

Title Protocol

The relation between cerebral blood flow, pain attenuation and autonomic parameters in rest, during and after exercise in patients with the chronic fatigue syndrome.

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ABBREVIATIONS

CFS	Chronic Fatigue Syndrome
FM	Fibromyalgia
CPM	Chronic Pain Modulation
HRV	Heart Rate Variability
HR	Heart Rate
LF	Low Frequency
HF	High Frequency
ANS	Autonomic Nervous System
BRS	Baroreflex Sensitivity
BP	Blood pressure
CA	Cerebral Autoregulation
CBF	Cerebral Blood Flow
ABP	Arterial Blood Pressure
BMI	Body Mass Index
VM	Vasalva Manoeuvre
PPT	Pressure Pain Threshold
NYHA	New York Heart Association
CIS	Checklist Individual Strength
PCS	Pain Catastrophizing Scale
TSK	Tampa Scale for Kinesiophobia
BVP	Blood Volume Pulse
SDNN	Standard deviation of NN intervals
RMSSD	square root of the mean squared difference of successive NNs
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MBP	Mean Blood Pressure

ICA	Internal Carotid Arteries
VA	Vertebral Arteries
IAPS	International Affective Picture Scale
SWA	Sensewear Pro3 Armband (activity monitoring)
MET	Metabolic equivalent

INTRODUCTION

Besides the characteristic fatigue, patients with Chronic Fatigue Syndrome (CFS) often suffer from chronic widespread and persistent pain (1, 2, 3). A population-based study revealed that 94 % of the persons diagnosed with CFS report muscle pain, and 84 % report joint pain (4). In fact, there is a great overlap between CFS and Fibromyalgia (FM), a disease particularly characterized by musculoskeletal pain (1).

Similar to FM (2, 3), previous studies define central sensitization as underlying mechanism maintaining chronic pain in patients with CFS (5,6,7). Central sensitization comprises hyper excitement of the central neurons, altered sensory processing in the brain and malfunctioning descending pain inhibitory mechanisms (8). Conditioned pain modulation (CPM) is a method for examining the descending inhibitory pathways. Meeus et al. (2008) already reported impaired CPM in CFS (9). Furthermore, exercise induced pain inhibition is not activated in patients with CFS, resulting in lower pain thresholds and pain exacerbation (10,11).

Descending pain inhibitory pathways arise mainly from the periaqueductal gray matter and the rostral ventral medulla in the brainstem (12). In addition, Pereira et al. 2010 reported an increase in parasympathetic activity by stimulating the periaqueductal gray matter in patients with chronic pain (13). This results in the hypothesis that malfunctioning of the descending pain inhibition in patients with CFS goes together with abnormal ANS responses, which are frequently reported in patients with CFS (14, 15). In order to measure autonomic cardiovascular responses, heart rate variability (HRV) analysis is often applied. HRV consists of a high frequency (HF) (0.15 to 0.4 Hz) and a low frequency (LF) (0.04 to 0.15 Hz) component. Heart rate (HR) fluctuations in the HF band reflect respiratory sinus arrhythmia, whereas fluctuations in LF range reflect baroreflex-mediated autonomic outflows (16). Several studies (17,18,19) used HRV analysis to reveal impaired Autonomic Nervous System (ANS) responses in patients with FM. Most of these studies observed increased sympathetic activity and decreased parasympathetic activity in patients with FM in rest.

Interestingly, in a recent pain study, Chalaye and colleagues (2012) observed in response to experimentally induced pain, besides the absent endogenous pain inhibition, greater sympathetic activity in FM. Healthy controls however showed higher parasympathetic activity (20). These results are partially in contrast with Reyes del Paso et al. (2011) who described the impaired autonomic cardiovascular regulation in FM as a reduction of both parasympathetic and sympathetic activity. Furthermore a reduced sympathetic reactivity to acute stress was noted. Although in this latter study a higher LF/HF ratio was observed in patients with FM, they also found reduced stroke volume and myocardial contractility in rest and less pronounced increase in stroke volume and myocardial contractility during cold pain stimulation in patients with FM, indicative for reduced sympathetic influences (21). There are some inconsistent findings about HRV in patients with CFS (22, 23), but not in relation to pain.

