Supplementary Online Content

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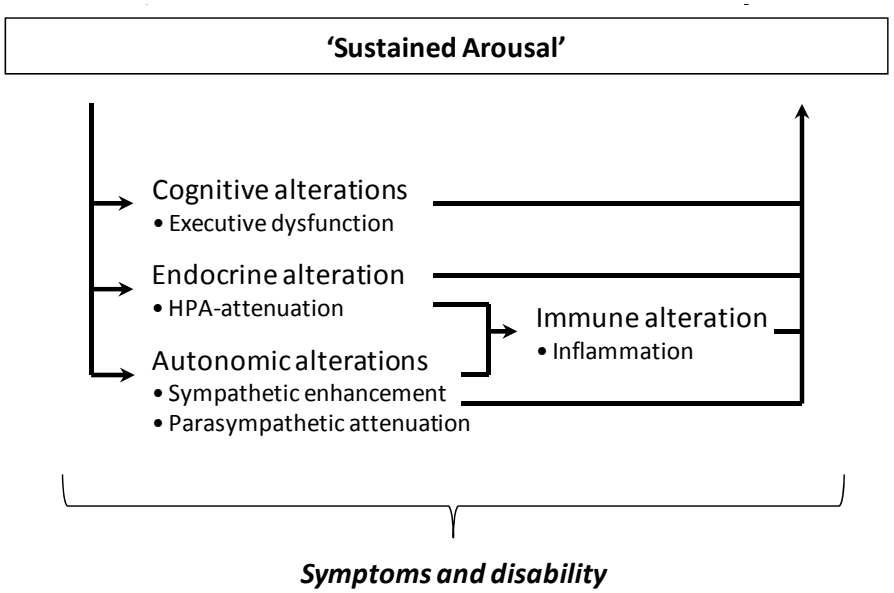
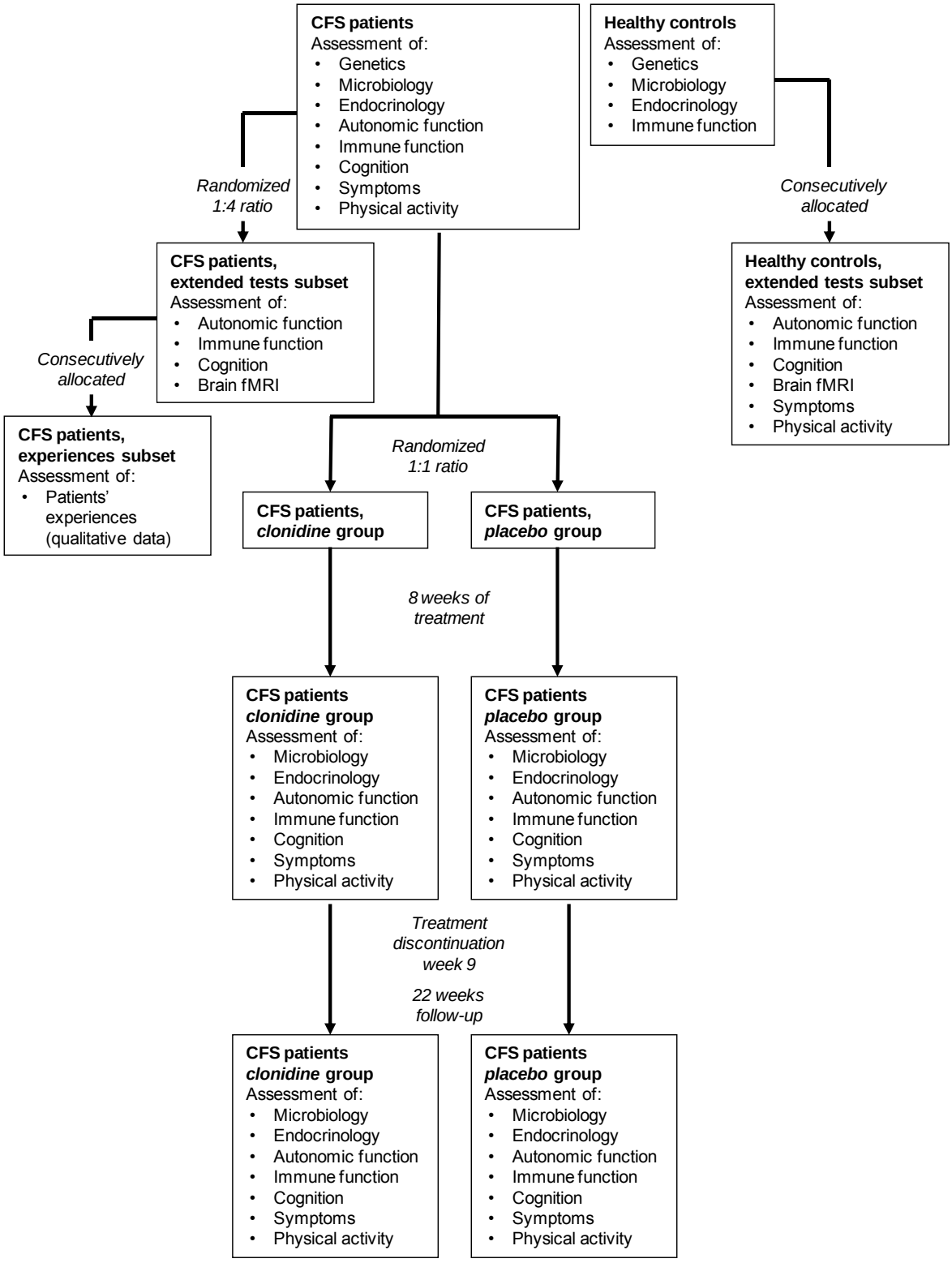
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Diagrammatic and simplified outline of the CFS sustained arousal model.



'Sustained arousal' is conceptualized as a chronic stress response causing alterations of cognitive, endocrine, and autonomic functions; the two latter in turn alters the immune function (2). These alterations in turn fuel the arousal, setting up a vicious circle.

eFigure 2. Overview of the NorCAPITAL project



eAppendix. List of investigators in the NorCAPITAL project

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eMethods. Study methods

The NorCAPITAL project

The NorCAPITAL-project (The Norwegian Study of Chronic Fatigue Syndrome in Adolescents: Pathophysiology and Intervention Trial; ClinicalTrials ID: NCT01040429) explores disease mechanisms, low-dose clonidine treatment effects and patients' experiences in adolescent chronic fatigue syndrome (CFS) (1). NorCAPITAL rests upon the 'sustained arousal'-model of CFS disease mechanisms (2) (eFigure 1), and encompasses a cross-sectional design, a double-blind, randomized, placebo-controlled design and a qualitative design (eFigure 2).

At baseline, all CFS patients underwent assessment of genetics, microbiology, endocrinology, autonomic function, immune function, cognition, symptoms and physical activity. In addition, a computer-based randomization procedure (randomization ratio 1:4, block size 4) allocated a subset of the CFS patients to extended baseline assessment of autonomic function, immune function, and cognition, as well as functional magnetic resonance imaging (fMRI) of the brain; 18 months disease duration served as stratification criterion. From this subset, a new subset of CFS patients was consecutively allocated to assessment of experiences by qualitative methodology. Similarly, all healthy controls underwent baseline assessment of genetics, microbiology, endocrinology and immune function. A subset of healthy controls was consecutively subjected to extended assessment of autonomic function, immune function, cognition, symptoms and physical activity, as well as brain fMRI.

