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RESEARCH ARTICLE

VALIDITY OF 2-DAY CARDIOPULMONARY EXERCISE TESTING IN FEMALE PATIENTS WITH MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

***C (Linda) MC van Campen and Frans C Visser**

Stichting Cardiozorg, Planetenweg 5, 2132 HN Hoofddorp, NL

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ABSTRACT

Introduction: Among the main characteristics of patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) are effort intolerance along with a prolonged recovery from exercise and post-exertional exacerbation of ME/CFS symptoms. The gold standard for measuring the severity of physical activity intolerance is cardiopulmonary exercise testing (CPET). Multiple studies have shown that peak oxygen consumption is reduced in the majority of ME/CFS patients. A consecutive day CPET protocol has shown a difference on day 2 in ME/CFS patients in contrast to sedentary controls. Because the studied numbers of female ME/CFS patients in the published literature, are not very extensive, the aim of this study was to examine whether the response to a 2-day CPET protocol in a larger sample of female ME/CFS patients was similar to that studied in other research teams. **Methods:** From 102 female patients, 70 female ME/CFS patients fulfilled the criteria of a 2-day CPET protocol for analysis. Measures of oxygen consumption (VO_2), heart rate (HR), systolic and diastolic blood pressure, workload (Work), and respiratory exchange ratio (RER) were made at maximal (peak) and ventilatory threshold (VT) intensities. Data were analysed using a paired t-test. **Results:** Baseline characteristics of the group were as follows. Mean age was 44 (12) years, median BMI was 27.1 (4.4)kg/m2. Median disease duration was 10 years (IQR 7-13). Heart rate, systolic and diastolic blood pressure at rest and the RER did not differ significantly between CPET 1 and CPET 2. All other CPET parameters at the ventilatory threshold and maximum exercise differed significantly (p-value between <0.005 and <0.0001). All patients experienced a deterioration of performance on CPET2 as measured by the predicted and actual VO2 and workload at peak exercise and ventilatory threshold. **Conclusion:** This study confirms that female ME/CFS patients have a reduction in exercise capacity in response to a consecutive day CPET. These results are similar to published results in female ME/CFS populations.

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INTRODUCTION

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a serious and potentially disabling chronic disease (Carruthers *et al.* 2011; Clayton 2015; Fukuda *et al.* 1994; IOM 2015). The exact patho physiology has not been established but there is considerable evidence that ME/CFS is associated with abnormalities of the central and autonomic nervous systems, and that an association with infectious agents and immunological abnormalities is often present (Arnett *et al.* 2011; Gerrity *et al.* 2004; Gur and Oktayoglu 2008; Klimas *et al.* 1990; Komaroff and Cho 2011; Naess *et al.* 2010; Okamoto *et al.* 2012; Ortega-Hernandez and Shoenfeld 2009; Stewart 2000).

Corresponding author: **C (Linda) MC van Campen,**
Stichting Cardiozorg, Planetenweg 5, 2132 HN Hoofddorp, NL.

Abnormalities of energy metabolism also have been described (Fluge *et al.* 2016; Naviaux *et al.* 2016; Tomas *et al.* 2018; Wong *et al.* 1992). An important symptom of patients with ME/CFS is exercise intolerance along with a prolonged recovery from exercise (physical as well as mental) and post-exertional exacerbation of ME/CFS symptoms(IOM 2015), termed post-exertional malaise (PEM)(Jones *et al.* 2010; Paul *et al.* 1999). The pathophysiology of the exercise intolerance is not exactly known but involves both metabolic abnormalities of skeletal muscles as well as central nervous system abnormalities (Fulle *et al.* 2007; Gur and Oktayoglu 2008; Jones *et al.* 2010; McCully *et al.* 2006; McCully *et al.* 2003; Siemionow *et al.* 2004; Wong *et al.* 1992). The gold standard for measuring the severity of physical activity intolerance is cardiopulmonary exercise testing (CPET). Studies on ME/CFS patients have shown conflicting results on test results.

