Exercise-induce hyperalgesia, complement system and elastase activation in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome : a secondary analysis of experimental comparative studies

TITLE: PARASYMPATHETIC ACTIVITY MIGHT ACCOUNT FOR REGULATING EXERCISE-INDUCED OXIDATIVE STRESS CHANGES IN PATIENTS WITH CHRONIC FATIGUE SYNDROME AND HEALTHY SUBJECTS: AN OBSERVATIONAL STUDY

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ABSTRACT

Purpose: Oxidative Stress has been proposed as contributor to pain in patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). During incremental exercise, oxidative stress enhances sooner and anti-oxidant response is delayed in patients with ME/CFS. We explored whether oxidative stress is associated to pain symptoms or pain changes following exercise, and the possible relations between oxidative stress and parasympathetic vagal nerve activity in patients with ME/CFS versus healthy, inactive controls.

Methods: Thirty-six participants were studied (women with ME/CFS and healthy controls). Subjects performed a sub-maximal exercise test with continuous cardiorespiratory monitoring. Thiobarbituric Acid Reactive Substances (TBARS) levels were taken as a measure of oxidative stress, and Heart Rate Variability (HRV) to assess vagal activity. Before and after the exercise, subjects were asked to rate their pain using visual analogic scale (VAS).

Findings: Significant between-groups differences were found for pain at both baseline and following exercise (both $p<.001$). In healthy controls, pain significantly improved following exercise ($p=.002$). Significant correlation between TBARS levels and pain were consistently found at baseline ($r= .540$, $p=.021$) and after exercise ($r=.524$, $p=.024$) in patients only. No significant correlations were found in either group between TBARS and HVR at baseline and following exercise. However, strong significant correlation was found between exercise-induced changes in HRV and TBARS changes ($r= -.725$, $p=.001$) in HCs.

Implications: Oxidative stress showed association to pain symptoms in people with ME/CFS. In addition, we propose that para-sympathetic activity following exercise partially accounts for decreases in oxidative stress in HCs. This result could be interpreted as a normal response to exercise, that is disrupted in patients with ME/CFS.

KEYWORDS: Chronic fatigue syndrome; pain; oxidative stress; autonomic nervous system; exercise

INTRODUCTION

Oxidative stress is the imbalance between an increased production and/or reduced clearance of reactive oxygen species that potentially leads to lipids, protein, and DNA damage.[1] Oxidative stress is capable of inducing deleterious changes, particularly to the central nervous system, given the intrinsic high vulnerability that neurons and glial cells have to metabolic changes.[2,3] Oxidative stress covers an important role in synaptic plasticity and many other mechanisms involved in nociceptive modulation and central sensitisation.[4,5] Elevated spinal levels of reactive oxygen species lead to peripheral and central sensitisation and alter nociception,[6] resulting in hyperalgesia mediated by both local and spinal oxidant mechanisms. Nitric oxide also holds the capability of inducing peripheral and central sensitisation by reducing receptor thresholds [7] and is able to reduce the inhibitory activity of the central nervous system leading to central sensitisation of dorsal horn neurons.[8] In line with the above observations, oxidative stress has also been associated with chronic pain and proposed as a possible contributor to the maintenance of pain symptoms.[9]

Central sensitisation – described as a state of generalised hyper-responsiveness of the central nervous system to a variety of stimuli – has been proposed as a key mechanism in many chronic pain conditions, including Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS),[10–12] a chronic condition in which widespread pain is very common and often reported as more disabling than fatigue itself.[13] Indeed, patients with ME/CFS show hyper-responsiveness to different stimuli and hyperalgesia to electrical and mechanical stimulations, when compared to healthy controls.[14]

In addition, one cardinal symptom of people with ME/CFS is post-exertional malaise – a worsening of pain and other symptoms in response to exercise, lasting up to 48 hours. While exercise normally induces hypoalgesic effects in healthy subjects, quickly increasing pain thresholds and improving subjective reports of pain symptoms,[15–17] people with ME/CFS actually show a decrease in pain thresholds following exercise.[18,19]