Furthermore, the baroreflex sensitivity (BRS) plays an important role in the autonomic cardiovascular response to pain (24). BRS is the change in R-R interval per unit change in systolic blood pressure (BP) (21). In acute pain a functional model exists where pain creates sympathetic arousal, which increases blood pressure (BP). This increased BP triggers baroreceptors, which in turn activates pain inhibitory descending pathways (24). Chronic pain induces alterations in this BP/pain sensitivity model due to changes in BRS, impairments in descending inhibitory pathways and/or activation of pain facilitatory pathways (24). Both in FM and in CFS, there is preliminary evidence for reduced BRS (21,25), but the relation to pain remains unclear.

Autonomic neural activity plays also an important role in the regulation of dynamic cerebral autoregulation (CA)(26). CA aims to maintain a stable and adequate cerebral blood flow (CBF). In rest, static or steady-state CA is active, dominated by vasomotor effects. Whereas autonomic effects are prominent in dynamic CA during exercise (27). For CA to be effective, the cerebral perfusion pressure must lie within a certain autoregulatory range (28). During moderate to heavy exercise, in healthy people, this range is possibly exceeded, making CA insufficient (29). In consequence, the baroreflex becomes an important mechanism for the regulation of arterial blood pressure (ABP) and CBF during exercise (28). Since decreased CBF is reported at rest in patients with CFS (30), it is hypothesized that patients with CFS don't exceed the autoregulatory range during exercise, making CA sufficient. Consequently, there would be less activation of the baroreceptors, reducing baroreceptor mediated pain inhibition during exercise.

To the best of our knowledge, studies examining the relation between ANS and pain in CFS are lacking. Likewise, studies investigating CBF in CFS are rare. Also in healthy controls, studies examining exercise induced analgesia in relation to autonomic function and CBF in response to exercise are lacking. Therefore the aim of the present study is examining the relation between CBF and autonomic functions (i.e. heart rate variability, blood pressure, skin conductance) in response to exercise in patients with CFS and healthy controls. Furthermore, pain in response to exercise will be measured.

This aim was based on several hypotheses with respect to pathophysiology. Firstly, it is hypothesized that there is a reduced CBF during and following exercise in patients with CFS compared to healthy controls. In addition, it is hypothesized that dysfunctional endogenous analgesia is correlated with decreased CBF. The third hypothesis is that impaired autonomic cardiovascular regulation is related to the (reduced) cerebral blood flow and the impaired pain inhibition.

Clinical significance of the results:

It has been well established that exercise therapy is effective for patients with CFS [50-52]. However, the effects are rather small and many patients show poor treatment adherence. The latter is due to the experience of symptom flares following exercises, a symptom often referred to as ‘post-exertional malaise’. In general, this study will contribute to our understanding of post-exertional malaise in patients with CFS, and hence the identification of new treatment targets for post-exertional malaise in CFS. More specifically, the following three targets will be addressed in the present study:

First, a substantial number of CFS patients have complaints of dizziness/lightheadedness during or immediately after exercise. In general, the origin of dizziness is multifactorial. In this study we hope to pinpoint the origin of these complaints to changes in cerebral blood flow. So far, the contribution of cerebral blood flow changes to the complaints of CFS patients have been underexposed in CFS research.

Secondly, a substantial number of CFS patients have pain complaints and flare-up of these symptoms after exercise. Although the relation between exercise and flare-up of pain symptoms is well known, the underlying mechanism of the flare-up is poorly understood. We hope to demonstrate that there is relation between decrease of cerebral blood flow and increased pain perception after exercise. If this relation is demonstrated in this study, further interventional studies can be designed to assess a cause (cerebral blood flow) – effect (increased pain) relation.

