Heart rate variability (HRV). Power spectral analysis will be performed after data detrending. Spectral decomposition of HRV is conducted using an autoregressive approach. The autoregressive spectrum is calculated by fitting a 16th-order model to the RR data. The autoregressive model parameters is solved using a forward-backward least squares method, and finally, the spectrum is obtained from the estimated autoregressive parameters. The power is calculated by measuring the area under the peak of the power spectra density curve, and corresponding bandwidths were interpreted as follows: HF region (0.15–0.40 Hz) indicative of parasympathetic modulation of heart rate; and LF region (0.04–0.15 Hz) mediated by both the sympathetic and parasympathetic arms of the autonomic nervous system. The power spectra is calculated in both raw and normalized units to represent the relative value of each power component as a proportion of total power. The ratio of LF/HF (which is independent of normalization) is then calculated and used as an index of sympathovagal balance (Cygankiewicz et al; 2013). All data acquisition and postacquisition analyses is carried out in accordance with standards put forth by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology (Camm et al. 1996).

Blood pressure. BP means will be calculated for the one week baseline period and the one week follow-up period. In addition BP will measured every minute for 10 min prior to each exercise test and for 20 min following each exercise test in order to assess the expected greater reductions in BP in females than males. In addition, baseline to follow-up BP data will be analyzed for sex differences (below).

Six minute walk test. Convergent validity of the 6MWT will be determined by assessing the correlation between the 6MWT and the SF-36 physical components summary scale (Finch et al; 2004). Discriminant validity (i.e., low associations with measures more distant from physical functioning) will be determined with associations with the SF-36 mental component scale and mental health index (Finch et al; 2004). If validity is established, this will support the use of this exercise protocol in ME/CFS research to study the biobehavioral characteristics of PEM influenced physical functioning.

Sex differences. Wilcoxon’s rank sum test and Fisher’s exact test will performed to determine sex differences in descriptive characteristics and in baseline questionnaires as continuous and categorical variables, respectively. LF and HF power will be transformed to their natural logarithm (ln) for statistical analysis because of their skewed distribution. Pearson’s correlation coefficient analysis will be performed between linear and nonlinear HRV parameters at rest and during exercise recovery. Correlation analyses will be conducted by including both male and female participants in a single group. All continuous data are reported as medians ± interquartile range unless otherwise specified. Linear mixed models for longitudinal daily data will be used to compare the differences in the heart rate variability (HRV) and BP parameters, activity level measured by actigraphy, and daily self-report web diary symptom and activity levels between the resting baseline week and the post-exercise follow-up week. These same baseline to follow-up comparisons will also be examined for sex differences by using linear mixed models for longitudinal data. Model assumptions will be diagnosed and data transformation may be needed to meet the assumptions. The advantage of this model is that it can naturally take into account missing data and give valid references as long as the missing data mechanism is missing at random which is believed to be true in this study. Sex will be the key explanatory variable in the regression models. Possible interactions between sex and symptom severity will be explored to check if the sex difference is moderated by symptom severity. Statistical significance will be set at $p < 0.05$. All data analysis will be carried out using SAS 9.4 (SAS institute, Cary, NC).
Furthermore, the immediate effects of the exercise tests on HRV, BP, and symptom severity will be analyzed. The changes in symptom intensity, HRV, and BP during and up to 20 min after each test will be assessed to identify the immediate post-exertional impacts that may vary by sex. As PEM may begin immediately or after a delay of several hours to one day, this assessment will reveal the range of individual post-exertional effects. As compared to healthy subjects who report improved well-being after exercise, this would appear unlikely to happen in the study sample, considering our pilot exercise data showing elevated fatigue ratings after a 6MWT (Preliminary Studies). Also the individual trajectories of PEM that unfold in the one week post-exercise period will be described and compared by sex. It is expected that women will show slower recovery patterns with respect to autonomic status, symptom severity, and physical functioning.