Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: June 30, 2022

Citation Campen C and Visser F., 2022. Comparison of the Degree of Deconditioning in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Patients with and without Orthostatic Intolerance, Medical Research Archives, [online] 10(6). https://doi.org/10.18103/mra.v10i6.2858

DOI
https://doi.org/10.18103/mra.v10i6.2858

ISSN: 2375-1924

RESEARCH ARTICLE

Comparison of the Degree of Deconditioning in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Patients with and without Orthostatic Intolerance

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ABSTRACT

Background: Orthostatic intolerance (OI) is a core finding in individuals with myalgic encephalomyelitis /chronic fatigue syndrome (ME/CFS). Deconditioning is often proposed as an important determinant for OI. Deconditioning can be objectively classified using the predicted peak oxygen consumption (%VO_2 peak) values as derived from cardiopulmonary exercise testing (CPET) and OI can be objectively quantified using cerebral blood flow (CBF) changes during tilt testing. Therefore, if deconditioning contributes to OI, a correlation between peak VO_2 and the %CBF reduction is expected.

Methods and results: 18 healthy controls (HC) and 122 ME/CFS patients without hypotension or tachycardia on tilt testing were studied. Deconditioning was classified as follows: %VO_2 peak ≥85%= no deconditioning, %VO_2 peak 65-85%= mild deconditioning, %VO_2 peak<65%= severe deconditioning. HC had higher %VO_2 peak compared to ME/CFS patients ($p<0.0001$). ME/CFS patients had significantly larger CBF reduction than HC ($p<0.0001$). No relation between the degree of deconditioning by the %VO_2 peak and the %CBF reduction in ME/CFS patients was found. Moreover, we separately analyzed ME/CFS patients without an abnormal CBF reduction. Despite equal CBF reductions compared to HC and large differences between these patients and the patients with an abnormal CBF reduction, cardiac index (CI) changes (measured by suprasternal Doppler) were significantly less compared to ME/CFS patients with an abnormal CBF reduction ($p<0.0001$) but larger than in HC ($p=0.004$). Despite these different hemodynamic findings, %VO_2 values were not different between the two patient groups, argumenting again against the causative role of hemodynamic abnormalities in deconditioning.

Conclusion: In ME/CFS patients without hypotension or tachycardia there is no relation between the %VO_2 peak during CPET and the %CBF and %CI reduction during tilt testing, whether or not patients have an abnormal CBF reduction during tilt testing. It suggests again that deconditioning does not play an important role in OI.

Keywords: chronic fatigue syndrome, myalgic encephalomyelitis, peak oxygen consumption, cardiopulmonary exercise test, deconditioning, cerebral blood flow, orthostatic hypotension, orthostatic intolerance, head-up tilt testing.

Introduction

Orthostatic intolerance (OI) is a core symptom in individuals with myalgic encephalomyelitis /chronic fatigue syndrome (ME/CFS), with a wide range of prevalence between 28 and 96% depending on patient population, used methodology of testing, and definitions [1]. In a recent study we found a prevalence of OI of 82% in adults with ME/CFS [2]. In that study we objectively quantified the degree of OI by measuring the cerebral blood flow (CBF) during tilt testing and found that the CBF reduction in ME/CFS patients was 26% versus 7% in healthy controls (HC). Moreover, during the tilt test we asked for OI symptoms and found that there was a linear relation between the degree of CBF reduction and the severity and number of OI complaints [2].

Some authors have proposed that deconditioning is an important pathophysiological mechanism in OI, including postural orthostatic tachycardia syndrome (POTS) [3-9].

Exercise tolerance and the degree of deconditioning can be quantified using cardiopulmonary exercise testing (CPET) [5, 10]. Parsaik et al. defined in a study on orthostatic intolerance in POTS patients, that a percentage peak oxygen consumption (VO_2) cut-off value of ≥ 85% of a reference population suggested absence of deconditioning, whereas 65-84% was considered mild deconditioning, and < 65% as severe deconditioning [5].

In a study describing the effects of tilt testing on stroke volume index (SVI) and cardiac index (CI) in ME/CFS patients and HC, a significantly larger decrease in SVI and CI was found: %SVI reduction in patients 35% vs 28% in HC; %CI reduction 20% in patients vs 10% in HC. The study also showed no differences in the decline in SVI and CI when ME/CFS patients with mild, moderate or severe disease severity were compared. It implicated that the degree of deconditioning (as indirectly inferred from the disease severity) had no influence on the observed changes in CI and SVI during tilting [11]. Another study correlated the degree of deconditioning in controls and ME/CFS patients with orthostatic intolerance during tilting and showed no differences in the degree of deconditioning or CBF reduction whether patients had orthostatic hypotension, postural orthostatic tachycardia syndrome or a normal heart rate (HR) and blood pressure (BP) response during tilt testing [12].

In this previous study [12] we compared the degree of deconditioning of patients with proven OI with HC. However, we demonstrated also in a previous tilt test study that 18% of patients with a normal HR and BP response showed a CBF decrease within the limits of normal (being a less or equal than 13% CBF reduction) [2]. To further demonstrate the absence of a relation between deconditioning and OI, we explored in the present study HC and ME/CFS patients with a normal HR and BP response during tilt testing and compared in this ME/CFS patient group those with and without an abnormal CBF reduction.