Multiple studies have shown that peak oxygen consumption is reduced in the majority of ME/CFS patients(De Becker *et al*. 2000; Fulcher and White 2000; Hodges *et al*. 2018; Jammes *et al*. 2005; Keller *et al*. 2014; Sargent *et al.* 2002; Sisto *et al.* 1996; Snell *et al.* 2013; Vanness *et al.* 2007; Vermeulen *et al*. 2010; Vermeulen and Vermeulen van Eck 2014; Wallman *et al*. 2004). However, studies have also shown that a single CPET in ME/CFS patients may show that peak VO_2 values are similar to or only slightly lower than those of healthy sedentary controls. A 2-day CPET protocol, with two CPET separated by 24 hours has confirmed that ME/CFS patients have significantly lower VO_2 and workload parameters on day 2 (CPET 2) compared to day 1 (CPET 1). In contrast, sedentary controls have unaltered or slightly improved VO_2 and workload(Keller *et al*. 2014; Lien *et al.* 2019; Nelson *et al*. 2019; Snell *et al.* 2013; Vanness *et al*. 2007; Vermeulen *et al*. 2010). Because of the relatively low number of female ME/CFS patients described in literature and a new recent finding in male ME/CFS patients on blood pressure differences in 2-day CPET protocols, the aim of this study was to examine the effect of a 2-day CPET protocol in female ME/CFS patients and to study blood pressure alterations on CPET day 2 compared to CPET day 1 in female ME/CFS patients.

Patients, material and methods: Participants were females with ME/CFS and performed a 2-day CPET protocol. All patients had a detailed clinical history to establish the diagnosis of ME/CFS according to the ME criteria (Carruthers *et al*. 2011) and CFS criteria of Fukuda(Fukuda *et al*. 1994). Of the 102 female patients undergoing CPET between June 2012 and August 2018, 4 were excluded because the ventilatory threshold could not accurately be determined, 25 only had a single CPET, and 3 patients had more than one test, but not on 2 consecutive days, leaving 70 female patients with data from a 2-day CPET protocol available for analysis. Alternative diagnoses which could explain the fatigue and other symptoms were ruled out in all patients. All patients gave informed consent to analyze their data. The use of clinical data for descriptive studies was approved by the ethics committee of the Slotervaart Hospital, the Netherlands (reference number P1736)

Cardiopulmonary exercise testing (CPET): Patients underwent a symptom-limited exercise test on a cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) according to a previously described protocol(Van Campen 2020). A RAMP workload protocol was used varying between 10-30 Watt/min increases, depending on sex, age, and expected exercise intolerance. Oxygen consumption (VO_2), carbon dioxide release (VCO_2), 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 blood pressures were measured using the Nexfin device (BMEYE, Amsterdam, The Netherlands)(Martina *et al*. 2012). Cycle seat height was positioned to approximately 175° of knee extension, and the same seat height was used for both tests. Expired gases were collected breath-by-breath through a two-way breathing valve, and analyzed using open circuit spirometry. The metabolic measurement system(Cortex, Biophysic Gmbh, Germany) was calibrated before each test with ambient air, standard gases 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 (Beaver *et al*. 1986). Ventilatory or anaerobic threshold is the exercise intensity at which metabolism transitions toward increased anaerobic energy production. The same experienced cardiologist supervised the test and performed visual assessment and confirmation of the algorithm-derived VT. Testing took place in a controlled environmentwith a temperature range of 20-24°C and 15-60% relative humidity. Patients were encouraged by standard phrases each minute to perform maximally to the point of exhaustion. The mean of the VO_2 measurements of the last 15 seconds before ending the exercise (peak VO_2) was taken. VO_2 at the peak and at the VT as well as the heart rate (HT) at the peak exercise were expressed as a percentage of the normal values of a population study: %peak VO_2, %VT VO_2 and %peak HR(Glaser *et al*. 2010). Also the mean respiratory exchange ratio (RER; VCO_2/VO_2) of the last 15 seconds was calculated. Immediately after the test the attending cardiologist noted the primary reason for termination of the exercise.

Statistical analysis: Data were analyzed using the statistical package of Graphpad Prism version 6.05 (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 with the IQR, where appropriate. Because of the multiple comparisons a conservative p value of <0.01 was considered significant.