All CFS patients were randomized to 9 weeks of treatment with low-dose clonidine or placebo in a 1:1 ratio, using a computer-based routine for stratified randomization (block size: 4); again, 18 months disease duration served as stratification criterion. Repeated assessment of microbiology, endocrinology, autonomic function, immune function, cognition, symptoms and physical activity was performed 8 weeks and 30 weeks after treatment initiation. Patients were encouraged not to start any other treatment during the study period.

In this article, no genetic, brain fMRI nor qualitative data are reported. From other categories of variables, only selected data are reported, as outlined below and in the Statistical analysis plan.

Recruitment of CFS patients

Referring units' assessment

All Norwegian hospital departments and primary care units which might be involved in caring for adolescent CFS patients were thoroughly and repeatedly informed of the NorCAPITAL project, and equipped with written information for distribution to potential study participants and their parents/next-of-kin. If consent was given, a standard form required the referral unit to confirm the result of clinical investigations considered compulsory to diagnose pediatric CFS (pediatric specialist assessment, comprehensive hematology and biochemistry analyses, chest x-ray, abdominal ultrasound, and brain MRI) (3), as well as provide other vital data (such as age and disease duration). Also, the referring units were required to confirm that the patient a) was unable to follow normal school routines due to fatigue; b) was not permanently bedridden; c) did not have any concurrent medical or psychiatric disorder that might explain the fatigue; d) did not experience any concurrent demanding life event (such as parents' divorce) that might explain the fatigue; e) did not use pharmaceuticals (including hormone contraceptives) regularly. Completed forms were consecutively conveyed to the study center at Oslo University Hospital (OUS). Each referral form was carefully evaluated by two of the authors (DS or EF) and supplementary patient information obtained, after which randomization was carried out.

Case definition

The diagnosis of CFS is based upon patients' reports of symptoms. Several different case definitions exist, reflecting unsettled controversies in the scientific community. The definition from the International Chronic Fatigue Syndrome Study Group at the Centers for Disease Control and Prevention (the Fukuda-definition) is most frequently used (4). This definition requires at least six months of unexplained, disabling chronic/relapsing fatigue of new onset combined with four or more of eight specific accompanying symptoms (headache, muscle pain, joint pain, sore throat, tender lymph nodes, impaired memory or concentration, unrefreshing sleep, and malaise after exertion). The validity of the Fukuda-definition has been questioned, in adults (5-7) as well as adolescents (8). In this study, we required only three months of unexplained, disabling chronic/relapsing fatigue of new onset, and no accompanying symptoms. In addition, no other chronic disorder was allowed. This case definition complies with authoritative clinical guidelines (3,9).

Randomization and blinding

Referred patients fulfilling pre-specified criteria (see eTable 1) were consecutively randomized after receipt of the referral form. The computer based routine (provided by the Dept. of Research Support at the Norwegian University of Science and Technology, Trondheim, Norway) was operated by a research nurse not affiliated with any other part of the study. The result of the clonidine/placebo allocation was forwarded to the hospital pharmacy (Sykehusapoteket, Oslo University Hospital, Norway).

In the production of the study drug (performed by Apoteket Produktion & Laboratorier, Sweden), Catapresan® tablets containing 25 µg clonidine hydrochloride (Boehringer Ingelheim, Germany) were enclosed in lactose capsules. Empty capsules were used as placebo comparator. The capsules were orange opaque, thus completely concealing whether they contained a tablet or not. They were demolition-restraint, and there were no differences in taste nor smell.

The study drug was imported by the hospital pharmacy (Sykehusapoteket, Oslo University Hospital, Norway) and provided to every study patient according to the allocation result. Two pharmaceutics were responsible for this procedure, and no one of them was otherwise affiliated with the study. Sealed envelopes providing information on allocation of every single patient (clonidine or placebo) was available in case of emergencies; however, no unblinding was performed during the course of the study.

All primary analyses of included variables were performed prior to unblinding, i.e. in the period from October 2012 until January 2013.

Investigational program

A one-day in-hospital assessment included clinical examination (with cardiovascular and pain assessment), blood sampling (antecubital venous puncture), autonomic testing (with 20^{o} head-up tilt test (HUT)) and cognitive tests, and always commenced between 7.30 and 9.30 a.m. Details of this program that are relevant for the present study are outlined below. All participants were instructed to fast overnight and abstain from tobacco products and caffeine at least 48 hours. Detailed information regarding pharmacotherapy and/or other therapeutic approaches was registered among CFS patients at all occasions. At week 8, CFS patients were told to postpone their prescribed morning study drug until after blood sampling and HUT. At week 30, one of the primary researchers (DS or EF) guessed at treatment allocation. All procedures were carried out in a quiet room in a fixed sequence and by three researchers only (DS, EF and AW). Participants that were allocated to extended baseline assessment underwent brain fMRI and extended cognitive testing the next day.

Following the in-hospital assessment, daily physical activity was monitored during seven consecutive days using the *activPAL* accelerometer device (PAL Technologies Ltd, Scotland), and a self-administered questionnaire was completed. Each participant received a gift certificate having the value of NOK 200 after each completed in-hospital assessment.

Clinical examination

Cardiovascular assessment

Blood pressures (oscillometric) and heart rate were measured automatically (Simens Medical Systems SC 7000, Danvers, MA, USA). Supine values were measured four times after at least 5 minutes of supine rest; the mean of the last three measurements is reported here. Upright values were measured once after 3 minutes of relaxed standing.

ECG was recorded using a standard, 12-lead device (Marquette MAC 5000 GE Medical Systems, Milwaukee, Wisconsin, USA); paper speed was 50 mm/sec. All recordings were manually inspected for abnormalities; in particular, the PR interval was compared with age-specific reference values (10), cf. exclusion criteria (eTable 2).

Pain assessment

The Brief Pain Inventory (BPI) (11) is validated for several chronic musculoskeletal pain disorders (12,13). During the clinical interview, each item from BPI was read aloud by one of the researchers (AW) and answered by the participant. Part 1 of the inventory consists of single items addressing different aspects of pain during the last week on 0-10 Likert scales; higher scores signalize more severe pain. In this study, the item for 'average pain' is selected as a secondary endpoint, in accordance with intervention trials in other chronic disorders (14,15).

Pressure pain threshold (16) was assessed by a commercially available algometer (Algometer Commander, JTECH Medical, Salt Lake City, USA). Results are not reported here.

Laboratory analyses

Sampling

Blood sampling was performed in the morning (between 8.00 and 10.00 a.m.). The participants were instructed to apply an ointment containing the local anesthetic lidocaine (Emla®) on the skin in the antecubital area one hour in advance. After at least five minutes supine rest in calm surroundings, blood samples for different laboratory assays were obtained in a fixed sequence from antecubital venous puncture.

Participants were instructed to bring a morning spot urine sample in a sterile container. Following the blood sampling procedure, samples of saliva and oral mucosa were collected for endocrinological and genetic analyses (results are not reported here).

Routine assays

Hematology and biochemistry routine assays were performed at the accredited laboratory at Oslo University Hospital, Norway.