Patients, material and methods

This was a retrospective study of patients referred between October 2012 and December 2021 to the Stichting CardioZorg, a cardiology clinic that specializes in the assessment and treatment of those with CFS and ME. A diagnosis of chronic fatigue syndrome (CFS) according to the Fukuda Criteria [13] and myalgic encephalomyelitis (ME) according to the international ME criteria [14] was established. In all patients, alternative diagnoses which could explain the fatigue and other symptoms were ruled out.

From our CPET database of 600 patients and HC, we selected for analysis HC and ME/CFS patients, who had a tilt test with supine and upright measurements of SVI, CI, and CBF, and who had no signs of hypotension, tachycardia, or syncope. CPET had been performed for a variety of reasons: assessment of the heart rate (HR) at the ventilatory threshold (VT), to guide exercise activity [15, 16], to demonstrate reduction of the exercise capacity on day two of a 2-day CPET protocol [17-19], and to assess the degree of disability for social security claims. The tilt test was performed for a variety of reasons: assessment of orthostatic stress, symptoms, hemodynamics (heart rate, blood pressure, as well as stroke volume index and cardiac index by Doppler echocardiography), and to demonstrate a reduction in CBF [2, 11].

To ensure that the clinical condition of the patient was relatively stable, a time interval of 1 year between the tests was taken. Additionally, ME/CFS patients were interviewed to ensure the stability of the disease severity. For comparison, HC who also underwent tilt testing and CPET within an interval of 3 month, were included.

In all patients we estimated the disease severity. Disease severity was graded using the International Consensus Criteria (ICC) with severity

scored as mild (approximately 50% reduction in activity), moderate (mostly housebound), or severe (mostly bedbound), very severe (bedbound and dependent on help for physical functions) [14]. Very severe patients were not included because the tilt test and CPET were too taxing.

The study was carried out in accordance with the Declaration of Helsinki. All ME/CFS participants and HC gave informed, written consent authorizing us to use their medical records for research purposes. The study of the use of clinical data was approved by the medical ethics committee of the Slotervaart Hospital, number P1736, Amsterdam, NL. The testing of HC was approved by the same ethics committee, number P1411.

Tilt test with SVI, CI, and CBF measurements

Measurements were performed as described previously [20]. Briefly, all participants were positioned for 20 min in a supine position before being tilted head-up to 70 degrees for a maximum of 30 minutes. HR, systolic BP (SBP)and diastolic BP (DBP) were continuously recorded by finger plethysmography [21, 22]. Internal carotid artery (ICA) and vertebral artery (VA) Doppler flow velocity frames were acquired by one operator in the supine position and twice during the upright phase, using a Vivid-I system (GE Healthcare, Hoevelaken, NL) equipped with a 6–13 MHz linear transducer. High resolution B mode images, color Doppler images, and the Doppler velocity spectrum (pulsed wave mode) were recorded in one frame. At least two consecutive series of six cardiac cycles per artery were recorded.

Time-velocity integral (VTI) frames were obtained in the resting supine position and while upright in the final minutes of the tilt test. The aortic VTI was measured using a continuous wave Doppler pencil probe connected to a Vivid I machine (GE, Hoevelaken, NL) with the transducer positioned in the suprasternal notch. A maximal Doppler signal was assumed to be the optimal flow alignment. At least 2 frames of 6 seconds were obtained. Echo Doppler recordings were stored digitally. The VTI was measured offline by manual tracing of at least 6 cardiac cycles, using the GE EchoPac post-processing software. This was performed by one operator (CMCvC). SVI was calculated from the VTI of the aortic valve, corrected for the aortic valve area as described previously [23, 24]. SVI was calculated by the equation: corrected left ventricular outflow tract (LVOT) cross-sectional area * the aortic VTI, divided by the body surface area (BSA; DuBois formula). SVI's of the separate cardiac cycles were averaged. CI was calculated as: HR * SVI.

Calculations of CBF were performed as described previously [20] by an independent operator, unaware of the clinical data. In one cardiac cycle CBF was calculated from the mean blood flow velocity * the mean surface area. To compensate for respiratory variation, flow in the four arteries was calculated in 6 cardiac cycles and data were averaged. Total CBF was calculated by adding the flow of the four arteries. For the present study the supine CBF and the CBF at the end of the upright phase of the tilt test were taken. The end-tilt CBF measurement was expressed as the percent reduction compared to the supine CBF. Based on our previous study, we considered OI to be confirmed by CBF measurements if a reduction greater than 2 SD below the mean of the healthy volunteers [25]. This defines an abnormal CBF result as a >13% CBF reduction during the tilt test.

Cardiopulmonary exercise testing

Patients underwent a symptom-limited exercise test on a cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) according to a previously described protocol [26]. Briefly, a ramp workload protocol was used, varying between 10-30 Watt/min. Oxygen consumption (VO_2 in ml/min/kg), carbon dioxide release (VCO_2 in ml/min/kg), and oxygen saturation were continuously measured (Cortex, Procare, The Netherlands), and displayed on screen using Metasoft software (Cortex, Biophysic Gmbh, Germany). An ECG was continuously recorded and HR and BP were measured using the Nexfin device (BMEYE, Amsterdam, The Netherlands) [22]. The metabolic measurement system (Cortex, Biophysic Gmbh, Germany) was calibrated before each test with ambient air, standard gasses of known concentrations, and a 3-L calibration syringe. The ventilatory threshold (VT), a measure of the anaerobic threshold, was identified from expired gases using the V-Slope algorithm [27]. An experienced cardiologist supervised the test and performed visual assessment and confirmation of the algorithm-derived VT. The peak VO_2 was defined as the mean of the VO_2 measurements of the last 15 seconds before ending the exercise. VO_2 at the VT and peak were expressed as a percentage of the normal values of a population study: $\%VO_2$ VT, $\%VO_2$ peak, respectively [28]. Also, the mean respiratory exchange ratio (RER; VCO_2/VO_2) of the last 15 seconds was calculated. [29]. As absolute oxygen consumption differs between