Finally, autonomic function abnormalities are also well known in CFS patients. These autonomic function abnormalities may change the autoregulation of cerebral blood flow in CFS patients compared to healthy controls. In this study we hope to demonstrate that the abnormal autonomic function will be related to the altered cerebral blood flow and thus the abnormal pain perception.

PATIENT POPULATION

Subjects

Fiftyone patients with CFS, and 51 healthy pain-free control subjects will be enrolled. Healthy women will be included in a ratio of 3 to 4, because the CFS population mainly consists of women (in the population of the Stichting Cardiozorg women account for 79% of the CFS patients, in line with the literature) Likewise, pooling of gender data of exercise physiology studies in CFS research has been identified as a common source of bias [54]. Each study participant should be aged between 18 and 65 years.

The sample size calculation was performed using G-Power, and based on the previously established differential effect of exercise on the pain perception in CFS patients versus normal the pain threshold being increased in normals and being unchanged in CFS patients. Therefore a one sided test (difference between two independent means (two groups)) was chosen with a power of 0.80 (beta = 0.20). Based on the assumption of an effect size of 0.50 on Conditioned Pain Modulation (CPM) functioning between controls and CFS patients, 51 participants per group are needed to obtain a significance level of $p=0.05$.

Inclusion criteria

CFS patients within the range of 18-65 years of age, who are diagnosed according to the Centre of Disease Control criteria (31). They will be recruited from the outpatient clinic of Stichting Cardiozorg, Amsterdam, which has an area of special interest in diagnosing and treating CFS patients. Sedentary healthy control subjects will be recruited by a public announcement on the websites of the Dutch CFS societies. Before being able to participate to the study, after signing the informed consent form, the International Baecke Questionnaire –Dutch version- will be sent by post to the control subject. Only those with categorical score: “low” will be asked to participate in the subsequent protocol. Healthy volunteers with higher scores will be screen failures.

Exclusion criteria:

- Pregnancy or being postnatal within 1 year.
- The current use of opioid medication.
- BMI >30.
- Unable to perform a maximal exercise test.
- Diabetes mellitus.
- Medical condition leading to chronic pain in sedentary controls and patients.
- Suspicion of ischemic complaints, regional wall motion abnormality or moderate to severe valvular disease (see cardiovascular screening further).

Protocol

After receiving information and signing the informed consent, demographical variables will be collected and the sedentary controls will undergo the cardiovascular screening. Thereafter, participants are asked to fill in some questionnaires assessing pain, pain cognitions, general well being, dizziness and CFS symptoms: the Pain Catastrophizing Scale, The Tampa Scale for Kinesiophobia, the Checklist Individual Strength, the Symptom List Chronic Fatigue Syndrome, the Dizziness questionnaire, and the International Baecke Questionnaire–Dutch version. These questionnaires will be filled in at the first visit, before randomization. (Of note: the Baecke will only be filled in at the first visit by CFS patients, because the questionnaire was already filled in by healthy volunteers as part of the inclusion screening).

Participants are asked not to undertake physical exertion at the day of tests, and are asked to refrain from analgesics, caffeine, alcohol or nicotine on the day of the tests. Furthermore participants are asked not to change or to start new medication or treatments 4 weeks prior and during study participation in order to obtain a steady state.

Subsequently they are subjected to the protocol presented in the flow chart. Participants are randomly (by lottery) allocated to a group in which first the below described data are collected while lying down and thereafter while being in the upright position. In the other group the order of being upright and lying down is reversed.

In the first group participants are asked to lie down and rest for 10 minutes. Thereafter, the Nexus and Nexfin recordings will be started, and carotid and vertebral artery data will be collected (1st flow measurement). Then, the Pressure Pain Threshold (PPT) and CMP protocol will be started. During and after this assessment carotid flow data (2nd and 3rd flow measurement) will also be collected.

