (0.81;1.71)	-1.71 (-2.74;-0.69)					
	Emo	2.95 ± 1.79 (2.20;3.71)	1.40 ± 1.23 (0.88;1.95)	-1.56 (-2.56;-0.55)					
M. Trapezius	Base	2.78 ± 1.36 (2.16;3.38)	1.71 ± 1.21 (1.18;2.29)	-1.06 (-1.90;-0.22)	Df = 2 F = 5.07 *P* = 0.009 η_p^2 = 0.121	NS	N/A	N/A	See Fig. 2. Data presented in supplementary material S1.
	Phys	3.30 ± 1.70 (2.55;4.11)	1.00 ± 1.15 (.53;1.59)	-2.30 (-3.25;-1.35)					
	Emo	3.00 ± 1.31 (2.38;3.59)	0.76 ± 1.08 (0.27;1.29)	-2.24 (-3.02;-1.46)					

Abbreviations: CFS = chronic fatigue syndrome; M.Quadriceps = temporal summation measured at the quadriceps site; M.Trapezius = temporal summation measured at the trapezius site; Base = baseline value; Phys = value measured directly after physical exercise; Emo = value measured directly after emotional stress test; η_p^2 = partial eta squared (effect size); NS = not significant; N/A = not applicable

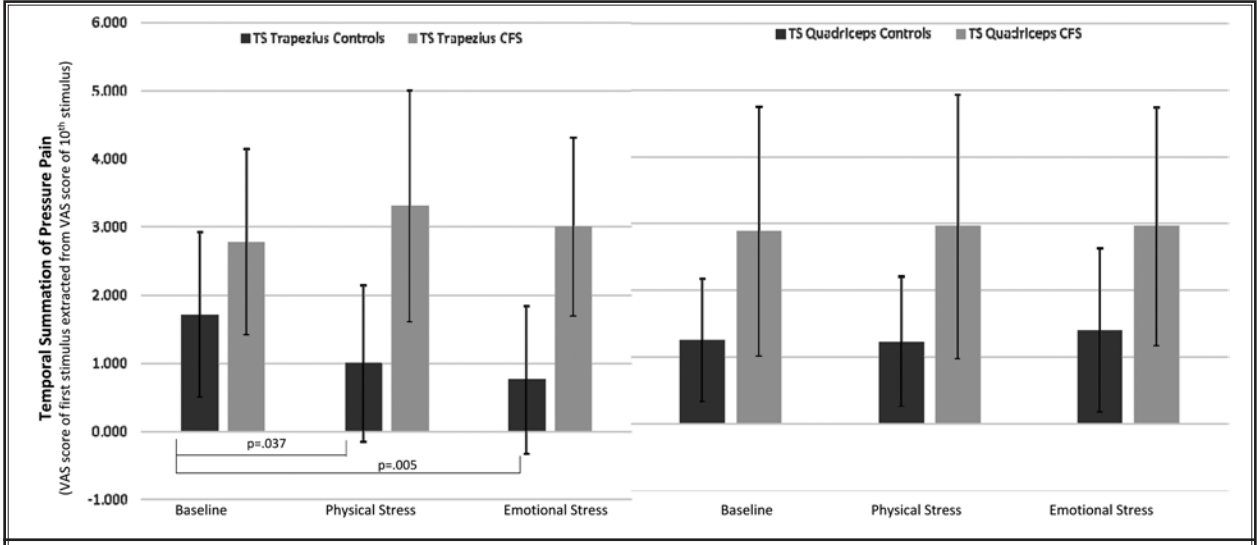

Fig. 2. *Changes in TS.*

age cohort ranges (27). These findings reject our hypothesis that a smaller CBF increase could explain the intolerance for physical exertion in CFS patients. This is in line with previous findings showing no altered CBF in CFS patients in supine position or in response to orthostatic stress (9,28). However, the study of Stewart et al (9) observed a lower absolute CBF velocity in response to incremental upright tilt combined with cognitive activation. There is some evidence for the influence of cognitive tasks on CBF alterations in chronic pain, providing an explanation for significantly lower CBF in CFS patients in Stewart et al (9), which was not observed in the present study. To conclude, it appears that HRV and CBF are within normal ranges at rest and in response to mild exercise in patients with CFS.

TS of Pressure Pain

TS of pressure pain at baseline was significantly higher in CFS patients when compared to healthy controls at both measurement sites, confirming previ-

ous findings of pressure hyperalgesia (6). During the TS protocol, 10 stimuli were given at a constant rate of one stimulus per second. In case of normal nociceptive processing, the pain score that a person gives to the consecutive stimuli will increase with every given stimulus. In case of abnormal nociceptive processing, this pain score will increase more intensely. This is reflected in the higher TS scores (visual analog scale [VAS] score at the tenth stimulus minus VAS score at the first stimulus) in CFS patients compared to the control group. Additionally, TS scores decreased after physical exercise in the control group, while they tended to increase in the CFS group). In healthy persons, the descending pain modulation becomes active because of physical exercise, which is reflected in a smaller increase of pain scores, resulting in a lower TS score. In CFS patients however, this score increased, which suggests the presence of EIH and dysfunctional pain modulation in CFS patients as reported previously (6).