males and females and are age related, results are shown in percent of a reference group to enable comparison of both genders and a broad age range [30-35]. For the definition of the degree of deconditioning the formula of Parsaik et al. was used [5]. In this study we considered subjects with a % VO_2 peak of ≥85% as normal (not deconditioned), and subjects with a %peak VO_2 of <85% as deconditioned, where subjects with %VO_2 peak between 65 and 85% were considered mildly deconditioned, and subjects with <65% of the %VO_2 peak were considered to be severely deconditioned.

Statistical analysis

Data were analyzed using Graphpad Prism version 8.2.4 (Graphpad software, La Jolla, California, USA). All continuous data were tested for normal distribution using the D'Agostino & Pearson omnibus normality test, and presented as mean (SD) or as median (IQR), where appropriate. Nominal data (gender, fibromyalgia, the presence/absence of an abnormal CBF reduction, oxygen consumption (no deconditioning, mild deconditioning, and severe deconditioning), were compared using the Chi-square test (up to a 3x3 table). For continuous data, groups were compared using the unpaired t-test or a Mann-Whitney test where appropriate. Within group comparison was performed using the ordinary one-way analysis of variance (ANOVA). Where significant, results were then explored further using the post-hoc Tukey's test. Linear regression was performed to assess the relation between measures (% CBF reduction from supine to end-tilt and %peak VO_2 for ME/CFS patients). Because of the multiple comparisons we elected to use a more conservative p-value of <0.01 to indicate statistical significance.

Results

Participants

In our database of 600 CPET studies between October 2012 and December 2021 570 patients met the criteria for ME/CFS. Of those 570 patients 277 ME/CFS patients had undergone a tilt test within the for stability required interval of 1 year (37 patients had an interval over one year and they were excluded from the study). Of the 277 patients, in 29 heart rate and/or blood pressure lowering drugs were present at the time of the test and they were excluded; 2 patients were excluded because of a body mass index (BMI) of ≥ 37 [28]. Another 79 patients had no upright measurements of the tilt test due to severity of disease and not being able to complete the standing period or had too poor image quality. These patients were also excluded, leaving 167 ME/CFS patients. Of those, 45 were excluded because of the presence of hypotension and/or tachycardia. This left 122 ME/CFS patients for analysis with a normal HR and BP response, with results of a CPET, and supine and upright measurements during a tilt test. In the analysed 122 patients the interval between the tilt test and CPET was less than 1 year (mean interval 5 (4) months). There were no differences in demographic data between ME/CFS patients who were included or excluded from the study (data not shown).

For comparison 18 HC fulfilled the inclusion criteria of undergoing a tilt test and CPET within the fixed study interval of three months and had no signs of hypotension or tachycardia during tilt testing. None of them used medication except for the occasional use of pain medication.

HC (7 males and 11 females) had a mean age of 51 (10) years and the ME/CFS patients (33 males and 89 females) 41 (10), which differed significantly (p=0.002). Other baseline characteristics were similar. Disease duration in patients was median 11 year (IQR 6-19.25 years). Table 1 shows the hemodynamic data of the tilt test: blood pressures did not differ between HC and ME/CFS patients, both supine and upright. SVI supine and CBF supine did not differ between the 2 groups. Supine HR was significantly higher in the patients, resulting in a higher CI supine. End-tilt HR was higher in patients, reductions in CI and CBF were significantly larger in the patient group (all three p<0.0001). Table 1 furthermore shows the hemodynamic data of the CPET. With a non-significantly different and high RER in both groups, indicating maximum effort, HR supine, and at the VT, but not at peak exercise, were significantly higher in patients compared to HC. Both the %VO_2 VT and the %VO_2 peak were significantly lower in patients (p=0.0006 and p<0.0001, respectively). Figure 1A shows the relation between %VO_2 peak and the %CBF reduction in the whole patient group. With a p value of 0.01 to be significantly different, only a trend of a larger %CBF reduction versus a lower %VO_2 peak, with a large variance, was observed.

Table 1: Hemodynamic results of the tilt test and cardiopulmonary exercise test of healthy controls and ME/CFS patients

	HC (n=18)	ME/CFS (n=122)	p-value
Tilt test data			
HR supine (bpm)	57 (8)	68 (11)	<0.0001
HR end-tilt (bpm)	70 (12)	85 (13)	<0.0001
SBP supine (mmHg)	140 (14)	140 (18)	0.99
SBP end-tilt (mmHg)	128 (17)	134 (18)	0.20
DBP supine (mmHg)	80 (5)	80 (9)	0.99
DBP end-tilt (mmHg)	82 (8)	86 (11)	0.10
SVI supine(ml/min/m^2)	40 (6)	38 (6)	0.31
SVI end-tilt (ml/min/m^2)	30 (5)	23 (4)	<0.0001
CI supine (L/min/m^2)	2.26 (0.41)	2.60 (0.44)	0.0008
CI end-tilt (L/min/m^2)	2.07 (0.40)	1.96 (0.33)	0.22
%change CI (%)	10 (5)	25 (9)	<0.0001
CBF supine (ml/min)	615 (89)	620 (99)	0.82
CBF end-tilt (ml/min)	576 (77)	477 (93)	<0.0001
%change CBF (%)	6 (4)	23 (11)	<0.0001
CPET data			
HR rest (bpm)	68 (11)	86 (15)	<0.0001
HR AT (bpm)	100 (11)	114 (16)	0.0007
HR peak (bpm)	144 (19)	148 (22)	0.43
Perc predicted VO_2 AT (%)	53 (15)	42 (13)	0.0006
Perc predicted VO_2 peak (%)	93 (20)	70 (21)	<0.0001
RER	1.11 (0.07)	1.09 (0.11)	0.37