The exact mechanisms underlying this lack of exercise-induced hypoalgesia in ME/CFS is largely unknown, as ME/CFS is heterogeneous in nature and several mechanisms co-occur. [20–22] However, oxidative stress may reasonably be proposed as one of the underlying mechanisms. Higher levels of oxidative stress have been reported in people ME/CFS,[23,24] and studies found that oxidative stress occurs earlier during exercise in people with ME/CFS compared to healthy controls.[25,26] In addition, people with ME/CFS present a delayed anti-oxidant response following exercise.[25,26]

A second important mechanism that has been proposed to explain the aforementioned exercise-induced hyperalgesia in people with ME/CFS is the alteration of the autonomic nervous system (ANS).[27] Following exercise, patients with ME/CFS show higher gene expression for receptors involved in ANS functions compared to healthy controls and gene concentrations are associated with pain symptoms. [28] A systematic review on the topic has shown that heart rate variability – a commonly used and reliable measure of ANS parasympathetic (vagal) activity [29,30] – differs between people with ME/CFS and controls.[31] In another recent paper of ours, we showed that exercise-induced hyperalgesia is associated with a reduced parasympathetic reactivation during the recovery phase following exercise.[32]

Interestingly, preliminary evidence suggests a direct link might exist between ANS activity and oxidative stress responses.[33] Increased oxidative stress in the rostral ventrolateral medulla in turn causes excitation of the sympathetic branch.[34] Similarly, angiotensin-II receptor blockade induces both inhibition of the ANS sympathetic branch and reduction of oxidative stress.[34] In addition, experimentally-induced increased levels of oxidative stress, in both animals and humans, will alter blood flow regulation and reduce muscle blood flow during exercise.[35] Although the link between ANS and oxidative stress appears to be clear, to the best of our knowledge this association has never been explored in ME/CFS or – more generally – chronic pain patients.

In the present study, we aimed to explore whether oxidative stress is associated with pain or with changes in pain after exercise, and whether a possible relationship exists between oxidative stress and ANS functions in people with ME/CFS and healthy controls. We believe that unravelling the mechanisms

associated with symptoms worsening following exercise will help identifying the targets for future treatments, therefore improving patients' care.

We hypothesised that oxidative stress is associated with higher pain levels, and that the change in pain symptoms induced by exercise is associated with the change in oxidative stress in response to exercise. In addition, we expected that oxidative stress and ANS functions are also associated; that is, the higher the level of oxidative stress, the lower parasympathetic activity in patients with ME/CFS.

METHODS

Subjects

Eighteen patients with ME/CFS and eighteen healthy inactive controls participated in the study. Patients' diagnosis, based on the 1994 Centre for Disease Control and Prevention criteria,[36] was established by two physicians specialized in internal medicine and with expertise regarding diagnosis and treatment of ME/CFS. Patients were excluded if they showed evidence of other neurological, endocrine, immune, and cardiovascular pathologies.

Healthy controls were friends of either patients or professionals involved in the research project and volunteers who responded to adverts. They were included if they had no known significant pathology and had inactive lifestyle. Inactivity was defined as having a seated occupation and performing a maximum of 3 h of moderate physical activity/week.[37]

All participants were women aged between 18 and 65 years, Dutch speaking and not pregnant, lactating or less than 1 year postnatal. Only women were included, as sex is an important source of bias in exercise physiology and pain studies [38–40] and ME/CFS is predominant prevalent in women.[41] Participants were asked to refrain from consuming caffeine, alcohol, nicotine and to avoid physical exertion on the day of the experiments. If possible, patients were also asked to abstain from medication acting on the cardiovascular system, the central nervous system, and the endocrine system in the 48 hours prior to the experiments.

The experiment was approved by the ethics committee of the University Hospital Brussels / Vrije Universiteit Brussel. All participants provided written informed consent before the start of the assessment.

Procedure

An initial familiarization session was organized at least one week before the actual start of the experimental session to inform participants about the procedures and devices used during the experiment. Demographic and clinical information was also collected during this first meeting.