Microbiological assays

The blood samples for microbiological analyses were collected in 4 mL EDTA tubes and gel-containing tubes, respectively. Detection of microbial DNA was performed by real-time polymerase chain reaction (PCR) in plasma (except EBV in whole blood), using in-house assays for adenovirus (17), human parvovirus B19 (18), and enterovirus (19), while commercial assays were used for Epstein-Barr virus (artus EBV, Qiagen, Hilden, Germany), human herpesvirus 6 (LightMix, TIB Molbiol, Berlin, Germany), and *Borrelia burgdorferi* sensu lato sp. (LightMix, TIB Molbiol, Berlin, Germany). Cytomegalovirus (CMV) DNA was analyzed by the Cobas Amplicor CMV monitor test (Roche, Branchburg, NJ, USA) during the first part of the study (278 samples in total). During the last part of the study (110 samples in total), CMV DNA was analyzed by an in-house real-time PCR assay using a LC480 PCR instrument (Roche), primers (GAACGTGTTGCGTTTCTTCG and AGCCTATCGGTGTCGCTGTA) and MGB probe (FAM-CACAGTAAAAGTAGCTGCGCT), detecting a conserved region in the CMV UL54 gene.

Specific antibody responses were assessed using anti-EBV EBNA IgG (Bio-Rad, Dreieich, Germany), anti-EBV VCA IgG and IgM (Hiss Diagnostics, Freiburg, Germany), anti-CMV IgG and IgM (Architect, Abbott, Illinois, USA), anti-Parvovirus B19 IgG and IgM (Biotrin, Dublin, Ireland), and anti-Borrelia burgdorferi IgG and IgM (Enzygnost, Siemens, Marburg, Germany).

Plasma norepinephrine assay

The blood samples for plasma norepinephrine (NA) analyses were obtained in vacutainer tubes treated with ethylene glycol tetraacetic acid (EGTA)–glutathione. The samples were placed on ice for approximately 30 minutes; thereafter, plasma was separated by centrifugation (3000 rpm, 15 min, 4 °C) and frozen at – 80 °C until assayed.

Samples were analyzed for plasma NA by high-performance liquid chromatography (HPLC) with a reversed-phase column and glassy carbon electrochemical detector (Antec, Leyden Deacade II SCC, Zoeterwoude, The Netherlands) using a commercial kit (Chromsystems, München, Germany) (20,21). All samples were measured in singlet, with serial samples from a given individual run at the same time to minimize run-to-run variability. The intra- and interassay coefficient of variation (CV) were 3.9 % and 10.8 %, respectively. The detection limit was 5.46 pM.

Urine free cortisol/creatinine ratio

The urine samples for cortisol and creatinine analyses were obtained in sterile plastic containers. Analyses were performed consecutively. For determination of urine free cortisol (non-conjugated cortisol), the urine samples were extracted with ether to avoid interference from other steroids, and thereafter assayed by solid phase competitive luminescence immunoassay (LIA) (type Immulite® 2000, Siemens Healthcare Diagnostics, NY, USA) (22). Intra- and interassay CV were <10 %. The urine levels of creatinine were analyzed using standard automatic analyzer techniques at the accredited laboratory at Oslo University Hospital, Norway. The cortisol/creatinine ratio was calculated in accordance with recent recommendations (23).

Serum C-reactive protein assay

The blood samples for C-reactive protein (CRP) analyses were collected in gel-containing vacutainer tubes. After centrifugation for 10 minutes (3600 rpm) at room temperature, the serum fraction was frozen at –80°C until analysis. All samples were analyzed in one batch featuring a high

sensitive assay (Roche Diagnostics, Indianapolis, IN, USA). The test principle is a particle-enhanced immunoturbidimetric assay (CRP Latex HS), where anti-CRP antibodies coupled to latex microparticles react with antigen in the sample; the following agglutination is measured turbidimetrically (24) The lower detection limit was 0.03 mg/L; the functional sensitivity was 0.11 mg/L. Interassay CV was 15.0 % and 5.0 % for low (0.6mg/L) and high levels (6.8mg/L), respectively.

Plasma clonidine assay

The blood samples for clonidine determinations were collected in 4 mL heparin tubes. After centrifugation for 12 minutes at 1000 g at room temperature, the plasma fraction was frozen at –20°C until analysis. A slight modification of the method described by Müller et al (25) was used for plasma clonidine assaying. The assay was validated based on FDA guidelines (26)

The samples were separated on an Alliance HT 2795 HPLC system and detected by a Micromass Quattro micro API MS/MS-instrument. System control, data acquisition and integration were performed by Masslynx software Ver 4.1.2008 (all from Waters, Milford, MA, USA). The MS/MS conditions were optimized by manual tuning during pump-infusion of neat solutions. The assay was set up to quantify from 0.10 μg/L to 5.00 μg/L clonidine in plasma. Quality control samples were included in all sample series, and placed both before and after the patient samples in each analytical run.

The median intra assay CV was 1 % at 5 μg/L, 5 % at 0.75 μg/L and 10 % at 0.10 μg/L. The inter assay CV was 6 % at 5 μg/L, 5 % at 0.75 μg/L and 12 % at 0.10 μg/L. Limit of detection, defined as a peak-to-peak signal to noise ratio of 5:1, verified by the Masslynx software, was 0.025 μg/L. Accuracy was 97 % (median) at 5 μg/L, 97 % at 0.75 μg/L, and 107 % at 0.10 μg/L.

Estimation of clonidine steady-state concentration

We measured plasma clonidine concentration 3 weeks and 8 weeks after therapy initiation. At each occasion, the participants were instructed to indicate the exact time span since intake of the last capsule.

The elimination of clonidine from plasma approximates a one-compartment model (27). During chronic oral administration, plasma half life varies considerably inter-individually (28,29), possibly due to genetic differences in hepatic metabolism (30). However, half life values remain fairly constant in each individual over time (28,29). For the purpose of estimating the rate constant of elimination (k_{elim}) in each individual, we assumed that both clonidine measurements were related to the same elimination process (ie., as if they were measured on the same day). We required a time span between the two measurements of ≥ 4 hours; then, the k_{elim} would be given from the following equation:

$$K_{elim} = -\frac{\ln C_2 - \ln C_1}{t}$$

where C_1 and C_2 are the two concentration measurements and t is the time (in hours) between them.

Trough levels of clonidine are considered a good estimate of steady-state concentration (29). In this study, a standardized trough level would be the plasma concentration measured exactly 12 hours after the last dosage. A deviation ± 2 hours from this ideal time point of blood sampling was regarded acceptable. If larger deviation, the measured plasma concentration was adjusted according to the equation above, applying individual estimates of k_{elim}. If an individual k_{elim} could not be estimated (e.g. due to lack of one concentration measurement), the median k_{elim} from all the individual estimates was used in the equation.

Following adjustments, the mean trough value was calculated in each participant and taken as an estimate of clonidine steady-state concentration during the intervention period. Concentration measurements below lower level of quantification but above lower limit of detection (8 in total) were included in the calculations.

Head up tilt-test

Heart rate responsiveness

The participants were subjected to a low-intensity head-up tilt test (HUT), as previously described (31) During HUT, they were attached to the Task Force Monitor® (Model 3040i, CNSystems Medizintechnic, Graz, Austria); a combined hardware and software device for noninvasive continuous recording of cardiovascular variables (32). They were positioned horizontally

on a tilt-table with foot-board support (Model 900-00, CNS-systems Medizintechnik, Graz, Austria). After 5 minutes of baseline recordings, they were head-up tilted 20° for 15 minutes, followed by another 5 minutes epoch in the horizontal position. Subjects were asked to relax; they did not speak and were not spoken to.