HC: healthy controls; ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome patients; HR: heart rate; DBP: diastolic blood pressure; SBP: systolic blood pressure; SVI: stroke volume index; CI: cardiac index; CBF: cerebral blood flow; AT: anaerobic ventilatory threshold; Perc predicted VO_2 AT: percent predicted oxygen consumption at the anaerobic ventilatory threshold; Perc predicted VO_2 peak: percent predicted oxygen consumption at maximum; RER: respiratory exchange ratio

In 26 patients the CBF reduction was within the normal range of HC (%CBF reduction of ≤ 13%) and were considered to have no OI. Ninety-six patients had a %CBF reduction >13%, and were designed as ME/CFS patients with OI. These two groups were analyzed separately and compared. Disease severity distribution (mild/moderate /severe) was significantly different between the two groups: the group with a ≤13% CBF reduction (without OI) comprised 19 (73%) patients with mild disease, 6 (23%) with moderate disease and 1 (4%) with severe disease, whereas the group above 13% CBF reduction (with OI) had a mild/moderate/severe disease severity distribution of 36 (38%), 43 (45%), and 17 (18%), respectively. This was a highly significant difference (chi-square 2x3 analysis: p=0.0009). Table 2 shows the results of the tilt test when comparing the two patient groups with and without a significant %CBF reduction. Patients with a %CBF reduction >13% had a significantly lower SVI at end-tilt and a higher %CI decrease than patients with a %CBF reduction ≤ 13% (p<0.0001). By definition, the %change in CBF was highly significantly different between the two groups. Table 2 furthermore shows the results of the CPET: although a trend to a larger %VO_2 peak seemed present in the patients without an abnormal %CBF reduction, this did not reach statistical significance. Figure 1B shows the relation between the %VO_2 peak and the %CBF reduction in the two patient groups. The slopes of the regression lines between %VO_2 peak and %CBF reduction did not differ between the two patient groups, and slopes were not significantly different from zero. In the patients with a %CBF reduction >13% the regression line equation was Y=0.04505*X - 0.3081, (r=0.14; p=0.16), in the patients' group with a %CBF ≤13%: Y=-0.003509*X – 5.119 (r=0.03; p=0.90).

We further analyzed the patients according the degree of deconditioning g as proposed by Parsaik et al. [5]. Table 3A shows the baseline characteristics of patients: no significant differences were found in baseline characteristics, except for disease severity. The majority of patients without deconditioning had mild disease, while severely deconditioned patients had more moderate and severe disease. Table 3B shows the hemodynamics of the tilt test and CPET. Tilt test data were not different between patients with no, mild and severe deconditioning. By definition the % VO_2 peak was different between the three groups. But also the %$V0_2$ at the VT was highly significantly different between the three groups, being lowest in

the severely deconditioned group. The same observation was present in the HR peak, being lowest in the most deconditioned group.

Table 2: Hemodynamic results of the tilt test and cardiopulmonary exercise test of ME/CFS patients with and without a CBF reduction >13%, indicating presence/absence of orthostatic intolerance

	Group 1 (n=96)	Group 2 (n=26)	p-value
Tilt test data			
HR supine (bpm)	69 (11)	66 (10)	0.15
HR end-tilt (bpm)	86 (13)	82 (12)	0.16
SBP supine (mmHg)	140 (18)	141 (19)	0.69
SBP end-tilt (mmHg)	134 (19)	137 (17)	0.41
DBP supine (mmHg)	81 (10)	79 (6)	0.56
DBP end-tilt (mmHg)	86 (12)	87 (8)	0.57
SVI supine(ml/min/m^2)	39 (6)	38 (4)	0.48
SVI end-tilt (ml/min/m^2)	23 (4)	26 (3)	0.0008
CI supine (L/min/m^2)	2.64 (0.41)	2.46 (0.36)	0.049
CI end-tilt (L/min/m^2)	1.92 (0.32)	2.09 (0.33)	0.022
%change CI (%)	27 (6)	15 (4)	<0.0001
CBF supine (ml/min)	626 (101)	600 (87)	0.23
CBF end-tilt (ml/min)	452 (79)	568 (85)	<0.0001
%change CBF (%)	28 (7)	5 (3)	<0.0001*
CPET data			
HR rest (bpm)	87 (14)	84 (17)	0.43
HR AT (bpm)	114 (16)	114 (19)	0.95
HR peak (bpm)	148 (22)	150 (21)	0.60
Perc predicted VO_2 AT (%)	41 (12)	43 (15)	0.41
Perc predicted VO_2 peak (%)	69 (21)	76 (19)	0.11
RER	1.09 (0.10)	1.10 (0.07)	0.89

