Additional file 2

Proposed study design

To test the hypothesis, a treatment is proposed that consists of adapted cold showers, 20°C, at a constant flow rate selected from the range 16 to 24 L/min, 3 minutes, preceded by a 5-minute gradual adaptation (expansion of the area of contact with cold water from the feet up, to make the procedure more comfortable), the whole procedure being repeated 2 times per day (morning and afternoon, no later than 7 p.m.).

58 patients with CFS diagnosed according to 1994 Centers for Disease Control criteria [157] would be needed to test if the proposed regimen can affect fatigue scores obtained by means of questionnaires [158-160] as well as other variables. The design of the proposed pilot study would be AB/BA cross-over with wait-list controls [161-163]. The wait-list control groups will consist of patients who receive no anti-fatigue treatments. The patients would be distributed randomly into 2 groups (29 patients each): AB or BA. Group AB will undergo twice daily adapted cold showers for 8 weeks, followed by 2 weeks of wash-out (no treatment), and then 8 weeks of control period (no treatment). The order is reverse for group BA: no treatment for 8 weeks, followed by 2 weeks of wash-out and then 8 weeks of cold hydrotherapy. The total duration of the study is 18 weeks. The fatigue questionnaires would be administered weekly, including immediately before initiation of the study. Additionally, urinary free cortisol (24-hour level) [164] and both baseline and cold-stress response measurements of plasma norepinephrine [165], heart rate and blood pressure [166], tilt table testing [167-170], and central activation failure assay [171] would be performed once a week. To compare these additional variables to those of normal subjects,

58 healthy volunteers (gender- and age-matched) would be recruited to an identical AB/BA crossover study and distributed randomly into 2 groups of 29 as described above for CFS patients. Fatigue questionnaires can be optionally administered to healthy volunteers as well and compared between treatment and control groups of the normal test subjects.

Sample size estimates for each of the above-mentioned variables are summarized in Table 1. All estimates are based on the expectation that the treatment will have an effect size (Cohen's *d*) of 0.5, which corresponds approximately to a change by one half of standard deviation (the after-treatment mean value of a variable in patients is 0.5 standard deviations away from the pre-treatment mean) [172]. This effect size is considered to be moderate [172]. Sample size estimates corresponding to Cohen's $d = 1$ are also shown (Table 2), and although this is usually considered a large effect size [172], it may not always translate into a significant clinical change [176] and therefore may also be considered if the planned study does not include all of the proposed variables.

While the smaller sample size is an attractive advantage of the cross-over design, the possibility of carry-over effects is a potential drawback [161-163]. The proposed 2-week wash-out period is expected to preclude detectable carry-over effects, but if the cross-over design is still unacceptable for some reason [173,174], a parallel design may be considered instead. The parallel trial would last a total of 8 weeks and include 4 groups: two groups of CFS patients, 77 each, and two groups of healthy volunteers, 77 each, according to Table 1; fewer participants if estimates from Table 2 are used and the study does not include all of the proposed variables. Weekly questionnaires and physiological measurements would be administered as described above for the cross-over trial. A "warm shower control", i.e. thermoneutral showers (34°C) performed in a manner similar to the adapted cold showers, may also be considered.

Table 1. Sample size estimates for variables included in the proposed study protocol (Cohen's *d* = 0.5).

The analysis was performed using PASS software [175]; t-test was two-tailed, the probability of Type I error, α, was 5%, and the probability of Type II error, β, was 20% in all calculations. Standard deviation (S.D.) estimates for predicted averages and for differences were calculated as described in [176]. "Group size" means the size of an experimental or control group (ideally, they would be equal). The total number of test subjects in a trial will equal group size times four (two groups of patients plus two groups of healthy volunteers). MFI-20 is Multidimensional Fatigue Inventory [158].