Association between EIH and CBF or HRV

Contrary to the a priori set hypotheses, no associations were found between EIH and CBF or HRV in CFS patients, neither at rest nor in response to physical/emotional stress. These results again seem to refute a role for CBF or HRV in explaining pain increases following exercise or emotional stress in CFS patients.

Cerebral autoregulation aims at maintaining the CBF adequate and stable. The perfusion pressure must be within autoregulatory range (60–160 mmHg) to be effective (29). This range may be exceeded during moderate or intense exercise. The baroreceptor reflex plays an important role in the regulation of CBF when this range is exceeded. One of the characteristics of CFS is a reduced baroreceptor reflex sensitivity (30), which may contribute to exaggerated pain sensitivity and typical EIH in these patients. In the present study, using a mild physical exercise, patients likely did not go beyond their autoregulatory range. This may explain why CBF parameters remained unaltered and why no association with EIH was found. Additionally, as evidence suggests that autonomic control is important for CBF regulation during exercise, it seems acceptable that when CBF is not related to EIH, this relation is also not seen for HRV (29).

Association between CBF or HRV and Self-Reported Symptoms

Although CFS patients reported high pain severity, fatigue severity, and concentration difficulties before, during, and following study participation, no association was found with CBF or HRV parameters at the different conditions. This is in line with other results reported in the present study, again not supporting a role for altered CBF or HRV in explaining pain increases and other symptoms following exercise or emotional stress in CFS patients.

Strengths, Limitations, and Recommendations for Further Research

This is the first study investigating CBF in CFS patients during physical exercise and emotional stress using transcranial Doppler, a method chosen for its real-time ability to monitor CBF across conditions and its superior time resolution.

Previous exercise physiological studies on CFS used case-control rather than experimental designs (31,32). This implies that previous observations regarding exercise physiology in CFS patients did not control for potential bias of emotional stressors or the fluctuating nature of CFS. Therefore, this study comprises a true experimental design (randomized cross-over) controlling for emotional stressors. Also, valid methods were used for examining TS of pressure pain, CBF, and HRV and for triggering emotional stress as control condition. The measurements' validity is also supported by the highly experienced staff within CBF and HRV measurements (experienced research nurses supervised by neurologists). Additionally, CBF data did not show relevant differences between the left and right side, and both CBF and HRV values fit within reported reference values (33). Last, the present study included only women, correcting for possible gender effects.

Some limitations are worth mentioning. Even though the effect sizes of results were medium to large, the study sample is relatively low. Also, the mild nature of the exercise bout can be discussed. However, this mild exercise was able to induce endogenous pain modulation in the healthy controls, which endorses the proper execution of the exercise bout. Moreover, mild exercises are more applicable to daily physical activities in CFS patients than vigorous exercises. Yet, further research may determine whether a higher effort induces disruption in CBF/HRV parameters, and if this is associated with EIH in CFS patients. Furthermore, the phase of the menstrual cycle, which is a potential influential factor of pain due to the potential influence of hormones on the pain experience, was not taken into account in the present study and should be considered in

future research. Additionally, although CFS patients share many symptom similarities with other chronic pain populations (34), they constitute a specific pain population. Hence, the study findings cannot be extrapolated to the general chronic pain population. Finally, the clinical relevance of these findings requires discussion. The aim of this study was to clarify the still largely unknown underlying pathophysiological mechanisms behind post-exertional malaise and EIH in CFS. Improved understanding of these mechanisms might lead to improved care when suitable treatments can restore dysfunctional mechanisms. Based on the present study findings, treatments that aim to improve CBF or dysautonomia are not indicated for treating EIH in people with CFS.

Conclusion

CFS patients do not show decreased CBF or HRV during mild physical exercise or emotional stress and no association was observed with EIH or self-reported symptoms. These results seem to reject a role for CBF and HRV in explaining pain increases during and following physical exercise in CFS patients.

Acknowledgments

The study was funded by ME Research United Kingdom, a national charity funding biomedical research into Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. The funder did not have any influence in the study design, the collection or analysis of the data, or the conception of this manuscript.

Anneleen Malfliet is a PhD research fellow funded by the Research Foundation Flanders (FWO) in Belgium. Kelly Ickmans is a postdoctoral research fellow of the Agency for Innovation by Science and Technology (IWT) – Applied Biomedical Research Program (TBM) in Belgium. Jo Nijs is holder of the Chair 'Exercise immunology and chronic fatigue in health and disease' funded by the Berekuyl Academy / European College for Decongestive Lymphatic Therapy in The Netherlands. Raf Brouns is a Senior Clinical Investigator of the FWO. The authors thank Saskia De Vos and Ellen Boels, research nurses at the department of Neurology, for aiding in the data collection.