HC: healthy controls; ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome patients; HR: heart rate; DBP: diastolic blood pressure; SBP: systolic blood pressure; SVI: stroke volume index; CI: cardiac index; CBF: cerebral blood flow; AT: anaerobic ventilatory threshold; Perc predicted VO_2 AT: percent predicted oxygen consumption at the anaerobic ventilatory threshold; Perc predicted VO_2 peak: percent predicted oxygen consumption at maximum; RER: respiratory exchange ratio; *: by definition significantly different

Table 3A: Baseline characteristics of the 3 ME/CFS patients groups divided by degree of deconditioning: no (group 1), mild (group 2) and severe (group 3)

	Gr 1 (n=30) No decond	Gr 2 (n=42) Mild decond	Gr 3 (n=50) Severe decond	Chi-square
Male/female	9/21 30/70%	8/34 19/81%	16/34 32/68%	0.35
Mild/moderate/severe disease	23/7/0 77/23/0%	19/20/3 45/48/7%	6/27/17 12/54/34%	<0.0001
Fibromyalgia yes/no	6/24 20/80%	12/30 29/71%	18/32 36/64%	0.31
Abnormal CBF reduction yes/no	23/7 77/23%	30/12 71/29%	43/7 86/14%	0.22
				ANOVA
Age (years)	44 (11)	42 (10)	39 (9)	F (2,119)=2.64; p= 0.08
Disease duration (IQR: years) #	11 (5-18)	11 (7-17)	11 (6-20)	X2(3)= 0.2115; p=0.90
Length (cm)	171 (10)	170 (10)	174 (8)	F (2,119)=2.18; p= 0.12
Weight (kg)	76 (16)	75 (15)	72 (16)	F (2,119)=0.52; p= 0.60
BMI (kg/m^2)	25.9 (4.8)	25.7 (4.9)	23.7 (4.3)	F (2,119)=2.93; p= 0.06
BSA (m^2)	1.87 (0.22)	1.85 (0.22)	1.86 (0.21)	F (2,119)=0.051; p= 0.95

IQR: interquartile range, Kruskal-Wallis statistical test; ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome patients; BMI: body mass index; BSA: body surface area (DuBois formula); decond: deconditioning

Table 3B: Hemodynamic results of the tilt test and cardiopulmonary exercise test of ME/CFS patients groups divided by degree of deconditioning: no (group 1), mild (group 2) and severe (group 3)

	Gr 1 (n=30) No decond	Gr 2 (n=42) Mild decond	Gr 3 (n=50) Severe decond	ANOVA
Tilt test data				
HR supine (bpm)	67 (11)	69 (10)	69 (12)	F (2,119)=0.19; p= 0.83
HR end-tilt (bpm)	81 (12)	87 (14)	86 (13)	F (2,119)=1.99; p= 0.14
SBP supine (mmHg)	143 (19)	139 (16)	139 (20)	F (2,119)=0.72; p= 0.49
SBP end-tilt (mmHg)	137 (20)	132 (16)	135 (19)	F (2,119)=0.76; p= 0.47
DBP supine (mmHg)	80 (8)	80 (7)	80 (11)	F (2,119)=0.004; p= 1.0
DBP end-tilt (mmHg)	85 (9)	86 (10)	86 (13)	F (2,119)=0.51; p= 0.60
SVI supine(ml/min/m^2)	39 (6)	38 (7)	39 (5)	F (2,119)=0.51; p= 0.60
SVI end-tilt (ml/min/m^2)	24 (4)	23 (4)	23 (4)	F (2,119)=1.19; p= 0.31
CI supine (L/min/m^2)	2.59 (0.36)	2.58 (0.40)	2.62 (0.42)	F (2,119)=0.17; p= 0.85
CI end-tilt (L/min/m^2)	1.95 (0.32)	1.99 (0.36)	1.94 (0.32)	F (2,119)=0.26; p= 0.77
%change CI (%)	25 (8)	23 (8)	26 (7)	F (2,119)=1.89; p= 0.16
CBF supine (ml/min)	622 (124)	611 (83)	626 (95)	F (2,119)=0.27; p= 0.76
CBF end-tilt (ml/min)	476 (88)	487 (91)	469 (98)	F (2,119)=0.44; p= 0.64
%change CBF (%)	23 (11)	20 (11)	25 (10)	F (2,119)=2.51; p= 0.09
CPET data				
HR rest (bpm)	81 (13)	88 (17)	87 (12)	F (2,119)=2.06; p= 0.13
HR AT (bpm)	115 (16)	117 (19)	111 (13)	F (2,119)=1.67; p= 0.19
HR peak (bpm)	160 (17)	156 (19)	135 (20)	F (2,119)=20.99; p<0.0001; post hoc 1 vs 3 p<0.0001 and 2 vs 3 p<0.0001
Perc predicted VO_2 AT (%)	54 (11)	44 (11)	32 (7)	F (2,119)=51.62; p<0.0001; post hoc 1 vs 2 p<0.0001 and 2 vs 3 p<0.0001 and 1 vs 3 p<0.0001
Perc predicted VO_2 peak (%)	98 (11)	74 (6)	51 (10)	F (2,119)=271.8; p<0.0001; post hoc 1 vs 2 p<0.0001 and 2 vs 3 p<0.0001 and 1 vs 3 p<0.0001
RER	1.10 (0.09)	1.08 (0.08)	1.06 (0.12)	F (2,119)=1.17; p= 0.31