RESULTS

Table 1 shows the characteristics of the study participants. Mean age was 41 (10) years, median BMI was 24.6(4.4)kg/m2. Median disease duration was 11 years (IQR 6-18). Thirty-eight female patients were diagnosed with fibromyalgia. According to the ICC criteria, 29 patients had mild disease, 27 patients had moderate disease and 14 patients had severe disease. Table 2 shows the parameters of the CPET of day 1 and day 2, the range of absolute differences between CPET 1 and 2 for CPET parameters at the ventilatory threshold and at peak exercise (VO_2 peak, %VO_2 peak, VO_2 VT, %VO_2 VT, WR peak and WR VT), and the percentage decline on day 2 compared to day 1. Only heart rate at rest and the RER did not differ significantly between CPET 1 and CPET 2. All other CPET parameters at the ventilatory threshold and maximum exercise differed significantly. Figure 1 shows the values of peak VO_2 at CPET1 and CPET2 (panel A), %predicted peak VO_2 at CPET1 and CPET2 (panel C), VO_2 at the ventilatory threshold at CPET1 and CPET2 (panel B) and the %predicted VO_2 at the ventilatory threshold for CPET1 and CPET2 (panel D). All values differed significantly (p<0.0001). Peak oxygen consumption changed from 19 (5) to 17 (6) ml/min/kg and oxygen consumption at the ventilatory threshold changed from 12 (2) to 9 (2) ml/min/kg. Figure 2 shows the workload graphs at peak exercise for CPET1 and CPET2 (panel A) and at the ventilatory threshold for CPET1 and CPET2 (panel B). Both values differed significantly (p<0.0001). Workload at peak exercise changed from 123 (29) to 108 (32) Watt and the workload at the ventilatory threshold changed from 62 (19) to 43 (18) Watt. Figure 3 is the graphic representation of the absolute differences in 6 CPET parameters from table 2. Figure 3 shows the differences in systolic and diastolic blood pressures both at rest and at peak exercise. All were significantly lower at the CPET test on day 2 compared to the

CPET test on day 1 (all $p<0.0001$). Systolic blood pressure at rest changed from 115 (30) to 109 (30) mmHg, diastolic blood pressure at rest changed from 78 (19) to 74 (19) mmHg. Systolic blood pressure at peak exercise changed from 150 (42) to 137 (41) mmHg and finally diastolic blood pressure at peak exercise changed from 93 (24) to 85 (23) mmHg.