Experiments took place in a quiet room with a constant temperature between 21-23°C. Pain symptoms were assessed using self-reports (see below). Prior to exercise testing (10 minutes at rest) and during the subsequent recovery period (again 10 minutes) physiological measures of autonomic function were obtained. Measures of autonomic functions were measured through ECG recording during 10 minutes of quite supine lying. The Nexus-10 wireless and portable telemetry data acquisition system (Mind media BV, The Netherlands) was used to calculate the Root Mean Square of Successive Differences between NN intervals (RMSSD). Patients wore an elastic belt around the chest, in which a stretch-sensitive piezoelectric sensor was placed to record respiratory peaks and *respiratory rate*.

A submaximal bicycle exercise test, known as the Aerobic Power Index, with continuous cardiorespiratory monitoring was then performed. The Aerobic Power Index is a standardized test, shown to be valid and reliable in both patients with ME/CFS and healthy people.[42] The detailed procedure for the Aerobic Power Index test can be found elsewhere.[19] In summary, cycling started at a workload of 25 Watt/min, increasing by 25 Watt/min until the patient reached 75% of their age-predicted maximal heart rate. Cycling rate was kept constant at 70 cycle/min. [43] After the test, subjects were asked the same self-reported measures of pain were taken as in baseline. Finally, subjects were asked to lie down for 10 minutes, after which a second blood sample was collected for measuring oxidative stress responses to exercise.

Outcome measures

Self-reported measures of pain

Pain was assessed with two different tools. The first one is the bodily pain subscale of the Short Form Health Survey (SF-36). It consists of two items assessing pain intensity and the influence that pain has had on one's life during the past 4 weeks.[44] The two items give a general score ranging from 0 to 100, with higher scores indicating more bodily pain. In addition, we also used a visual analogue scale (VAS), for assessing bodily pain intensity. The VAS comprised of 100 mm horizontal lines with the outer points labelled as 'no pain' and 'unbearable pain'. In order to control for clinical features that might have influenced our results, *depression and anxiety* were assessed using the Hospital Anxiety and Depression Scale (HADS).[45] HADS is a valid and reliable tool to assess psychological distress in both patients and healthy subjects.[46]

Determination of oxidative stress

Two tubes of blood were collected 10 minutes before and following the exercise test. Samples were stored at ambient temperature for 1 hour and were then centrifuged for ten minutes at 4000 rpm. The resulting serum was stored at –80°C. Each sample was labelled with a code so that the researcher in charge of the analysis was blinded to the group allocation. Biochemical analysis was performed at the RED Laboratories NV (Belgium). *Oxidative stress* was evaluated measuring Thiobarbituric Acid Reactive Substances (TBARS) (OxiSelect TBARS Assay Kit for malondialdehyde (MDA) quantitation from Cell Biolabs). TBARS is a well-established assay for screening and monitoring lipid peroxidation – a generally accepted marker of oxidative stress.[47] The TBARS assay provides relevant information concerning free radical activity in disease states and measurement of many anti-oxidant compounds' characteristics.[48,49]

Physiological measures of autonomic function

Data from the ECG recording were analysed offline with the BioSig toolbox in MATLAB software (The MathWorks, USA). The mean of measurements over the 10-minute periods were then used for further analysis. From these measures, heart rate, HRV, and the high-frequency component of HRV (HF-HRV,

power range 0.15-0.40Hz) were calculated after Fast Fourier transformation. Further details regarding raw data handling, artefacts removal, and analysis are described in a previous paper.[32]

Although international guidelines recommended (in 1996) to report the low-frequency component of HVR (LF-HVR) and the LF/HF ratio, [50] more recent work questions the validity and reliability of these measures.[51–53] For this reason, we are not reporting these parameters in the present paper; however, a detailed report of all autonomic measures from this study – including both LF-HRV and the LF/HF ratio – has already been reported in another paper.[32] Here we report measures of heart rate, HRV, and HF-HRV, expressed by calculating the RMSSD.