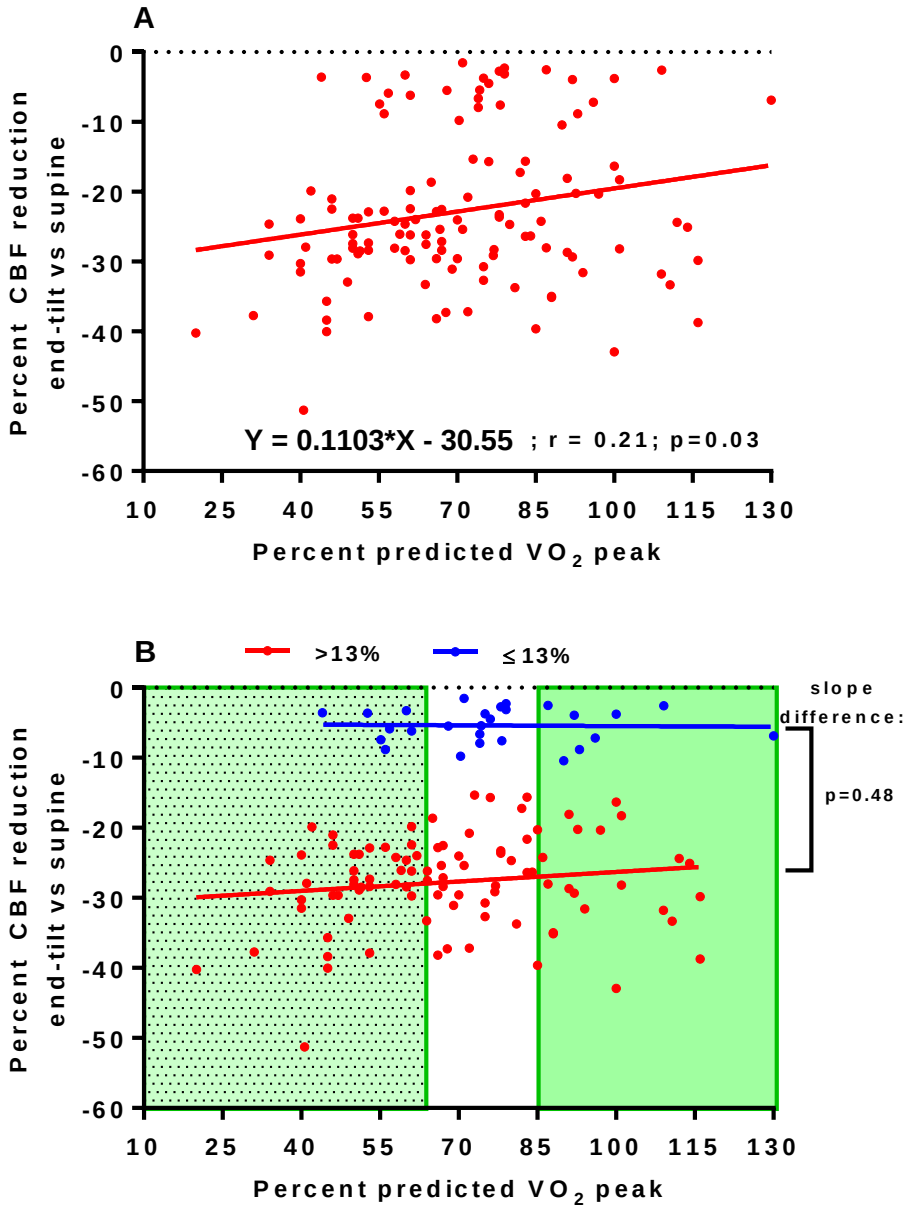
ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome patients; HR: heart rate; DBP: diastolic blood pressure; SBP: systolic blood pressure; SVI: stroke volume index: CI: cardiac index; CBF: cerebral blood flow; AT: anaerobic ventilatory threshold; Perc predicted VO_2 AT: percent predicted oxygen consumption at the anaerobic ventilatory threshold; Perc predicted VO_2 peak: percent predicted oxygen consumption at maximum; RER: respiratory exchange ratio

As the %CBF reduction of the patients with a %CBF reduction ≤13% was similar to that of HC, we performed a subgroup analysis comparing these 26 patients with HC. Table 4 shows the results: HR supine and end-tilt were higher in patients than in HC: p=0.004 and p=0.003, respectively. SVI supine was similar between the two groups, but SVI end-tilt was lower in the patient group: p=0.002. Although CI supine was higher in the patients than in HC (due to the higher HR supine), the difference did not reach significance. The %CI change was larger in patients compared to HC: p<0.0001. CPET data showed a higher HR at rest and at the VT in patients compared to HC: p=0.00009 and p=0.007, respectively. %VO_2 peak was significantly higher in HC compared to patients: p=0.008. Figure 2 illustrates the CI and CBF changes, as well as the VO_2 peak differences between HC, and patients with and without a significant CBF reduction during tilt testing.