For the Valsalva maneuver (VM), participants are positioned in the upright position. In the upright position, Nexus and Nexfin measurements will continuously be recorded and carotid flow data will be collected before, during and after the Valsalva maneuver (4th-6th flow measurement). The Valsalva maneuver will be performed twice to allow flow measurement of the left and right carotid artery. After the Valsalva maneuver the PPT will be determined. In the second group the order of lying down and being upright for the Valsalva maneuver is reversed.

Study Design

Subsequently, participants are randomized to either the submaximal bicycle exercise test, or to the tilt table test. This is done to prevent bias due to the standard procedure of tests. During both tests cerebral flow (7th flow measurement) and Nexfin blood pressures will be recorded.

Immediately after the tests the Borg scale (ratings of perceived pain, fatigue and concentration difficulties) will be obtained.

Within 5 minutes after the tests (either bicycle or emotional stressor) the Nexus, Nexfin and PPT measurements will be performed. Twenty four, 48 hours and 1 week after completing the tests, the post-exertional Borg scale score will be asked by telephone.

Three to six weeks later participants are crossed over both for the order of lying down and being upright for the Valsalva maneuver and for the bicycle test and emotional stressor. Immediately after the tests, the same Borg scale scores will be obtained.

Within 4 weeks after completion of the studies, but stabilized to baseline values with respect to fatigue, pain and concentration (according to the Borg scale) participants will wear the SenseWear activity armband (SWA) in order to establish capability of exercise.

METHODS

Assessments:

Collection of demographic data (at screening):

The following data will be collected from all participants: age, length, weight, BMI, the risk factors smoking, hypertension, hypercholesterolemia, diabetes mellitus and a family history of cardiovascular disease in first degree relatives will be noted, as well as the highest educational level, professional status, medication use, previous diseases, hospitalizations, and surgical operations.

Cardiovascular screening

History taking will include the presence dyspnea and angina complaints, palpitations and dizziness.

- Dyspnea symptoms will be scored according to the NYHA criteria and graded I-IV.
- Chest pain symptoms will be scored as non-specific chest pain, atypical angina or typical angina. Those participants with a suspicion of ischemic complaints will be excluded.

- Physical examination: any abnormality of the central venous pressure, heart and lung auscultation, a liver enlargement and peripheral edema will be noted.
- Standard 12 lead ECG: any abnormality of the rhythm, AV or IV conduction abnormality, repolarization abnormality and morphological abnormalities will be noted.
- Echocardiogram: a standard 2D and Doppler echocardiogram will be obtained. Any abnormality of clinical significance will be noted. Participants with any regional wall motion abnormality or with moderate to severe valvular disease will be excluded.

Questionnaires:

Participants are asked to fill-in some questionnaires assessing pain, pain cognitions, general well being, dizziness and CFS symptoms.

- the International Baecke Questionnaire –Dutch version. This questionnaire will be used as a screening tool for in- or exclusion of healthy volunteers. Also patients will be asked to fill-in this questionnaire to compare the physical activity with that of healthy volunteers. Thus for CFS patients the questionnaire will be filled in prior to randomization.
- CFS Symptom List (32). Filled in by CFS patients and healthy volunteers prior to randomization.
- Checklist Individual Strength (CIS). The CIS is a 20-item self-report questionnaire that captures four dimensions of fatigue, including subjective experience of fatigue, reduction in motivation, reduction in activity and reduction in concentration. Respondents rate the extent to which each statement is true for them in the past two weeks on a seven-point Likert scale ranging from 1 = “Yes, that is true” to 7 = “No, that is not true.” (33) Filled in by CFS patients and healthy volunteers prior to randomization.
- Pain Catastrophizing Scale (PCS). The PCS is a 13-item instrument. The PCS instructions ask participants to reflect on past painful experiences, and to indicate the degree to which they experienced each of 13 thoughts or feelings when experiencing pain, on 5-point scales with the end points (0) not at all and (4) all the time. The PCS yields a total score and three subscale scores assessing rumination, magnification and helplessness (34). Filled in by CFS patients and healthy volunteers prior to randomization.
- Tampa Scale for Kinesiophobia (TSK) – version CFS. The TSK version CFS is a 17-item instrument with scores ranging from 1-4 to quantify fear of physical movement and activity (32).
- Scaling dizziness/light-headedness. Filled in by CFS patients and healthy volunteers prior to randomization. Borg Score: Post-exertional malaise will be measured by asking the patients to give a Borg score between 0 and 10 for pain, fatigue and concentration difficulties immediately after the interventions (exercise test or emotional stressor), and 24 hours later and 48 hours later (interview by phone).