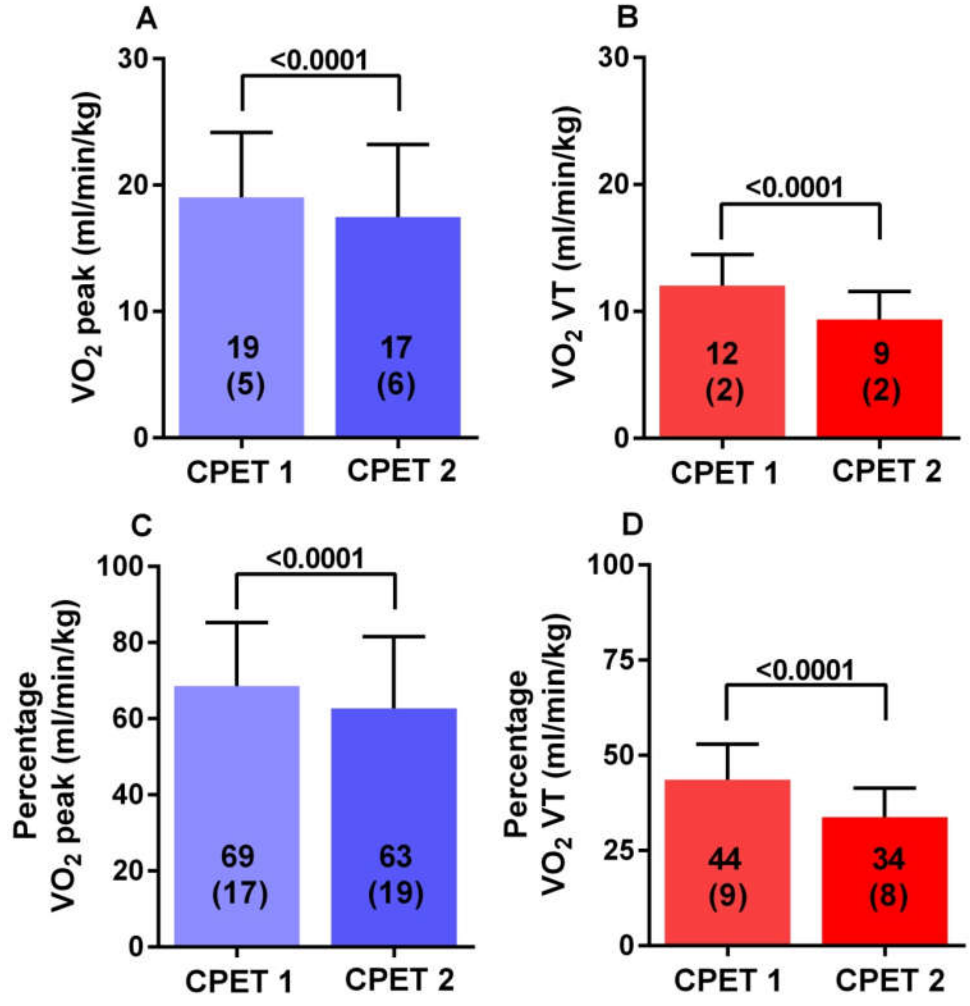
DISCUSSION

A two day CPET protocol in ME/CFS patients shows a unique feature of the disease: reduced CPET parameters as the VO_2 peak and at the ventilatory threshold on the second day of the protocol, which is in contrast to the VO_2 data in sedentary controls (Lien *et al*. 2019; Nelson *et al*. 2019; Snell *et al*. 2013; Vanness *et al*. 2007; Vermeulen *et al*. 2010). These findings of the lower VO_2 at peak exercise on the second day in ME/CFS patients, compared to sedentary controls, makes it unlikely that this phenomenon is due to deconditioning (Nijs *et al*. 2004; Vanness *et al*. 2007), and suggests metabolic abnormalities. It may represent an early sign of post-exertional malaise (PEM)(IOM 2015), a cardinal feature of the disease. In studies analyzing the difference between day 1 and day 2 CPET in ME/CFS patients, a relatively limited numbers of patients have been studied, varying from 6 to 18 female patients (Keller *et al*. 2014; Lien *et al*. 2019; Nelson *et al*. 2019; Vanness *et al*. 2007; Vermeulen *et al*. 2010) and one somewhat larger female study population(Snell *et al*. 2013). We recently reported on a male ME/CFS patient (n=25) population as literature only reported 12 (5 and 7 respectively) male patients in two studies (Keller *et al*. 2014; Nelson *et al*. 2019; Van Campen 2020). To investigate whether males have a different CPET phenotype, we analyzed the response to CPET in a larger male ME/CFS patient sample. The main finding of this recent study was that in male ME/CFS patients, all measurements of VO_2 and workload at the ventilatory threshold and at peak exercise were significantly lower on the second day CPET compared to the first day, similar to published findings in females. We studied a larger female ME/CFS patient population to additionally validate published results in female ME/CFS patients and to study the recently described changes in blood pressure differences between day 1 and 2 CPET studies in female ME/CFS patients. In the studied females similar significant findings on decline in systolic and diastolic blood pressure were found as described in male ME/CFS patients(Van Campen 2020). Additionally, we observed a lower heart rate at the ventilatory threshold, apart from lower heart rate at peak exercise on day 2 compared to day 1, in female ME/CFS patients as had been described earlier by Nelson *et al*.(Nelson *et al*. 2019) and as we described in male ME/CFS patients(Van Campen 2020).

Cardiopulmonary exercise testing 2-day protocols: comparison to literature: VanNess *et al*. (Vanness *et al*. 2007) studied 6 female CFS patients and 6 female sedentary controls in a two day CPET protocol. The first day peak VO_2 was 26.2 (4.9) ml/min/kg for CFS patients and 28.4 (7.2) ml/min/kg for sedentary controls. The results for patients are higher than reported in our study. Age was not reported in this study, so differences might be due to a younger study population with less intense disease. This study documented a significant decline in VO_2 peak and VO_2 at ventilatory threshold at the second day. Our results in female ME/CFS patients are consistent with these findings.