Instantaneous heart rate (HR) was obtained from the R-R interval (RRI) of the electrocardiogram. Photoplethysmography on the right middle finger was used to obtain a non-invasive, continuous recording of arterial blood pressure (33). Impedance cardiography was used to obtain a continuous recording of the temporal derivate of the transthoracic impedance (dZ/dt) (34). Blood pressure and impedance recordings are not reported in this article.

All recorded signals were on-line transferred to the built-in recording computer of the Task Force Monitor® that was running software for real-time data acquisition. HR data were exported to Microsoft Excel for further analyses. From each experimental run of HUT, we calculated the median in two epochs: From 270 to 30 seconds prior to tilt (Baseline) and from 30 to 270 seconds after being tilted (Tilt). We thereafter computed the delta value (Tilt – Baseline), which reflects the HR response to the tilt maneuver.

Cognitive tests

Test routines

All participants underwent cognitive testing in the following sequence: The digit span test from the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV), (35), the Color-Word Interference test from the Delis-Kaplan Executive Function System (D-KEFS) (36), and the Hopkins Verbal Learning Test-Revised (HVLT-R) (37). The Behavior Rating Inventory of Executive Function (BRIEF) was self-administered by the parents/next-of-kin (38). Only result from the digit span backward subtest from WISC-IV is reported here.

Digit span backward

Previous studies of CFS patients have demonstrated subtle cognitive deficits in working memory as well as other executive functions (39,40). Digit span tests are widely used for assessment of verbal or auditory working memory (35), and have been successfully applied in previous CFS research (39), as well as in intervention trials of other medical conditions (41,42).

The digit span test can be performed in a “forward” and “backward” manner (35). At both occasions, the examiner reads aloud strings of random digits (approximately one digit per second). The first two strings consist of 2 digits, the next two strings of 3 digits, etc. The digit span forward test requires the test person to repeat the digits in the same order as heard; for digit span backward, the test person is required to repeat the digits in reverse order. Each answer is scored 1 (correct) or 0 (incorrect). When both strings in a pair (ie. two strings of equal length) are answered incorrectly, the test is discontinued. Total scores are the sum of correct answers.

The digit span backward tests requires more mental manipulation of information as compared to the digit span forward test, and is assumed to assess central executive functions in a broader sense (43). Therefore, in this study, the digit span backward score is selected as a secondary endpoint. Total range is from 0 to 14; higher scores imply better working memory.

Daily physical activity

Accelerometers are widely used devices for accurate measurements of physical activity (44). They provide reliable and valid data among patients with impaired physical capacity (45), and have been successfully applied in previous CFS studies (46,47).

In this study, we used the *activPAL* accelerometer device (PAL Technologies Ltd, Glasgow, Scotland) for monitoring of daily physical activity during seven consecutive days. *ActivPAL* provides reliable and valid data on step number and cadence as well as time spent on walking, standing and sitting/lying during everyday activities (48,49). The device has also been validated in an adolescent population (50), and it is sensitive for changes of step number with time (51).

A recording period of seven consecutive days was selected, according to present recommendation (44). The recording unit (weight: 15 grams, size: 53 x 35 x 7 mm), was attached midline on the anterior aspect of the thigh by specially designed adhesive strips (PAL*stickies*), according to the manufacturer’s instruction. The participants were instructed to wear the unit permanently (ie, also during the night); however, they were shown how to remove it during showering/bathing and re-apply it afterwards. After the recording period, the unit was returned by mail in a pre-stamped envelope.

Data from the recording units was transferred to a computer running producer developed software. For each participant, all recording epochs were carefully and independently reviewed by two

of the authors (DS and EF). If one recording day was considered to contain erroneous or incomplete data, that entire day was removed from further calculation. Doubtful cases were discussed until consensus was reached.

Finally, the mean number of steps per day was calculated for all recording epochs. The mean number of steps per day is the primary endpoint in this study.

Questionnaires

Overview

In NorCAPITAL, a total of four separate questionnaires were applied. A comprehensive CFS questionnaire on background, symptoms and functions, that combines several separate inventories, was self-administered by all participants and returned in pre-stamped envelopes; details of this questionnaire are outlined below. Two other questionnaires (the Brief Pain Inventory (BPI) and the Behavior Rating Inventory of Executive Function (BRIEF)) were administered to the participants and the parents/next-of-kin, respectively, during the one-day in-hospital assessment; in addition, a questionnaire addressing adverse events was administered to CFS patients at week 8. Details of these three questionnaires are described elsewhere.

CFS questionnaire construction

In accordance with a CFS symptom inventory for adults (52), we have previously developed a CFS symptom inventory for adolescents, assessing the frequency of 24 common symptoms during the preceding month. Each symptom is rated on a 5-point Likert scale, ranging from 'never/rarely present' to 'present all of the time'. The inventory includes the eight accompanying symptom of the Fukuda-definition. In addition, it assesses other illness related aspects (such as fatigue duration and social consequences), as well as psychosocial aspects (such as family characteristics and alcohol/drug usage). The CFS symptom inventory for adolescents has been applied in routine clinical practice and in previous studies (8,31,53,54), and slightly modified according to experience.

In NorCAPITAL, we constructed a comprehensive CFS questionnaire by combining the CFS symptom inventory for adolescents with several other validated inventories assessing fatigue (Chalder Fatigue Questionnaire (55)), sleep disturbances (Karolinska Sleep Questionnaire (56)), symptoms of autonomic dysfunction (Autonomic Symptom Profile (57)), depressive symptoms (Mood and Feelings Questionnaire, child version (58)), and functional disability (Functional Disability Inventory (59)). At week 30, the questionnaire distributed to the CFS patients asked them to guess which treatment (clonidine or placebo) they had received.

CFS questionnaire: Background and subgrouping variables

The educational level among parents was categorized according to the International Standard Classification of Occupations (ISCO-88) (60). School absenteeism was estimated as the ratio between mean days absent from school last month and days supposed to be at school last month.

Questions from the CFS symptom inventory (52) were used to subgroup the CFS patients as to whether they adhered to the Fukuda-criteria or not; each accompanying symptoms was considered to be 'present' if scored 2 ('present 2-3 times per month') or higher on the 1-5 Likert scale. The Mood and Feelings Questionnaire (MFQ) has been thoroughly validated in children and adolescents (61). MFQ consists of 34 items, each scored on a 0-2 Likert scale; thus, the total sum score is from 0 to 68. A score ≥ 20 implies presence of depressive symptoms to a degree that suggests mood disorder (61), and was in this study used to subgroup all participants.

CFS questionnaire: Functional disability

The Functional Disability Inventory (FDI) was designed as a global measure of children's and adolescent's physical and psychosocial functioning in everyday social roles (59). FDI has been thoroughly validated in different patient populations, and is sensitive for change with time (59,62,63). Also, FDI has been used successfully in previous studies of adolescent CFS (64). FDI consists of 15 items, each scored on a 0-4 Likert scale. In this study, the FDI total sum across all items is selected as a secondary endpoint. Total range is from 0 to 60; higher scores imply more severe disability.