Valsalva Manoeuvre (VM):

After a resting period of 5 minutes, a normocapnic standardized VM will be performed. To perform the VM, subjects blow into a mouthpiece connected to a modified sphygmomanometer and maintained a pressure of 40 mm Hg for 15 seconds (27). The VM will be performed twice to obtain left and right cerebral flow recordings.

Nexus measurements:

Continuous recordings of skin conductance and heart rate variability will be obtained using the Nexus 10 device with a blood volume pulse (BVP) and skin conductance sensors (NeXus 10, Mind Media BV, The Netherlands), and processed using the Bio Trace+ software version V2010A (Mind Media BV). All sensors will be attached at the participants' right hand. The skin conductance sensor uses two Ag-AgCL electrodes that are secured by velcro straps to the tip of the index and ring finger. The sensor is sensitive to very small (1/1000 micro-siemens) relative changes in skin conductance. The BVP sensor uses finger-tip photoplethysmography to measure heart rate and monitor relative blood flow. The BVP sensor will be placed on the little finger. Heart rate variability (HRV) can be acquired through this sensor (35). HRV measures in time domain include standard deviation of inter-beat intervals (SDNN) and root mean square of successive differences between NN intervals (RMSSD). In addition, power spectrum analysis of the pulse intervals, the time interval between two consecutive pulses can be derived by Fast Fourier transformation using Kubios HRV 2.0. It is suggested that LF (0.04-0.15Hz) power of HRV is mediated by both sympathetic and parasympathetic modulations (36). HF (0.15-0.4Hz) power of HRV is mainly under control of the vagal nerve (36). The LF/HF ratio is an indicator of cardiac sympathetic modulation and sympathovagal balance (36). In every assessment period Nexus data will be calculated from a 2-minute period.

Pressure pain threshold (PPT) and temporal summation (TS):

Pressure algometry has been found to be efficient and reliable in the exploration of pathophysiological mechanisms involved in pain (37,38) and for the evaluation of treatment outcome, as reviewed by Fischer (39).

PPT is measured with an analogue Fisher *algometer* (FDK 40, Wagner Instruments, Greenwich). For this purpose a rubber tip of 1cm² is placed in the skin web between thumb and index finger (40), at the trapezius

muscle, and at the proximal third of the calf, in order to test pain thresholds on non-specific locations both on the extremities and the trunk. These three sites are assessed in random order. The force is gradually increased at a rate of 1 kg/s until the subject indicates that the pain level has been reached. At a specific site the procedure is performed three times with 10s intervals in between. The threshold is determined as the mean of the two last values out of three consecutive measurements. This procedure has found to be reliable in healthy controls (41). TS is elicited by 10 applications (pulses) of the algometer at pressure pain detection threshold intensity on the dorsal surface of the right hand middle finger midway between the first and the second digital joints, and at the middle of the right-hand side trapezius belly. For each pulse, pressure is increased at a rate of 2 kg/s to the previously determined PPT, where it is maintained for 1 second before being released. Pulses are presented with an inter stimulus interval of 1 second. Subjects are instructed to rate the pain level of the 1st, 5th and 10th pulse according to a visual analogue scale (VAS).(42)

Nexfin measurements:

Heart rate (HR) and blood pressure (BP) will be continuously measured by finger plethysmography using the Nexfin device (BMeye, Amsterdam, NL). This is a validated device and has been tested against non-invasive and invasive blood pressure measurements (43,44).