Statistical analysis

We explored our data using between and within group comparisons. Normality of the variables was tested in each group (patients and healthy controls) with the Shapiro-Wilk test and appropriate descriptive statistics were calculated. Group comparisons at baseline and following the exercise test were examined using the Independent Samples T-test or the Mann-Whitney U test, according to the results of normality analysis. Chi-Square tests were used to analyse categorical data. Paired-samples t-test or Wilcoxon signed rank test were performed to analyse the effect of exercise in each group. Pearson or Spearman's correlation analysis was used to examine the relation between self-reported pain and TBARS measures. Correlation analyses were also performed to analyse associations between ANS function and oxidative stress. The significance level was set at 0.05. All data were analysed using SPSS 23.0© for Mac (Headquarters, Chicago, Illinois, USA).

RESULTS

Subjects' characteristics

Thirty-six subjects (18 patients with ME/CFS and 18 healthy inactive controls) completed the assessment. Table 1 summarises demographic and clinical characteristics of the groups. No statistically significant differences at baseline were found for age, BMI, anxiety and depression (see table 1). Patients with ME/CFS scored significantly higher in the SF-36 pain subscale (see table 1). People with ME/CFS

also reported significantly more pain than controls, as expressed by the VAS ($p < .001$). No differences at baseline were found for either the level of oxidative stress or the measures of ANS activity (i.e., heart rate, respiratory rate, HRV and HF-HRV; all p-values > .05; table 2).

Effect of the exercise challenge

Within-group analysis revealed that p*ain* (VAS) improved following exercise only in healthy controls ($p= .002$), while it did not change in people ME/CFS ($p= .393$). *Oxidative stress*, measured using MDA-TBARS level, did not change in response to exercise in either group. In comparison, *HRV* and HF-HVR (measure through RMSSD calculation) decreased significantly only in patients ($p= .039$ and $p= .034$, respectively). The exercise test induced similar changes in both groups for the other measures, e.g., heart rate and respiratory rate. The effect of exercise on ANS measures (HVR, HF-HVR, and heart rate) in ME/CFS have been already presented in a recent paper of ours [32] and are reported here only to provide clearer general picture of the results. Data are summarised in table 2.

Associations between pain and oxidative stress

No correlations between MDA-TBARS and pain symptoms were found in healthy people. On the contrary, in people with ME/CFS, MDA-TBARS levels were related to VAS bodily pain, both at baseline and after exercise. Figure 1 summarises visually the most relevant correlations between MDA-TBARS levels and bodily pain (baseline: $r= .540$; $p= .021$; post-exercise: $r= .524$, $p= .026$) in ME/CFS. Linear regression analysis revealed that MDA-TBARS significantly explained pain symptoms in MC/CFS ($r^2= .259$ $p=.03$ before exercise; $r^2= .254$ $p=.03$ after exercise).

Regarding the SF-36 bodily pain score and MDA-TBARS levels no significant correlations were found. The change in MDA-TBARS from baseline to post-exercise did not correlate with the changes in pain measures.

Associations between oxidative stress and ANS activity

No association between MDA-TBARS and ANS activity was found in either group at baseline or after the exercise challenge. In the healthy control group, strong associations were found between exercise-induced changes in MDA-TBARS levels and changes in parasympathetic activity. More specifically, exercise-induced decrease in MDA-TBARS levels were strongly associated with the increase in HRV (r= -.720, p= .001), and HF-HRV (r= -.674, p= .002). Linear regression analysis demonstrated that the change in parasympathetic activity can explain over 36% of the change in MDA-TBARS levels (r^2= .366, p= .008). Also, the change in MDA-TBARS levels were correlated with HR (r= -.522, p=.026). No such correlations were found in the ME/CFS group. The most relevant associations are presented in figure 2.

DISCUSSION

In the present paper, we aimed to investigate possible associations between oxidative stress and pain symptoms in healthy subjects and people with ME/CFS, and assess whether oxidative stress levels and ANS parasympathetic (vagal) activity were linked. We set up a design in which subjects underwent a sub-maximal exercise test and we recorded pain symptoms, oxidative stress level, and ANS activity before and after the test.