CFS questionnaire: Symptoms

The Chalder Fatigue Questionnaire (CFQ) (55) is regarded a valid outcome measure in CFS research among adults (4,65) as well as adolescents (66,67). In this study, the CFQ total sum score is selected as a secondary endpoint (ie. the sum across all 11 CFQ items, each of which is scored on a 0-3 Likert scale). Total range is from 0 to 33; higher scores imply more severe fatigue.

Besides fatigue, insomnia is one of the most prevalent symptoms in adolescent CFS patients (54). The Karolinska Sleep Questionnaire (KSQ) has been applied in epidemiological studies of fatigue (68). An insomnia subscale, based upon factor analyses (57,69), is constructed as the arithmetic mean across four items addressing insomnia problems during the preceding month (each of which is scored on a 1-6 Likert scale). Total range is from 1 to 6; lower scores imply poorer sleep. The insomnia subscale is selected as a secondary endpoint in this study.

The CFS symptom inventory for adolescents contains two items addressing unpleasantness due to loud sounds and normal indoor lighting, respectively, scored on 1-5 Likert scales. In a previous study of adolescent CFS, a composite score assumed to reflect sensory hypersensitivity was computed by taking the arithmetical mean across these two items (8). In a multivariate regression analysis featuring multiple symptom dimensions, this hypersensitive score was significantly and independently related to disability as well as indices of autonomic cardiovascular control (8). The hypersensitivity score was therefore selected as a secondary endpoint in the present study. Total range is from 1 to 5; higher scores imply more severe hypersensitivity.

Statistical analyses

Analyses set

A full analysis set of the CFS patients was defined as all patients who were randomized to clonidine/placebo and subsequently included (n = 120). The full analysis set was used for the modified intention to treat-analyses of clonidine effects (cf. Statistical analysis plan). All missing values were imputed by multiple imputation using the procedure implemented in SPSS. Variables statistically significantly associated ($p<0.05$) or with a correlation coefficient $r>0.3$ were used as predictors in the imputation model. A total number of 25 repetitions was applied, and the median estimate of the treatment difference was reported along with the corresponding 95% confidence interval and p-value. As a sensitivity analysis, missing values were imputed by the 'last observation carried forward'-principle, and missing data at baseline were imputed 'backwards' if valid follow-up data existed.

A per protocol analysis set was defined as all patients in the 'full analysis set' that completed the follow-up period without any protocol deviations: Interruption of therapy/drop-out; primary endpoint measurements missing; low index of compliance (more than 3 SD below mean value); low plasma concentration of clonidine at week 8 (more than 3 SD below mean value); diagnosed with another chronic disorder during the study period; commencing other treatment during the study period; experiencing trauma/severe illness during the study period. Missing data were not imputed in the per protocol analysis set.

A safety analysis set was defined as equivalent with the full analysis set. However, missing values were not imputed for safety endpoints.

Factor analysis

In order to confirm construct validity of the selected symptom variables for subgrouping and endpoint evaluation, a factor analysis (Principal Component Analyses with Varimax Rotation) on baseline questionnaire data in CFS patients (per protocol analysis set) was performed. The following variables were included:

- MFQ – total sum score (subgrouping variable)
- CFQ – total sum score (endpoint variable)
- BPI – average pain score (endpoint variable)
- KSQ – insomnia score (endpoint variable)
- CFS symptom inventory - sensitivity towards sounds (part of the hypersensitivity score, an endpoint variable)
- CFS symptom inventory - sensitivity towards light (part of the hypersensitivity score, an endpoint variable)

eResults and Discussion

Validity assessments

For the great majority of participants completing the investigational program, the *activ*PAL registration yielded 7 consecutive days of valid activity recordings (eTable 3). At baseline, *activ*PAL registrations were missing in two CFS patients (both in the placebo group); thus, these cases were excluded from modified intention to treat-analysis of the primary endpoint (cf. above).

The factor analysis of the symptom variables confirmed a five-factor construct (eTable 4). The items addressing unpleasantness due to loud sounds and normal indoor lighting loaded heavily on one factor (0.85 and 0.88, respectively), and did not load on any other factors. The four scores from CFQ, BPI, KSQ and MFQ loaded on one factor each (loadings from 0.94 to 0.97).

Protocol deviation

In the intervention part of the study, 14 CFS patients dropped out prior to week 8, and an additional 3 prior to week 30 (eTable 5). At week 8, a total of 26 protocol deviations were registered, leaving 94 patients for the per protocol-analyses. At week 30, 17 additional protocol deviations were registered; thus, at this stage, 77 patients were available for the per protocol-analyses.

Additional therapy

Throughout the study period, 2 CFS patients used thyroid hormone supplement, 1 used melatonin and 1 used bronchodilators occasionally (eTable 6). A total of 7 CFS patients regularly participated in cognitive behavioral therapy or other variants of psychotherapy. In the period from baseline to week 8, an intensive rehabilitation program was initiated in 3 patients. In the period from week 8 to week 30, 2 patients received antibiotics, 1 patient started using a proton pump-inhibitor, 5 patients commenced psychotherapy and 8 patients started intensive rehabilitation.

Power calculation

The primary endpoint (step/day) had a standard deviation (SD) of 2386 in the CFS patients, whereas the statistical power calculation assumed a SD of 4000. The drop-out rate was somewhat higher than assumed; still, our initial power calculation probably over-estimated the number needed to be included, which would result in higher power to detect effects of clonidine treatment.

As for safety endpoint, the study was not powered to detect rare events. However, clonidine is an old drug with a well known safety profile, and no new safety signals would be expected.

Guessing at treatment allocation

Both patients' guess and primary researchers' guess seemed to be randomly related to allocation groups (eTable 7), indicating successful double blinding.

eTable 1. Criteria for randomization—CFS patients

Based on written information from the referring unit, there is evidence of ...	*Based on written information from the referring unit, there is **no** evidence of ...*
Persisting or constantly relapsing fatigue lasting 3 months or more.	Another disease process or current demanding life event that might explain the fatigue
Functional disability resulting from fatigue to a degree that prevent normal school attendance	Another chronic disease
Age ≥ 12 years and < 18 years	Permanent use of drugs (including hormones)
	Permanently bed-ridden

eTable 2. Criteria for inclusion and exclusion

	Inclusion criteria	*Exclusion criteria*
CFS patients	Persisting or constantly relapsing fatigue lasting 3 months or more. Functional disability resulting from fatigue to a degree that prevent normal school attendance Age ≥ 12 years and < 18 years	Another current disease process or demanding life event that might explain the fatigue Another chronic disease Permanent use of drugs (including hormones) possibly interfering with measurements Permanently bed-ridden Positive pregnancy test Pheocromocytoma Evidence of reduced cerebral and/or peripheral circulation due to vessel disease Polyneuropathy Renal insufficiency Known hypersensitivity towards clonidine or inert substances (lactose, saccarose) in capsula Abnormal ECG (apart from ectopic beats) Supine heart rate < 50 beats/min Supine systolic blood pressure < 85 mmHg Upright systolic blood pressure fall > 30 mmHg
Healthy control subjects	Age ≥ 12 years and < 18 years	Another chronic disease Permanent use of drugs (including hormones)

eTable 3. Number of *activ*PAL registrations* with days of valid recordings

	CFS patients			*Healthy controls*
Days of valid recordings	*Baseline*	*Week 8*	*Week 30*	*Baseline*
7 days	114	97	87	32
6 days	3	3	4	3
5 days	1	1	3	0
4 days	0	2	2	1
3 days	0	1	2	1
2 days	0	0	0	1
All missing†	2	16	22	1