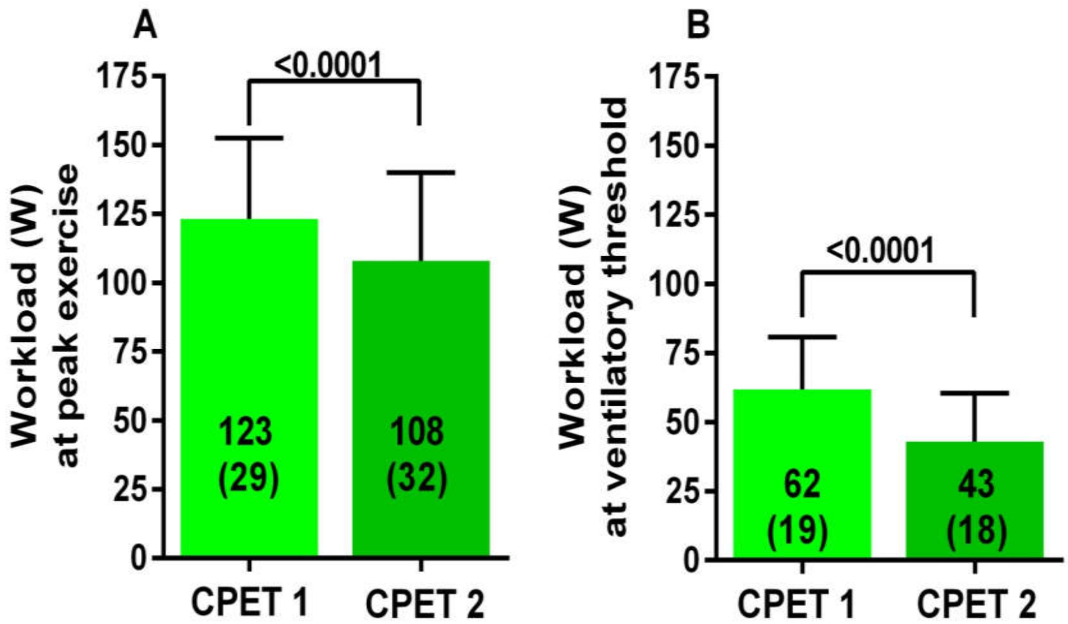
Vermeulen *et al*.(Vermeulen *et al*. 2010) studied 15 female ME/CFS patients and 15 female controls in a two day CPET protocol. The peak VO_2 was 22.3 (5.7) ml/min/kg for CFS patients and 31.2 (7.0) ml/min/kg in controls, patient results were somewhat higher in the female ME/CFS patient population of our study. The mean age of studied subjects was lower in this study (35.5 (11.9) years) which – together with hypothetically less intense diseased population – might account for the difference in peak oxygen consumption. .At both day 1 and day 2 a significantly lower peak VO_2 and VO_2 at the ventilatory threshold was found in ME/CFS patients compared to controls. In ME/CFS patients there was a decrease between day 1 and day 2 in peak VO2 and an unaltered VO2 at the ventilatory threshold. Snell *et al*.(Snell *et al*. 2013) studied 51 female ME/CFS patients and 10 female controls. The peak VO_2 in ME/CFS patients was 21.51 (4.09) ml/min/kg at day 1 and 20.44 (4.47) ml/min/kg at day 2. The most important difference between day 1 and day 2 was found to be a decrease in workload at the ventilatory threshold: for ME/CFS patients from 49.41 (20.40) Watt to 22.20 (18.05) Watt. The patient population included was older (46.29 (8.01) years) than the patient population in the present study, The higher value of oxygen consumption is probably different due to the inclusion from a less intense diseased patient population. Multivariate analysis showed no significant differences between control participants and participants with CFS for test 1. However, for test 2, participants with CFS reached significantly lower values for oxygen consumption and workload at peak exercise and at the ventilatory or anaerobic threshold.