Table 4 Hemodynamic results of the tilt test and cardiopulmonary exercise test of healthy controls (group 1) and ME/CFS patients with a %CBF reduction ≤ 13% (group 2)

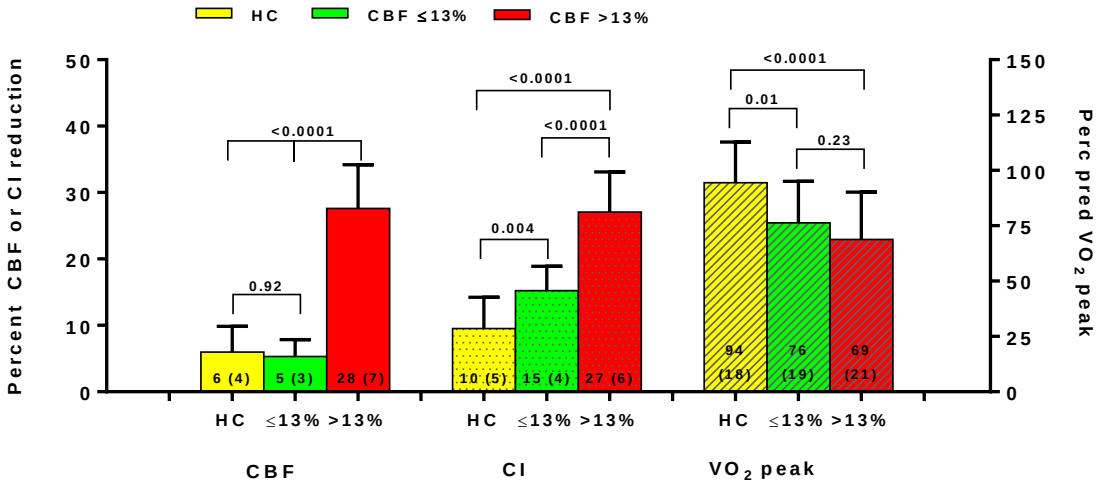
	Group 1 (n=18)	Group 2 (n=26)	p-value
Tilt test data			
HR supine (bpm)	57 (8)	66 (10)	0.004
HR end-tilt (bpm)	70 (12)	82 (12)	0.003
SBP supine (mmHg)	140 (14)	141 (19)	0.95
SBP end-tilt (mmHg)	128 (17)	137 (17)	0.28
DBP supine (mmHg)	80 (5)	79 (6)	0.93
DBP end-tilt (mmHg)	82 (8)	87 (8)	0.15
SVI supine(ml/min/m^2)	40 (6)	38 (4)	0.11
SVI end-tilt (ml/min/m^2)	30 (5)	25 (3)	0.002
CI supine (L/min/m2)	2.26 (0.41)	2.46 (0.36)	0.08
CI end-tilt (L/min/m^2)	2.07 (0.40)	2.09 (0.35)	0.83
%change CI (%)	10 (5)	15 (4)	<0.0001
CBF supine (ml/min)	615 (89)	600 (87)	0.58
CBF end-tilt (ml/min)	576 (77)	568 (85)	0.75
%change CBF (%)	6 (4)	5 (3)	0.41
CPET data			
HR rest (bpm)	68 (11)	84 (17)	0.0009
HR AT (bpm)	100 (11)	114 (19)	0.007
HR peak (bpm)	144 (19)	150 (21)	0.31
Perc predicted VO_2 AT (%)	53 (15)	43 (15)	0.04
Perc predicted VO_2 peak (%)	93 (20)	76 (19)	0.008
RER	1.11 (0.07)	1.10 (0.07)	0.28

Figure 1 Correlations between percent predicted maximal oxygen consumption of the cardiopulmonary exercise test and the percent cerebral blood flow reduction during tilt testing of ME/CFS patients.



Linear regression analysis of the percent predicted maximal oxygen consumption as determined by CPET and the percent cerebral blood flow reduction during tilt testing. Fig 1A shows the whole ME/CFS group, Figure 1B the subdivision of ME/CFS patients with a percent CBF reduction >13% (red dots and line) and with a percent CBF reduction ≤13% (blue dots and line). Green area right side: ME/CFS patients without deconditioning, middle white area: patients with mild deconditioning; dotted green area right side: patients with severe deconditioning, as determined by the criteria of Parsaik et al.5. Both slopes are non-significant from zero. ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome; CBF: cerebral blood flow; Percent VO2 peak: maximal oxygen consumption of the cardiopulmonary exercise test as percent of a reference group

Figure 2 ANOVA analysis of cerebral blood flow changes, cardiac index changes, and percent predicted maximal oxygen consumption in healthy controls, ME/CFS patients without and with an abnormal cerebral blood flow reduction (cut-off value 13%)



CBF: cerebral blood flow; CI: cardiac index; Perc: percent; pred: predicted; ME/CFS: myalgic encephalomyelitis/chronic fatigue syndrome

Discussion

The main finding of this study is that in patients with an abnormal %CBF reduction (group 1 of Table 2), there is no relation between the %VO_2 peak (as a measure of deconditioning) and the reduction in CBF. Furthermore, we also analyzed the opposite: if there was a supposed relation between the %VO_2 peak and the CBF reduction, the patients without a significant CBF reduction should have a higher %VO_2 peak than the patients with a significant CBF reduction. However, there were no significant differences in %VO_2 peak and other CPET data between the patients with and without a significant CBF reduction. Also, when categorizing the %VO_2 peak as a) absence of deconditioning, b) mild, and c) severe deconditioning [5], no significant differences were found in the three patient categories (see Table 3B and Fig 2B). Taken together, these findings provide no support for the hypothesis that deconditioning is a determining factor in the pathogenesis of orthostatic intolerance in ME/CFS.

Studies on deconditioning in ME/CFS show contrasting results: some studies suggest evidence for physical deconditioning [36-39], while others found that the degree of deconditioning was not sufficient to explain the exercise intolerance in ME/CFS [40-44].