Variables (and references)	**Minimal group size**		**Expected effect size (and clinical change [176])**
	AB/BA cross-over design	**Parallel design**	
MFI-20 General Fatigue Scale [158,177,178]	23	73	Cohen's *d* = 0.5 (mean is 3.6 S.D. away from normal mean)
MFI-20 Physical Fatigue Scale [158,177,178]	20	56	Cohen's *d* = 0.5 (mean is 2.6 S.D. away from normal mean)
MFI-20 Mental Fatigue Scale [158,177,178]	13	55	Cohen's *d* = 0.5 (mean is 2.1 S.D. away from normal mean)
24-hour urinary free cortisol[1] [164]	27	**77**	Cohen's *d* = 0.5 (mean is 0.2 S.D. away from normal mean)
Change in systolic blood pressure in tilt testing[2] [167]	26	64	Cohen's *d* = 0.5 (mean is 5.8 S.D. away from normal mean)
Central activation failure [171]	24	50	Cohen's *d* = 0.5 (mean is 12.1 S.D. away from normal mean)
Norepinephrine, plasma level[3] [179]	25	59	Cohen's *d* = 0.5 (change in the response to cold)
Heart rate[3] [167,180]	27	64	Cohen's *d* = 0.5 (cold stress-induced change)
Systolic blood pressure[3] [167,180]	**29**	71	Cohen's *d* = 0.5 (cold stress-induced change)

[1] The normal mean and CFS mean are less than 1 S.D. apart.

[2] These data vary widely in literature (Additional file 1); only one report (not necessarily the most representative) was selected randomly for these calculations.

[3] It is not known if these variables will differ significantly between patients and normals (Additional file 1).

Table 2. Sample size estimates with the expected effect size (Cohen's *d*) equal to unity.

The analysis was performed as described in the caption of Table 1. Some of the variables have a modest expected effect size judging by the clinical change (clinical change may be considered insignificant if the mean of the treatment group is 2 standard deviations or farther away from the mean of the normal population [176]). If the planned study only includes variables with a modest expected clinical change, then the sample size can be substantially reduced compared to estimates from Table 1. "Group size" means the size of an experimental or control group (ideally, they would be equal). The total number of test subjects in a trial will equal group size times four (two groups of patients plus two groups of healthy volunteers). MFI-20 is Multidimensional Fatigue Inventory [158].

Variables (and references)	**Minimal group size**		**Expected effect size (and clinical change [176])**
	AB/BA cross-over design	**Parallel design**	
MFI-20 General Fatigue Scale [158,177,178]	7	19	**Cohen's *d* = 1** (mean is 3.2 S.D. away from normal mean)[1]
MFI-20 Physical Fatigue Scale [158,177,178]	6	15	**Cohen's *d* = 1** (mean is 1.9 S.D. away from normal mean)
MFI-20 Mental Fatigue Scale [158,177,178]	5	15	**Cohen's *d* = 1** (mean is 1.3 S.D. away from normal mean)
24-hour urinary free cortisol[2] [164]	8	20	**Cohen's *d* = 1** (mean is 0.2 S.D. away from normal mean)
Change in systolic blood pressure in tilt testing[3] [167]	8	17	**Cohen's *d* = 1** (mean is 5.3 S.D. away from normal mean)
Central activation failure [171]	7	14	**Cohen's *d* = 1** (mean is 10.2 S.D. away from normal mean)
Norepinephrine, plasma level[4] [179]	11	25	**Cohen's *d* = 1** (change in the response to cold)
Heart rate[4] [167,180]	5	11	**Cohen's *d* = 1** (cold stress-induced change)
Systolic blood pressure[4] [167,180]	6	13	**Cohen's *d* = 1** (cold stress-induced change)

[1] A large value of effect size as expressed by Cohen's *d* greater than 0.8 may not always produce a significant clinical change and vice versa [176]. Different variables listed in this table have a great variability of the ratio of S.D. to the difference between the mean of the (healthy) population and the mean of CFS patients.

[2] The normal mean and CFS mean are less than 1 S.D. apart.

[3] These data vary widely in literature (Additional file 1); only one report (not necessarily the most representative) was selected randomly for these calculations.

[4] It is not known if these variables will differ significantly between patients and normals (Additional file 1).

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