Keller *et al*. (Keller *et al*. 2014) studied 22 CFS patients (17 females and 5 males) in a two day CPET protocol. No controls were included. The first day results for peak VO_2 were 21.9 (4.75) ml/min/kg and percentage predicted VO_2 peak 77.1 (20.22)%. Those results are slightly higher when compared to our study, probably due to gender differences in exercise values and the inclusion of several male ME/CFS patients. On day 2 results for peak VO_2 were 18.6 (4.06) ml/min/kg and percentage predicted VO_2 peak 65.2 (15.74)%. Differences between CPET 1 and CPET 2 from this study population show similar significant declines as we have found in the present study. Also, VO_2 at the ventilatory threshold, peak workload and workload at the ventilatory threshold were all significantly lower on day 2. Nelson *et al*. studied 16 ME/CFS patients (9/7 female/male) and 10 controls (5/5 female/male)(Nelson *et al*. 2019). The peak VO_2 results reported for patients were 27.3 (9.2) ml/min/kg which is higher than the results in the present study. The biggest change this study reported was a decline in workload at the ventilatory threshold (from 87.8 (29.6) to 72.5 (27.7) Watt). Decreases in maximum workload and peak oxygen consumption were non-significant, maybe due to the study population and the gender mix. This may also account for the differences in results compared to the present study, as well as maybe a less intense diseased study population. They concluded that a decrease of the workload at the ventilatory threshold in ME/CFS patients may represent an objective biomarker for the diagnosis of ME/CFS. Finally, Lien *et al*. included ME/CFS patients and controls: 18 patients and 15 controls completed the total study protocol(Lien *et al*. 2019). Peak VO_2 and VO_2 at the ventilatory threshold were significantly lower in ME/CFS patients on day 2 compared to day 1. Peak workload decreased significantly in ME/CFS patients comparing day 2 with day 1.



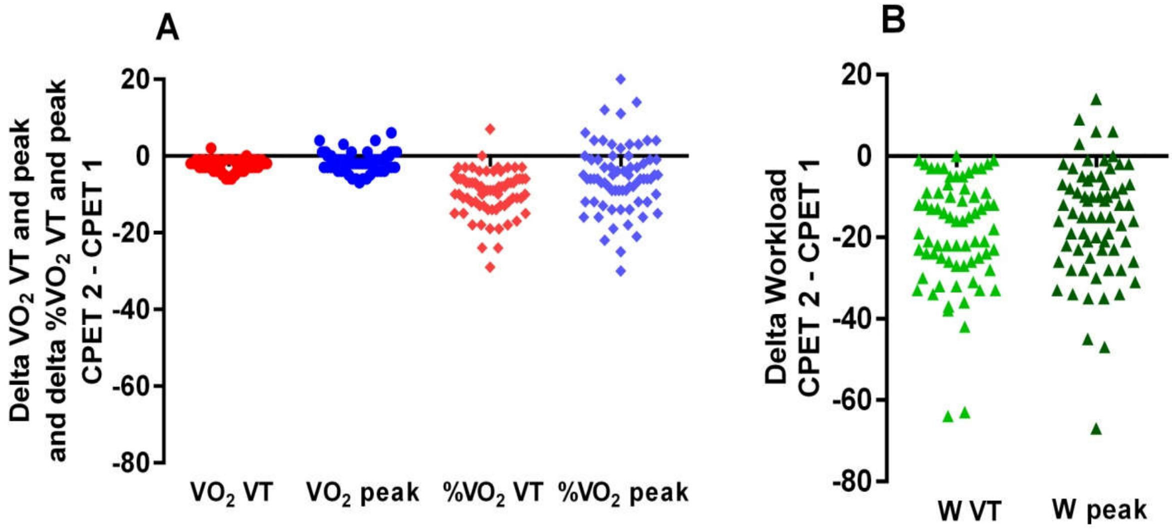
CPET: cardiopulmonary exercise test; VT: ventilatory (or anaerobic) threshold.