* Number of steps per day, derived from the *activ*PAL registrations, served as primary endpoint in the clonidine intervention part of the study

† Numbers include all drop-outs, cf. Figure 1

eTable 4. Confirmative factor analysis of questionnaire symptom variables

	Factor 1	*Factor 2*	*Factor 3*	*Factor 4*	*Factor 5*
MFQ - total sum score	-	-	-	-	0.94
CFQ - total sum score	-	-	0.98	-	-
BPI - average pain score	-	0.97	-	-	-
KSQ - insomnia score	-	-	-	0.97	-
CFS symptom inventory - hypersensitivity score	-	-	-	-	-
Sensitivity towards sounds	0.85	-	-	-	-
Sensitivity towards light	0.88	-	-	-	-

Based on principal component analysis with varimax rotation. Factor loadings < 0.20 have been removed for clarity

eTable 5. Protocol deviations during the intervention part of the study. Number of cases

	Week 8		*Week 30*	
	Clonidine group	*Placebo group*	*Clonidine group*	*Placebo group*
Interruption of therapy/drop-out	5	9	1	2
Primary endpoint measurement missing	1	2	2	1
Low index of compliance*	2	1	n.a.	n.a.
Low plasma concentration of clonidine*	1	n.a.	n.a.	n.a.
Diagnosed with another chronic disorder	1	1	-	-
Commencing other treatment	1	2	5	6
Experiencing trauma/severe illness	-	-	-	-
Total	*11*	*15*	*8*	*9*

*More than 3 SD below mean value.
n.a.=not applicable

eTable 6. Pharmacotherapy and other therapeutic approaches during the course of the study. Number of cases*

	Throughout the study period		*Commenced during intervention period (Baseline-Week 8)*		*Commenced during follow-up period (Week 8-Week 30)*	
	Clonidine group	*Placebo group*	*Clonidine group*	*Placebo group*	*Clonidine group*	*Placebo group*
Thyroid hormone supplement	2	-	-	-	-	-
Bronchodilators, occasionally	-	1	-	-	-	-
Antibiotics	-	-	-	-	1	1
Melatonin	-	1	-	-	-	-
Proton pump inhibitor	-	-	-	-	1	-
Intensive rehabilitation	-	-	1	2	4	4
Cognitive behavioral therapy/other psychotherapy	2	5	-	-	3	2

*Some patients commenced two or more therapies during the study period
Unconventional therapies, such as homeopathics, diets and 'The lightning process' etc. is not included

eTable 7. Guessing at treatment allocation

	CFS patients' allocation		*p-value**
	Clonidine	*Placebo*	
CFS patients' guess – no. (%)			
Clonidine	19 (37)	15 (33)	.674
Placebo	32 (63)	31 (67)	
Primary researchers' (DS or EF) guess – no. (%)			
Clonidine	29 (57)	23 (50)	.545
Placebo	22 (43)	23 (50)	

* Chi-square test

eTable 8. Background characteristics—supplement

	CFS patients, baseline	*Healthy controls*	*P-value*
Ethnicity - no. (%)			
Scandinavian	118 (98)	62 (91)	.027
Not scandinavian	2 (1.7)	6 (8.9)	
Lives with ... - no. (%)			
... both parents	85 (73)	26 (70)	.800
... one parent	31 (26)	11 (30)	
... alone	1 (0.8)	0 (0)	
Parents' highest education - no. (%)			
Primary	5 (4.3)	0 (0)	.387
Secondary	30 (26)	8 (23)	
Lower university	34 (29)	8 (23)	
Higher university	48 (41)	19 (54)	
Siblings - no. (%)			
0	14 (12)	9 (24)	.321
1	61 (52)	16 (43)	
2	33 (28)	9 (24)	
≥ 3	10 (8.5)	3 (8.1)	
Alcoholic beverages - no. (%)			
Never	90 (78)	28 (78)	.951
Occationally	25 (22)	8 (22)	
Tobacco products - no. (%)			
Never	95 (83)	29 (78)	.494
Occationally	19 (17)	8 (22)	
Narcotics/illigal drugs - no. (%)			
Never	114 (100)	34 (92)	.014
Occationally	0 (0)	3 (8.1)	
School absenteism - %, mean (SD)	65 (30)	2.1 (6.8)	<.001
Blood hematology and biochemistry			
Hemoglobin - g/dL, mean (SD)	13.4 (1.0)	13.4 (1.1)	.989
Leucocytes - 10^9 cells/L, mean (SD)	5.9 (1.5)	5.9 (1.7)	.874
Lymphocytes - 10^9 cells/L, mean (SD)	2.2 (0.6)	2.1 (0.6)	.263
Neutrophils - 10^9 cells/L, mean (SD)	3.0 (1.1)	3.1 (1.5)	.680
Trombocytes - 10^9 cells/L, mean (SD)	286 (62)	292 (55)	.468
Sodium - mmol/L, mean (SD)	141.5 (1.7)	141.2 (1.6)	.212

eTable 8. Background characteristics—supplement (continued)

	CFS patients, baseline	*Healthy controls*	*P-value*
Potassium - mmol/L, mean (SD)	3.83 (0.23)	3.87 (0.20)	.276
Creatinine - µmol/L, mean (SD)	55.5 (9.2)	56.7 (9.0)	.378
ALT - U/L, mean (SD)	17.4 (10.4)	15.8 (7.8)	.237
Albumin - g/L, mean (SD)	44.9 (2.4)	45.4 (2.5)	.139
Blood microbiology			
B. burgdorferi PCR - no (%)			
Negative	120 (100)	65 (100)	n.a.
Positive	0 (0)	0 (0)	
Anti-*B. burgdorferi* IgM - no. (%)			
Negative	111 (100)	56 (97)	.116
Positive	0 (0)	2 (3.4)	
Anti-*B. burgdorferi* IgG - no. (%)			
Negative	115 (98)	62 (95)	.350
Positive	2 (1.7)	3 (4.6)	
EBV PCR - no. (%)			
Negative	117 (99)	65 (98)	>.999
Positive	1 (0.8)	1 (1.5)	
Anti-EBV EBNA IgG - no. (%)			
Negative	52 (48)	35 (58)	.206
Positive	56 (52)	25 (42)	
Anti-EBV VCA IgM - no. (%)			
Negative	107 (97)	60 (98)	>.999
Positive	3 (2.7)	1 (1.6)	
CMV PCR - no. (%)			
Negative	119 (100)	65 (100)	n.a.
Positive	0 (0)	0 (0)	
Anti-CMV IgM - no. (%)			
Negative	116 (100)	64 (98)	.359
Positive	0 (0)	1 (1.5)	
Anti-CMV IgG - no. (%)			
Negative	63 (53)	36 (56)	.711
Positive	55 (47)	28 (44)	
Parvovirus B19 PCR - no. (%)			
Negative	115 (98)	60 (95)	.345
Positive	2 (1.7)	3 (4.8)	
Anti-Parvovirus B19 IgM - no. (%)			
Negative	114 (98)	64 (98)	>.999
Positive	2 (1.7)	1 (1.5)	