For the resting period of 10 minutes, prior to the interventions, the mean of the last minute of HR, systolic (SBP), diastolic (DBP), and mean BP (MBP) data will be taken. For the Valsava maneuver the mean of 10 seconds of HR, systolic, diastolic, and mean BP, prior to the VM, at the last 10 sec period of the 2 VM's will be taken. Similarly, the last 10 seconds of the one minute after the last VM will be used to determine HR, SBP, DBP and MBP. In a similar manner before, during and after the pain pressure threshold determination and during the emotional stressor, HR and blood pressures will be measured with the same time specified intervals as during the VM.

Cerebral blood flow (CBF):

Blood flow will be measured in the internal carotid arteries (ICA) and vertebral arteries (VA) with a Vivid-I system (GE Healthcare, Hoevelaken, NL) equipped with a 10MHz linear transducer. Measurements of the ICA's will be performed ~1.0–1.5 cm distal to the carotid bifurcation and alternated with flow measurements in the vertebral arteries at the C2-C5 level. When obtaining blood flow velocity measurements, care will be taken to ensure that the probe position is stable, that the insonation angle will be less than 60 degrees, and that the sample volume is positioned in the center of the vessel and adjusted to cover the width of the vessel diameter. B mode images, color Doppler images and the Doppler velocity spectrum (obtained from the pulsed wave Doppler) will be recorded in one frame.

All CBF measurements will be performed by the same operators (45). Blood flow of one cardiac cycle will be calculated from the mean blood flow velocity (by manual tracings of the velocity contour) x the mean surface area. Mean surface area is calculated as proposed by Sato et al. (2011). In detail, the peak systolic and end diastolic diameters will be measured, and then the mean diameter (cm) will be calculated: mean diameter = [(systolic diameter×1/3)] + [(diastolic diameter×2/3)]. From the mean diameter mean surface area will be calculated. In each frame of the four arteries blood flow will be calculated in at least 6 consecutive cardiac cycles to eliminate the effects caused by breathing variation. Furthermore the time interval between the start of the study and the measured flow in an artery will be calculated. Measured flow data of the 4 arteries will be plotted against time using GraphPad Prism 5, (GraphPad Software Inc, San Diego, USA). Flow data of the 4 arteries over time will be fitted with a second order polynomial fit and total cerebral flow will be calculated from the raw and fitted flow data. In a similar manner relative oxygen consumption will be fitted against time. Finally, for inter individual comparison total cerebral flow will be plotted against the relative oxygen consumption data.

Tilt Table stressor:

Previous exercise physiology studies in the field of CFS used case-control rather than experimental designs. This implies that previous observations regarding exercise physiology (including exercise immunology, exercise gene studies and exercise pain physiology studies) in CFS patients did not control for potential bias due to non exercise stressor or the fluctuating nature of CFS. Therefore, the present study applies a true experimental design (randomized cross-over study design) controlling for non exercise stressors.

In line with this reasoning, it is unknown whether the hemodynamic changes of physical exercise or the stress of the exercise (or the combination of the two) is responsible for the altered pain perception as previously observed following exercise in patients with CFS. This holds true for both the patients and the healthy volunteers. In an attempt to unravel these two separate contributions, patients and healthy volunteers will undergo a non exercise stressor, in which the contribution of the hemodynamic changes are far less than during exercise, namely a tilt table test.

Subjects will be lying down on the tilt table for 15 minutes, after which they will be tilted at 70° for a maximum of 30 minutes after which they will be returned to lying down and the test is completed.

Spiro-ergometry:

Exercise will be performed on a Corival Recumbent ergometer (Lode BV, Groningen, NL) with special adjustments allowing the measurement of cerebral flow during exercise. Volunteers and patients will undergo a submaximal exercise test according to the following protocol: after 3 min rest and 3 min of unloaded cycling at a rate of 55-65 cycles/min, resistance will gradually increase using a ramp workload protocol using 7.5-20 Watt/min increases. A workload protocol will be chosen, which allows exercise duration of 12-15 minutes. Participants will exercise to a level of approximately 75% of their peak oxygen consumption. In patients peak oxygen has been previously established and healthy volunteers will be asked to perform a symptom-limited exercise before participation to the study to determine their peak oxygen consumption.