Figure 1. Peak oxygen consumption for CPET1 and CPET2 (panel A), the % predicted peak oxygen consumption for CPET1 and CPET2 (panel B), the oxygen consumption at ventilatory (anaerobic) threshold for CPET1 and CPET2 (Panel C) and the % predicted oxygen consumption at ventilatory threshold for CPET1 and CPET2 (Panel D)



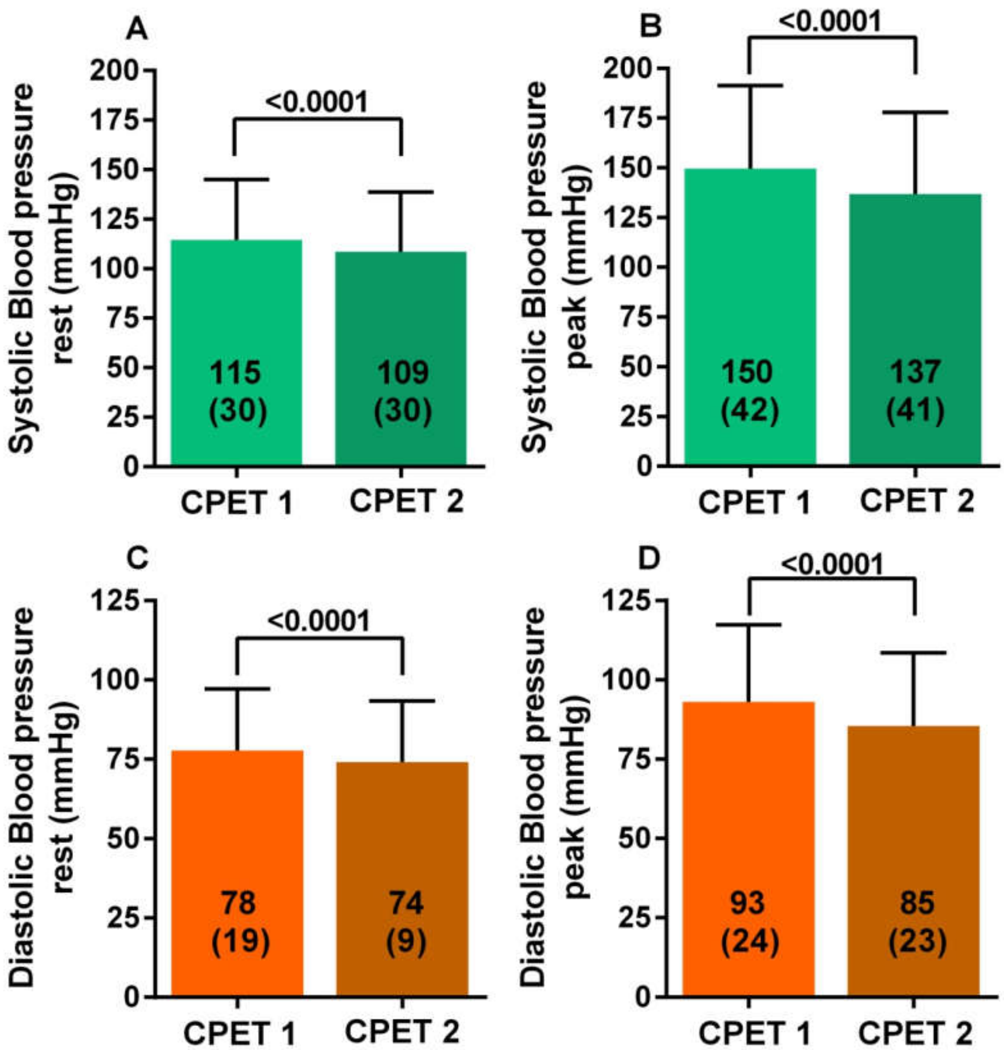
CPET: cardiopulmonary exercise test; VT: ventilatory (or anaerobic) threshold.

Figure 2. Workload at peak exercise for CPET1 and CPET2 (panel A) and at the ventilatory threshold for CPET1 and CPET2 (panel B).



CPET: cardiopulmonary exercise test; VT: ventilatory (or anaerobic) threshold.