Anti-Parvovirus B19 IgG - no. (%)			
Negative	59 (52)	34 (55)	.739
Positive	54 (48)	28 (45)	
HHV-6 PCR - no. (%)			
Negative	110 (93)	57 (88)	.205
Positive	8 (6.8)	8 (12)	
Enterovirus PCR - no. (%)			
Negative	117 (100)	65 (100)	n.a.
Positive	0 (0)	0 (0)	
Adenovirus PCR - no. (%)			
Negative	118 (100)	65 (100)	n.a.
Positive	0 (0)	0 (0)	

P-values are based on Chi-square test, Fisher's exact test or Student t-test, as appropriate. SD=standard deviation, ALT=alanine aminotransferase, PCR=polymerase chain reaction, EBV=Epstein Barr-virus, CMV=cytomegalovirus, HHV-6=Herpes Hominis-virus 6, n.a.=not applicable

eTable 9. Outcome of clonidine intervention: per-protocol analyses

Efficacy variables	*Baseline*	*Week 8 (during treatment)*	*Week 30 (after treatment)*
Steps per day - number			
Clonidine group, mean	4670	4568	4560
Placebo group, mean	4653	5173	4406
Difference (95 % CI)		-605 (-1356 to 145)	155 (-836 to 1145)
p-value (clonidine vs. placebo)		.112	.757
FDI - total sum score			
Clonidine group, mean	24.0	22.8	20.0
Placebo group, mean	23.0	21.8	20.3
Difference (95 % CI)		0.9 (-2.5 to 4.3)	-0.3 (-4.6 to 4.1)
p-value (clonidine vs. placebo)		.588	.901
CFQ - total sum score			
Clonidine group, mean	19.2	16.6	13.7
Placebo group, mean	19.4	13.9	14.6
Difference (95 % CI)		2.7 (0.4 to 5.1)	-0.9 (-3.6 to 1.8)
p-value (clonidine vs. placebo)		.023	.493
BPI - average pain score			
Clonidine group, mean	4.7	3.8	3.8
Placebo group, mean	4.3	3.6	3.2
Difference (95 % CI)		0.3 (-0.5 to 1.0)	0.5 (-0.3 to 1.4)
p-value (clonidine vs. placebo)		.512	.218
KSQ - insomnia score			
Clonidine group, mean	3.4	3.8	3.7
Placebo group, mean	3.5	3.8	3.6
Difference (95 % CI)		0.0 (-0.3 to 0.4)	0.0 (-0.4 to 0.5)
p-value (clonidine vs. placebo)		.906	.946
Symptom inventory - hypersensitivity score			
Clonidine group, mean	2.9	2.5	2.3
Placebo group, mean	2.8	2.4	2.4
Difference (95 % CI)		0.2 (-0.2 to 0.5)	-0.1 (-0.5 to 0.4)
p-value (clonidine vs. placebo)		.400	.689
Digit span backward - total sum score			
Clonidine group, mean	5.6	6.1	6.1
Placebo group, mean	6.0	6.1	6.8
Difference (95 % CI)		0.0	-0.7 (-1.5 to 0.2)
p-value (clonidine vs. placebo)		.974	.111
Heart rate responsiveness - beats/min			
Clonidine group, mean	5.2	5.1	4.5
Placebo group, mean	4.8	4.9	6.0
Difference (95 % CI)		0.1 (-1.2 to 0.8)	-1.5 (-3.1 to 0.1)
p-value (clonidine vs. placebo)		.848	.066
Plasma norepinephrine - pmol/L			
Clonidine group, mean	2040	1458	1696
Placebo group, mean	1942	1711	1891
Difference (95 % CI)		-253 (-490 to -17)	-195 (-515 to 125)
p-value (clonidine vs. placebo)		.036	.229

eTable 9. Outcome of clonidine intervention: per-protocol analyses (continued)

Efficacy variables	*Baseline*	*Week 8 (during treatment)*	*Week 30 (after treatment)*
Urine cortisol/creatinine ratio - nmol/mmol			
Clonidine group, mean	4.25	3.41	3.77
Placebo group, mean	3.07	3.72	3.75
Ratio (95 % CI)		0.91 (0.67 to 1.26)	1.01 (0.71 to 1.42)
p-value (clonidine vs. placebo)		.580	.976
Serum CRP - mg/L			
Clonidine group, mean	0.59	0.45	0.63
Placebo group, mean	0.58	0.64	0.57
Ratio (95 % CI)		0.69 (0.51 to 0.95)	1.10 (0.72 to 1.70)
p-value (clonidine vs. placebo)		.022	.649
Safety variables			
Supine SBP - mm Hg			
Clonidine group, mean	112	112	113
Placebo group, mean	114	111	112
Difference (95 % CI)		1.4 (-1.4 to 4.2)	1.2 (-2.1 to 4.5)
p-value (clonidine vs. placebo)		.326	.459
Supine DBP - mm Hg			
Clonidine group, mean	62	62	60
Placebo group, mean	65	61	60
Difference (95 % CI)		1.3 (-1.5 to 4.1)	0.6 (-2.6 to 3.9)
p-value (clonidine vs. placebo)		.363	.707
Supine heart rate - beats/min			
Clonidine group, mean	75	70	75
Placebo group, mean	76	73	73
Difference (95 % CI)		-3.0 (-5.9 to 0.05)	1.9 (-1.6 to 5.3)
p-value (clonidine vs. placebo)		.054	.284
Upright SPB - mm Hg			
Clonidine group, mean	116	116	117
Placebo group, mean	117	117	117
Difference (95 % CI)		-1.2 (-5.2 to 2.7)	0.3 (-4.0 to 4.5)
p-value (clonidine vs. placebo)		.528	.897
Upright DPB - mm Hg			
Clonidine group, mean	74	70	70
Placebo group, mean	75	71	71
Difference (95 % CI)		-0.3 (-4.5 to 4.0)	-0.8 (-4.5 to 2.8)
p-value (clonidine vs. placebo)		.902	.647
Upright heart rate - beats/min			
Clonidine group, mean	97	92	97
Placebo group, mean	95	96	99
Difference (95 % CI)		-3.5 (-10.1 to 3.1)	-2.0 (-8.3 to 4.4)
p-value (clonidine vs. placebo)		.294	.535

Means and differences at week 8 and week 30 are estimated from the parameters of the general linear model. For Urine cortisol/creatinine ratio and Serum CRP, modeling was performed on ln-transformed variables; all means are based on back-transformation of the variables, and ratios instead of differences are reported. CI=confidence interval, FDI=Functional Disability Inventory, CFQ=Chalder Fatigue Questionnaire, BPI=Brief Pain Inventory, KSQ=Karolinska Sleep Questionnaire, CRP=C-reactive protein, SBP=systolic blood pressure, DBP=diastolic blood pressure