At rest and during cycling minute ventilation ($\dot{V}E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide release ($\dot{V}CO_2$) and oxygen saturation are continuously measured using the Cortex Metalyzer 3B, and together with a 3 lead ECG, displayed on screen using Metasoft software (Cortex Biophysik GmbH, Leipzig, Germany). $\dot{V}O_2$ at rest and during exercise will be expressed as % of the previously determined peak $\dot{V}O_2$.

Assessment of post-exertional malaise:

The Borg score for pain, fatigue and concentration difficulties (see section questionnaires) will be measured immediately after the interventions (exercise test or emotional stressor), and 24 hours later, 48 hours and 1 week later (interview by phone).

Real-time activity monitoring:

The SenseWear® Pro3 Armband (SWA) (BodyMedia Inc., Pittsburgh, PA, USA) wireless multisensor accelerometer will be used for real-time monitoring of physical behavior of all participants during 5 consecutive days. This activity monitor has a two-axis accelerometer along with several other physiological sensors (heat flux, skin temperature, near-body ambient temperature, body position, movements of the upper arm, and galvanic skin response) from which data are integrated and subsequently can be uploaded and analyzed using computer software. Energy expenditure is estimated based on gender, age, height, and weight, together with the information collected from all sensors. Good validity and reliability of the SWA has been shown in healthy adults under laboratory (33) and free-living conditions (34). The SWA is lightweight and comfortable to wear. It is worn on the back of the left upper arm over the triceps muscle. From the SWA data, the hours of lying down and sleep, the hours of being inactive in the upright position, the total hours of physical activity, the hours of moderate activity, the hours of vigorous and very vigorous exercise, the number of steps and the estimated total energy expenditure and estimated active expenditure are taken and normalized to 24 hrs. Moderately active is defined as an estimated energy expenditure ≥ 3 METS, vigorous exercise as an estimated energy expenditure ≥ 6 METS and very vigorous exercise ≥ 9 METS (48).

Patients and healthy volunteers will wear the armband during 7 days (Monday – Sunday) in a period between 2 and 4 weeks after the last assessment. The Sensewear data will be used as a validation tool for the physical activity questionnaire in healthy volunteers. At present there are no established data of the relation between the questionnaire and estimated energy expenditure of the Sensewear. If in an interim statistical analysis a large discrepancy between the physical activity questionnaire (the score being low), and the estimated energy expenditure is found in some healthy volunteers, this factor will be taken into account in the final statistical analysis.

OUTCOMES**Primary Outcome**

The primary outcome of this study is the relationship between decreased cerebral blood flow during exercise and increased pain sensation after exercise.