Data was collected by Robbert-Jan Van Hooff, Raf Brouns, and Joris De Win. Data was processed by Roselien Pas and Anneleen Malfliet. Data was analyzed by Anneleen Malfliet. All authors made substantial contributions to the design of the study. Anneleen Malfliet wrote the first draft of this manuscript, but all authors made contributions to finalize it in terms of grammar and intellectual content. Robbert-Jan van Hooff and Jo Nijs contributed equally as last author. All authors gave final approval of the manuscript. The authors have no conflict of interest to report.

S1. *Temporal summation (TS) – detailed interaction effects.*

		Comparison	Significance (effect size)
CFS patients	TS Quadriceps	Base vs Phys	.065 (.091)
		Base vs Emo	.145 (.091)
	TS Trapezius	Base vs Phys	.108 (.198)
		Base vs Emo	.473 (.198)
Controls	TS Quadriceps	Base vs Phys	.223 (.056)
		Base vs Emo	.162 (.056)
	TS Trapezius	Base vs Phys	.037 (.070)
		Base vs Emo	.005 (.070)
Between Groups Comparison		At Baseline	.162 (.051)
		After Physical Stress	.621 (.006)
		After Emotional Stress	.417 (.017)

Effect sizes are derived from the ANOVA-analyses and presented as partial eta squared.
Abbreviations: CFS= Chronic Fatigue Syndrome; Con= Healthy controls; Base= Baseline value; Phys= TS measured directly after physical exercise; Emotional stressor= TS measured directly after emotional Stress test;

References

1. National Collaborating Centre for Primary Care UK. National Collaborating Centre for Primary Care (UK). London; 2007.
2. Meeus M, Nijs J, Van Oosterwijck J, Van Alsenoy V, Truijen S. Pain physiology education improves pain beliefs in patients with chronic fatigue syndrome compared with pacing and self-management education: A double-blind randomized controlled trial. *Arch Phys Med Rehabil* 2010; 91:1153-1159.
3. Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; 66:355-474.
4. Kwon M, Altin M, Duenas H, Alev L. The role of descending inhibitory pathways on chronic pain modulation and clinical implications. *Pain Pract* 2014; 14:656-667.
5. Nijs J, Kosek E, Van Oosterwijck J, Meeus M. Dysfunctional endogenous analgesia during exercise in patients with chronic pain: To exercise or not to exercise? *Pain Physician* 2012; 15:ES205-ES213.
6. Meeus M, Roussel NA, Truijen S, Nijs J. Reduced pressure pain thresholds in response to exercise in chronic fatigue syndrome but not in chronic low back pain: An experimental study. *J Rehabil Med* 2010; 42:884-890.
7. Micieli G, Tassorelli C, Bosone D, Cavallini A, Viotti E, Nappi G. Intracerebral vascular changes induced by cold pressor test: A

model of sympathetic activation. *Neurol Res* 1994; 16:163-167.

8. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: A comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994; 121:953959.
9. Stewart JM, Medow MS, Messer ZR, Baugham IL, Terilli C, Ocon AJ. Postural neurocognitive and neuronal activated cerebral blood flow deficits in young chronic fatigue syndrome patients with postural tachycardia syndrome. *Am J Physiol Heart Circ Physiol* 2012; 302:H1185-H1194.
10. Nijs J, Van Oosterwijck J, Meeus M, Lambrecht L, Metzger K, Frémont M, Paul L. Unravelling the nature of postexertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: The role of elastase, complement C4a and interleukin-1beta. *J Intern Med* 2010; 267:418-435.
11. Meeus M, Hermans L, Ickmans K, Struyf F, Van Cauwenbergh D, Bronckaerts L, De Clerck LS, Moorken G, Hans G, Grosemans S, Nijs J. Endogenous pain modulation in response to exercise in patients with rheumatoid arthritis, patients with chronic fatigue syndrome and comorbid fibromyalgia, and healthy controls: A double-blind randomized controlled trial. *Pain Pract* 2015; 15:98-106.
12. Jones JB, Shatat IF, Egan BM, Paulo RC. Decreased heart rate variability is associated with increased transcranial Doppler velocities in children with sickle cell disease. *Ethn Dis* 2014; 24:451-455.
13. Tracy LM, Ioannou L, Baker KS, Gibson SJ, Georgiou-Karistianis N, Giummarra MJ. Meta-analytic evidence for decreased heart rate variability in chronic pain implicating parasympathetic nervous system dysregulation. *Pain* 2016; 157:7-29.
14. De Becker P, Dendale P, De Meirleir K, Campine I, Vandenborne K, Hagers Y. Autonomic testing in patients with chronic fatigue syndrome. *Am J Med* 1998; 105:22S-26S.
15. Boneva RS, Decker MJ, Maloney EM, Lin JM, Jones JF, Helgason HG, Heim CM, Rye DB, Reeves WC. Higher heart rate and reduced heart rate variability persist during sleep in chronic fatigue syndrome: A population-based study. *Auton Neurosci* 2007; 137:94-101.
16. Burton AR, Rahman K, Kadota Y, Lloyd A, Vollmer-Conna U. Reduced heart rate variability predicts poor sleep quality in a case-control study of chronic fatigue syndrome. *Exp Brain Res* 2010; 204:71-78.
17. Ghione S. Hypertension-associated hypalgesia: Evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension* 1996; 28:494-504.
18. Newton JL, Davidson A, Kerr S, Bhala N, Pairman J, Burt J, Jones DE. Autonomic dysfunction in primary biliary cirrhosis correlates with fatigue severity. *Eur J Gastroenterol Hepatol* 2007; 19:125-132.
19. Faro M, Sàez-Francás N, Castro-Marrero J, Aliste L, Fernández de Sevilla T, Alegre J. Gender differences in chronic fatigue syndrome. *Reumatol Clin* 2016; 12:72-77.
20. Ekelund U, Sepp H, Brage S, Becker W, Jakes R, Hennings M, Wareham NJ. Criterion-related validity of the last 7-day, short form of the International Physical Activity Questionnaire in Swedish adults. *Public Health Nutr* 2006; 9:258-265.
21. Chen G, Meng L, Alexander B, Tran NP, Kain ZN, Cannesson M. Comparison of noninvasive cardiac output measurements using the Nexfin monitoring device and the esophageal Doppler. *J Clin Anesth* 2012; 24:275-283.
22. Yperzeele L, van Hooff RJ, De Smedt A, Nagels G, Hubloue I, De Keyser J, Brouns R. Feasibility, reliability and predictive value of in-ambulance heart rate variability registration. *PLoS One* 2016; 11:e0154834.
23. Sorond FA, Hollenberg NK, Panych LP, Fisher ND. Brain blood flow and velocity: Correlations between magnetic resonance imaging and transcranial Doppler sonography. *J Ultrasound Med* 2010; 29:1017-1022.
24. Cathcart S, Winefield AH, Rolan P, Lushington K. Reliability of temporal summation and diffuse noxious inhibitory control. *Pain Res Manag* 2009; 14:433-438.
25. Meeus M, Ickmans K, Struyf F, Hermans L, Van Noesel K, Oderkerk J, Declerck LS, Moorkens G, Hans G, Grosemans S, Nijs J. Does acetaminophen activate endogenous pain inhibition in chronic fatigue syndrome/fibromyalgia and rheumatoid arthritis? A double-blind randomized controlled cross-over trial. *Pain Physician* 2013; 16:E61-E70.
26. Lang P, Bradley M, Cuthbert B (2008). *International Affective Picture System (IAPS): Affective ratings of pictures and instruction manual* (Technical Report No. A-8). Gainesville, FL: University of Florida, Center for Research in Psychophysiology.
27. Zhang J. Effect of age and sex on heart rate variability in healthy subjects. *J Manipulative Physiol Ther* 2007; 30:374-379.
28. Razumovsky AY, DeBusk K, Calkins H, Snader S, Lucas KE, Vyas P, Hanley DF, Rowe PC. Cerebral and systemic hemodynamics changes during upright tilt in chronic fatigue syndrome. *J Neuroimaging* 2003; 13:57-67.
29. Ogoh S. Autonomic control of cerebral circulation: Exercise. *Med Sci Sports Exerc* 2008; 40:20462054.
30. Reyes del Paso GA, Garrido S, Pulgar Á, Duschek S. Autonomic cardiovascular control and responses to experimental pain stimulation in fibromyalgia syndrome. *J Psychosom Res* 2011; 70:125-134.
31. Jones DE, Hollingsworth KG, Jakovljevic DG, Fattakhova G, Pairman J, Blamire AM, Trenell MI, Newton JL. Loss of capacity to recover from acidosis on repeat exercise in chronic fatigue syndrome: A case-control study. *Eur J Clin Invest* 2012; 42:186-194.
32. White AT, Light AR, Hughen RW, Bateman L, Martins TB, Hill HR, Light KC. Severity of symptom flare after moderate exercise is linked to cytokine activity in chronic fatigue syndrome. *Psychophysiology* 2010; 47:615-624.
33. Sammito S, Böckelmann I. Reference values for time- and frequency-domain heart rate variability measures. *Heart Rhythm* 2016; 13:1309-1316.
34. Staud R. Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. *Expert Rev Neurother* 2012; 12:577-585.