eTable 10. Dose response relationships

Efficacy variables	*Clonidine concentration (µg/L), week 8*	*Clonidine concentration (µg/L), steady state*
Steps per day - number		
Regression coefficient, B (95 % CI)	-3862 (-7655 to -70)	-5378 (-10458 to -297)
p-value	.046	.039
FDI - total sum score		
Regression coefficient, B (95 % CI)	10.3 (-11.8 to 32.3)	21.1 (-7.3 to 49.4)
p-value	.352	.141
CFQ - total sum score		
Regression coefficient, B (95 % CI)	15.2 (3.4 to 27.0)	19.3 (3.5 to 35.0)
p-value	.013	.018
BPI - average pain score		
Regression coefficient, B (95 % CI)	-0.3 (-4.8 to 4.2)	1.7 (-4.3 to 7.6)
p-value	.905	.577
KSQ - insomnia score		
Regression coefficient, B (95 % CI)	-1.9 (-4.1 to 0.4)	-2.9 (-5.8 to -0.04)
p-value	.106	.047
Symptom inventory - hypersensitivity score		
Regression coefficient, B (95 % CI)	0.1 (-2.4 to 2.6)	0.8 (-2.4 to 4.0)
p-value	.917	.620
Digit span backward - total sum score		
Regression coefficient, B (95 % CI)	-0.3 (-3.4 to 2.9)	-0.3 (-4.5 to 3.9)
p-value	.865	.888
Heart rate responsiveness - beats/min		
Regression coefficient, B (95 % CI)	1.0 (-7.5 to 9.6)	3.1 (-8.2 to 14.4)
p-value	.808	.580
Plasma norepinephrine - pmol/L		
Regression coefficient, B (95 % CI)	-704 (-2010 to 601)	-239 (-1986 to 1509)
p-value	.282	.784
Urine cortisol/creatinine ratio - nmol/mmol		
Regression coefficient, B (95 % CI)	-0.8 (-2.7 to 1.1)	-0.2 (-2.8 to 2.4)
p-value	.403	.891
Serum CRP - mg/L		
Regression coefficient, B (95 % CI)	1.2 (-0.7 to 3.0)	0.5 (-2.0 to 3.0)
p-value	.223	.698

eTable 10. Dose response relationships, continued

Safety variables	*Clonidine concentration (µg/L), week 8*	*Clonidine concentration (µg/L), steady state*
Supine SBP - mm Hg		
Regression coefficient, B (95 % CI)	-5.6 (-23.4 to 12.2)	2.4 (-20.6 to 25.4)
p-value	.531	.834
Supine DBP - mm Hg		
Regression coefficient, B (95 % CI)	-6.6 (-24.3 to 11.2)	-4.0 (-27.4 to 19.4)
p-value	.461	.731
Supine heart rate - beats/min		
Regression coefficient, B (95 % CI)	-6.0 (-21.6 to 9.5)	2.6 (-18.9 to 24.0)
p-value	.440	.812
Upright SPB - mm Hg		
Regression coefficient, B (95 % CI)	-4.8 (-27.5 to 17.9)	3.9 (-24.3 to 32.1)
p-value	.673	.782
Upright DPB - mm Hg		
Regression coefficient, B (95 % CI)	10.8 (-15.3 to 36.9)	7.2 (-25.7 to 40.0)
p-value	.408	.662
Upright heart rate - beats/min		
Regression coefficient, B (95 % CI)	12.9 (-31.7 to 57.4)	18.0 (-39 to 74.9)
p-value	.562	.526

Regression coefficients are calculated from multivariable linear regression analyses, controlling for baseline values of each outcome variable. For urine cortisol/creatinine ratio and serum CRP, values were ln-transformed prior to analyses. CI=confidence interval, FDI=Functional Disability Inventory, CFQ=Chalder Fatigue Questionnaire, BPI=Brief Pain Inventory, KSQ=Karolinska Sleep Questionnaire, CRP=C-reactive protein, SBP=systolic blood pressure, DBP=diastolic blood pressure

eTable 11. Adverse effects, self-reported

	Clonidine group	*Placebo group*	*p-value*
Total - no. (%)			
No	14 (25)	18 (35)	.223
Yes	43 (75)	33 (65)	
Drowsiness - no. (%)			
No	47 (82)	46 (90)	.246
Yes	10 (18)	5 (10)	
Dry mouth - no. (%)			
No	38 (67)	37 (73)	.508
Yes	19 (33)	14 (27)	
Unwellness - no. (%)			
No	49 (86)	47 (92)	.307
Yes	8 (14)	4 (8)	
Constipation - no. (%)			
No	49 (86)	43 (84)	.809
Yes	8 (14)	8 (16)	
Sleepiness - no. (%)			
No	43 (75)	47 (92)	.020
Yes	14 (25)	4 (8)	
Loose stool - no. (%)			
No	51 (89)	46 (90)	.901
Yes	6 (11)	5 (10)	
Rash - no. (%)			
No	49 (86)	49 (96)	.098
Yes	8 (14)	2 (4)	
Itching - no. (%)			
No	54 (95)	47 (92)	.587
Yes	3 (5)	4 (8)	
Sadness - no. (%)			
No	52 (91)	49 (96)	.443
Yes	5 (9)	2 (4)	
Headache - no. (%)			
No	44 (77)	46 (90)	.070
Yes	13 (23)	5 (10)	
Breast development in men - no. (%)			
No	22 (100)	22 (100)	n.a.
Yes	0 (0)	0 (0)	
Dry nasal mucus membranes - no. (%)			
No	51 (89)	50 (98)	.117
Yes	6 (11)	1 (2)	

eTable 11. Adverse effects, self-reported (continued)

	Clonidine group	*Placebo group*	*p-value*
Hallucinations - no. (%)			
No	56 (98)	51 (100)	>.999
Yes	1 (2)	0 (0)	
Confusion - no. (%)			
No	52 (93)	50 (98)	.366
Yes	4 (7)	1 (2)	
Nigthmares - no. (%)			
No	51 (89)	50 (98)	.117
Yes	6 (11)	1 (2)	
"Tingling" in extremities - no. (%)			
No	48 (84)	46 (90)	.355
Yes	9 (16)	5 (10)	
Pain in the extremitites - no. (%)			
No	53 (93)	49 (96)	.682
Yes	4 (7)	2 (4)	
Dizziness when rising - no. (%)			
No	41 (72)	46 (90)	.017
Yes	16 (28)	5 (10)	
Blurred vision - no. (%)			
No	53 (93)	49 (96)	.682
Yes	4 (7)	2 (4)	
Dry eyes - no. (%)			
No	52 (91)	43 (84)	.270
Yes	5 (9)	8 (16)	
Edema in the extremities - no. (%)			
No	56 (98)	51 (100)	>.999
Yes	1 (2)	0 (0)	
Other - no. (%)			
No	49 (86)	42 (82)	.607
Yes	8 (14)	9 (18)	

P-values are based on Chi-square test and Fisher's exact test, as appropriate. n.a.=not applicable.

eTable 12. Associations between physical activity, heart rate responsiveness and plasma norepinephrine in CFS patients at baseline

	Heart rate responsiveness - beats/min	*Plasma norepinephrine - pmol/L*
Steps per day - number		
Regression coefficient, B (95 % CI)	-70 (-170 to 30)	0.003 (-0.57 to 0.58)
p-value	.166	.993

Regression coefficients are calculated from multivariable linear regression analyses.
CI=confidence interval

eTable 13. Potential clonidine effects on markers of plasma volume: per-protocol analyses

	Baseline	*Week 8 (during treatment)*
Hemoglobin - g/dL		
Clonidine group, mean	13.4	13.3
Placebo group, mean	13.5	13.4
Difference (95 % CI)		-0.05 (-0.28 to 0.19)
p-value (clonidine vs. placebo)		.691
Weight – kg		
Clonidine group, mean	60.3	61.8
Placebo group, mean	61.3	62.2
Difference (95 % CI)		-0.4 (-2.1 to 1.4)
p-value (clonidine vs. placebo)		.680

Means and differences at week 8 are estimated from the parameters of the general linear model.
CI=confidence interval

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