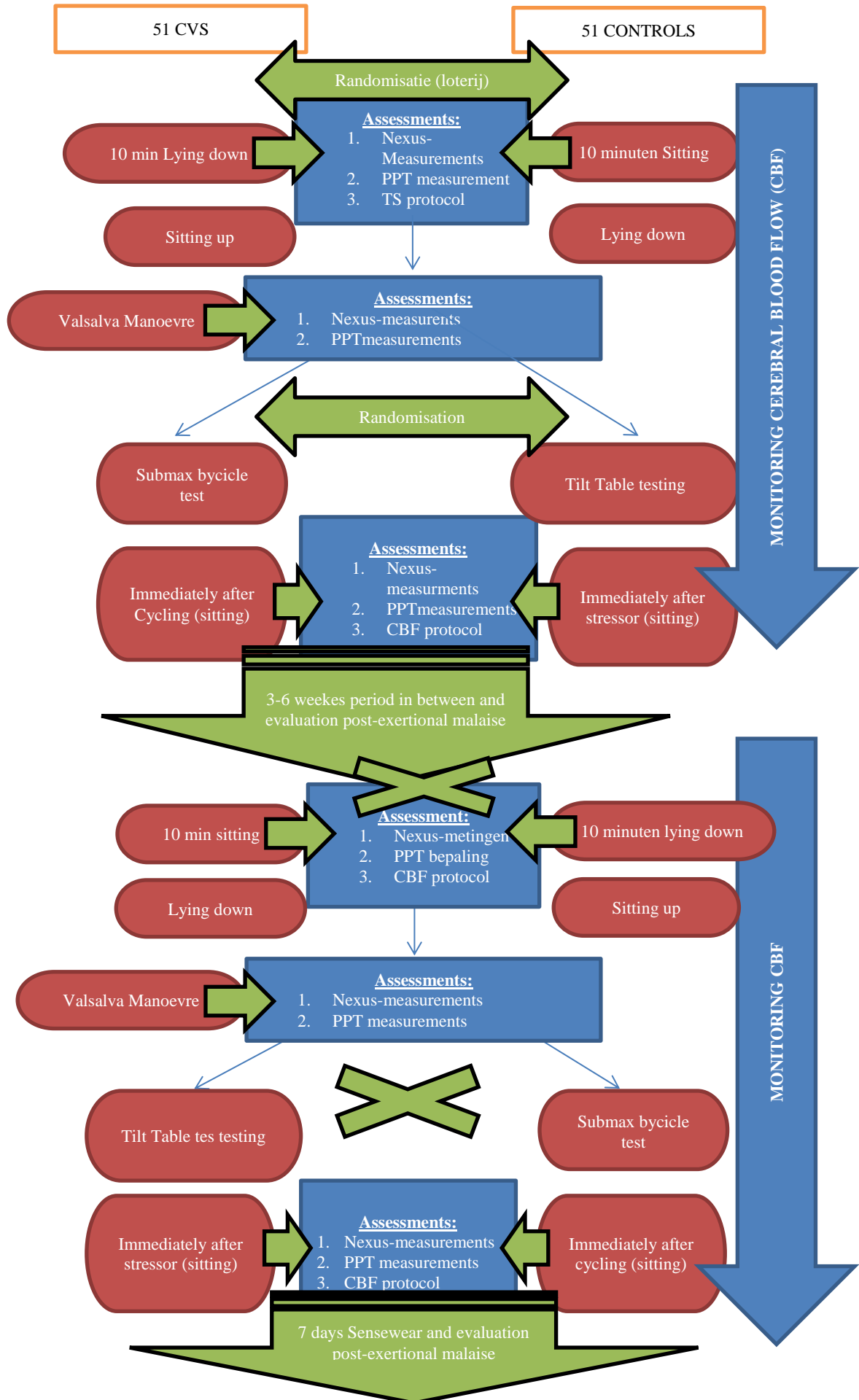
Secondary Outcomes:

- Cerebral blood flow before and after non exercise stressor
- Comparison of CBF changes between exercise and non exercise stressor
- Comparison of CBF changes between patients and healthy subjects
- Comparison of PPT between patients and healthy subjects
- Comparison of peak oxygen consumption between patients and healthy subjects
- Relation of CBF and an increase in oxygen consumption during exercise.
- Comparison of Vasalva manoeuvre measurements between patients and healthy subjects
- Comparison of questionnaires between patients and healthy subjects.

Statistical analysis

All data will be analyzed using the Statistical Package for Social Sciences 20.0© for Windows (SPSS Inc. Headquarters, Chicago, Illinois, USA). Normality of the variables will be tested and appropriate descriptive statistics will be used. In case of normality, comparability of the groups before intervention will be studied with an independent t-test. Pearson correlation coefficients will be calculated between CPM efficacy, autonomous

parameters and cerebral blood flow. Repeated measures ANOVAs will be used to evaluate the effect of lying down, Valsalva maneuver, exercising and an emotional stressor on CPM, autonomous parameters and cerebral blood flow (p interaction).



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