Deconditioning can be defined as reversible changes/loss of function in body systems due to physical inactivity, including the cardiovascular system and muscles as the most important ones. Decline in muscle strength and muscle bulk are the most important and consistently reported findings when deconditioning is studied. Reduced VO_2 peak and decreased cardiac output during exercise are also linked to deconditioning but can be assumed to be the result from the reduction in muscle bulk.

As muscle bulk and muscle strength are difficult to obtain during exercise, measuring exercise capacity by VO_2 peak during CPET is considered to be the gold standard for assessing deconditioning [5]. Franklin et al. reviewed in a meta-analysis the available literature on VO_2 peak in ME/CFS patients versus controls [45], showing that ME/CFS patients have a significantly lower VO_2 peak compared to controls. But within the ME/CFS patient group also differences in VO_2 peak are present. We have previously shown that oxygen consumption is dependent on the disease severity: more severely affected patients have a VO_2 peak than mildly affected patients [18, 46]. The same observation can be inferred from the present study: in patients without deconditioning the majority of patients had mild disease, and severely deconditioned patients showed predominantly moderate and severe disease (Table 3A).

Possible explanations of a reduced exercise performance in ME/CFS patients can involve both skeletal muscle fatigue and an altered central nervous system innervation as reviewed by Jammes and Retornaz [47]). Moreover, chronotropic

incompetence during exercise in ME/CFS patients [48], and cardiovascular deconditioning [37] have also been shown to contribute to the reduction in VO_2 peak.

In two previous studies we addressed the relation between exercise intolerance/ deconditioning and orthostatic stress abnormalities. One study addressed mainly SVI and CI changes during tilt testing [11]. A significant difference was found in the SVI and CI reduction during tilt when ME/CFS patients were compared to controls. However, no differences in SVI and CI reductions were found when ME/CFS patients with mild, moderate or severe disease were compared. As severe ME/CFS patients (being bed bound) are very probably more deconditioned than mild or moderate patients, this difference in conditioning may not play a role in the abnormal hemodynamic response during tilt testing. In another study we compared ME/CFS patients with hypotension, postural orthostatic tachycardia syndrome, and with a normal HR and BP response during a tilt test with HC [12]. ME/CFS patients had a significantly poorer exercise intolerance as shown by the CPET compared to controls. No differences in %VO peak were observed in those three patient groups, and also no differences were documented in the CBF reduction during tilt testing. The similarities in OI and CBF reduction between the groups, when divided into no deconditioning, mild deconditioning and severe deconditioning by CPET also suggest that deconditioning cannot be an important determining factor for the CBF reduction/OI.

Although the majority of ME/CFS patients show an abnormal CBF reduction during tilt testing, an interesting group are those with a CBF reduction within the limits of normal of HC. In the patients with a CBF reduction within the limits of normal, the %CI reduction during tilt testing is significantly less than in patients with an abnormal CBF reduction ($p<0,0001$), but significantly more compared to HC: $p<0.004$ (Table 4 and Figure 2). Nevertheless, although differences in CI reduction are present in the patient groups, they have similar $\%VO_2$ peak data. It again highlights the finding that deconditioning is not related to hemodynamic findings (CBF and CI reductions).

The results also indicate that the relation between cardiac output and cerebral flow (cardio-cerebral coupling) may be different between HC and ME/CFS patients. Moreover, there may be differences of this coupling in the subsets of the ME/CFS patient population. Finally, Castle-Kirszbaum et al. concluded from their meta-analysis that "current literature is insufficiently robust to confirm an independent relation between cardiac output and CBF" [49]. Thus, further studies are needed to address the relation between cardiac output and CBF (cardio-cerebral coupling) both in HC and ME/CFS patients.

The present study shows again that ME/CFS patients with a "seemingly" normal tilt test, i.e. absence of a pathological HR or BP response, do show CBF abnormalities in the majority of these patients during tilt testing. This may have diagnostic and therapeutic implications.

Limitations

The patients included in this study were a subset of stable ME/CFS patients with a tilt test and a CPET within a 1 year interval. Stability of disease was confirmed by review of patient charts by an experienced clinician. Furthermore, in this study patients were studied who did not use OI medication, or used compression stockings. This may have introduced bias. For this analysis we only studied ME/CFS patients with a tilt test result without hypotension, tachycardia or syncope. We cannot comment on whether our results can be extended to those with POTS or orthostatic hypotension. This question deserves attention in future studies. In the present study we compared the OI/CBF reduction data, as obtained during a tilt test (being a passive test), with the CPET data of a dynamic exercise test in ME/CFS patients. It can be speculated that the degree of CBF reduction may be different between the two tests. This also needs to be studied in future.

Conclusion:

This study showed in ME/CFS patients without hypotension or tachycardia, that there is no relation between the %VO2 peak during CPET and the %CBF reduction during tilt testing, whether or not patients have an abnormal CBF reduction during tilt testing. It suggests again that deconditioning does not play an important role in OI. Despite similarities in %CBF reduction between HC and the subset of patients without a significant CBF reduction, %CI reductions are significantly larger in this ME/CFS subset than in HC, but significantly less than in the patients with a significant %CBF reduction. It suggests that cardio-cerebral coupling may be different in subsets of ME/CFS patients.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest.

Funding
This research has no funding

Author Contributions
CMCVC and FCV conceived the study, CMCVC and FCV collected the data, CMCVC performed the primary data analysis and FCV performed secondary data analyses. All authors were involved in the drafting and review of the manuscript.

Data Availability Statement
The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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