Figure 3. Range of absolute differences of CPET parameters, peak VO2, predicted % peak VO2, VO2 at the ventilatory threshold, predicted % VO2 at the ventilatory threshold (panel A), workload at the ventilatory threshold and workload at peak exercise (CPET 2 – CPET 1)(panel B)



CPET: cardiopulmonary exercise test.

Figure 4 Systolic blood pressure in rest for CPET1 and CPET2 (panel A), systolic blood pressure at peak exercise for CPET 1 and CPET 2 (panel B), diastolic blood pressure in rest for CPET1 and CPET2 (Panel C) and the diastolic blood pressure at peak exercise for CPET1 and CPET2 (Panel D)

Table 1. Baseline criteria

	Female ME/CFS (n=70)
Age in years	41(10)
Height in cm	170 (7)
Weight in kg	71 (13)
BSA in m^2	1.4 (0.2)
BMI in kg/m^2	24.6 (4.4)
Disease duration in years	11 (6-18)

BMI: body mass index (DuBois formula); BSA: body surface area. Mean (SD); Median (IQR)

Table 2. CPET1 and CPET2 variables for female ME/CFS patients

Peak exercise	CPET 1	CPET 2	Range of absolute differences CPET 2 – CPET 1	% difference CPET 2 – CPET 1	p-value
VO_2 peak in ml/min/kg	19 (5)	17 (6)	-7 to 6	-9 (14)	<0.0001
%pred VO_2 peak	69 (17)	63 (19)	-30 to 20	-9 (13)	<0.0001
HR rest in bpm	89 (13)	88 (12)		0 (9)	ns
HR peak in bpm	152 (19)	145 (22)		-5 (6)	<0.0001
SBP rest in mmHg	122 (13)	116 (14)		-5 (7)	<0.0001
SBP peak in mmHg	160 (19)	147 (22)		-8 (10)	<0.0001
DBP rest in mmHg	83 (9)	79 (9)		-4 (8)	<0.0001
DPB peak in mmHg	99 (12)	91 (11)		-7 (11)	<0.0001
Workload peak in Watts	123 (29)	108 (32)	-67 to 22	-13 (13)	<0.0001
RER peak	1.1 (0.1)	1.1 (0.1)		-3 (8)	ns
Ventilatory threshold					
VO_2 VT in ml/min/kg	12 (2)	9 (2)	-6 to 2	-22 (11)	<0.0001
%pred VO_2 VT	44 (9)	34 (8)	-29 to 7	-22 (11)	<0.0001
HR VT in bpm	117 (14)	106 (12)		-9 (6)	<0.0001
Workload VT in Watts	62 (19)	43 (18)	-64 to 0	-30 (19)	<0.0001

VT: ventilatory threshold; CPET: cardiopulmonary exercise test; DBP: diastolic blood pressure; HR: heart rate; pred: predicted; RER: respiratory exchange ratio; SBP: systolic blood pressure; VO_2: oxygen consumption

Also, the workload at the ventilatory threshold decreased significantly in ME/CFS patients on day 2. This is in line with the present study.

Limitations: First, we did not include female sedentary controls for comparison in this study. Second, this was not a prospective trial, as most patients underwent consecutive day CPET for clinical management reasons. Thirdly, differences between the previously discussed studies and the present study might be in the demographic characteristics and illness severity of the study population, but also in the exact methodology of the CPET used in the different study centers. Finally, reference values for predicted VO_2 can differ between studies as well.

Conclusion

This larger size study in female ME/CFS patients shows that exercise capacity expressed in peak VO_2, VO_2 at the ventilatory threshold and workload both at peak and at the ventilatory threshold decreased significantly on day 2 compared to day 1. These results are similar to published results in female ME/CFS populations, but replication in a larger sample of studied subjects in another research center increases external validation of the results. Furthermore, we were able to show that blood pressure differences were similar in female ME/CFS patients as in male ME/CFS patients, a novel finding in literature. As known from the discussed 2 day CPET protocols in literature, showing the significant difference on day 2 in ME/CFS patients is of clinical importance in showing impairments and signs of post-exertional malaise. Further comparisons are needed to explore whether the absolute or relative changes in exercise parameters are similar or different between male and female ME/CFS patients.

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