Our results only partially confirm results from available literature. First, we did not find higher levels of oxidative stress in ME/CFS and chronic pain when compared to healthy people at baseline, as previously reported.[6,14,23] A possible explanation might be that, in the present study, we controlled for physical activity levels, enrolling only sedentary healthy controls. Studies focussing on oxidative stress in patients with chronic pain rarely account for physical activity. However, patients are likely less active than healthy subjects.[54–56] Our results might suggest that lower physical activity accounts for higher oxidative stress levels and should be assessed in patients with chronic pain. Our results are in line with what has been shown by Jammes et al. (2012), that did not find any increase in oxidative stress at baseline in patients without acute infection or high-level of physical stress.[26]

Secondly, exercise did not increase oxidative stress levels. However, oxidative stress responses to exercise might be mediated by exercise intensity. A maximal exercise test appears sufficient to induce changes in TBARS levels in patients with ME/CFS.[9] It is possible that the submaximal exercise test we used was not sufficiently intense to induce oxidative stress responses.

However, exercise was intense enough to exert a hypo-analgesic effect on healthy people, confirming existing literature.[15,19,32,57,58] Patients with ME/CFS, in contrast, did not show hypoalgesia following exercise.[19,22,34,59] Oxidative stress was consistently found associated with patients' pain symptoms both prior and following exercise in our study, explaining more than 25% of patient's pain.

The mechanisms underlying this exercise-induced hypoalgesia have been explored but are still not fully understood.[22,25,58,59] We found very interesting results when exploring the association between parasympathetic activity and oxidative stress and we therefore propose that exercise-induced oxidative stress responses might be finely regulated by parasympathetic activity. We already showed that HRV is lower in patients with ME/CFS [31] and that it further decreased in people with ME/CFS, but not in healthy subjects.[32]

Now we have found that exercise-induced changes in parasympathetic activity were strongly associated with oxidative stress changes in healthy people, and that changes in the vagal activity can explain up to 36% of the change in oxidative stress levels. In particular, oxidative stress decrease was associated with an increase of both HRV and HF-HRV following exercise. Increased HRV reflects an increase of parasympathetic (vagal) activity.[60] That is, the higher the parasympathetic activity during exercise, the lower the oxidative stress levels. This confirms animal and preliminary human studies, suggesting an interplay between oxidative stress and the ANS,[34,35] and calls for further research on this potentially very relevant link. This association was only found in healthy controls, suggesting that it might be a normal physiological response, that can be disrupted in patients. Unfortunately, we failed to find association between pain reduction and oxidative stress changes – which would have made our results more

coherent. However, we did not take any measure of anti-oxidant capacity, which might be more relevant than pro-oxidant products when moderate exercise is employed.[61]

Our data should be interpreted with caution. In this article, we included measures of parasympathetic activity only: HF-HRV and RMSSD. HVR measures reflecting sympathetic activity such as LF-HVR and the LF/HF ration, have been extensively criticized and deemed unreliable,[51–53] and we decided not to include them. Other measures have been recently proposed to assess sympathetic activity, in particular blood pressure variability.[62–64] A link between blood pressure and pain sensitivity has been found in chronic pain patients.[65] Blood pressure changes have been linked to exercise-induced hypoalgesia [66] – suggesting a relevant role for the baroreceptor reflex.[67] However, beat-to-beat blood pressure registration is required to assess this relationship,[68] something that our devices could not measure.

In addition, other mechanisms could play a role in the pathophysiology of ME/CFS. Immune system alteration and inflammation is definitely a major one. Evidence shows that elevated levels of inflammatory cytokines released by degenerative peripheral tissues and immune cells can be part of the pathophysiology of ME/CFS as well as other persistent and widespread pain syndromes, such as fibromyalgia.[69–72] However, whether these mechanisms play a role in exercise-induced fatigue or hyperalgesia has yet to be investigated in detail. To date, available data on the link between immune system changes and post-exertional malaise is inconclusive.[22] Future research exploring immune system responses are warranted, as it might be of outstanding clinical interest.

CONCLUSION

Trying to transfer basic knowledge into clinical research is always challenging, especially when using indirect measures of complex phenomena such as ANS activity and oxidative stress. However, we believe our results are interesting and exciting insights into the yet under-explored link. We found consistent associations between pain symptoms and oxidative stress in patients with ME/CFS, but not in healthy people. On the contrary, there was a strong association between exercise-induced changes in ANS and oxidative stress in healthy controls. The more the para-sympathetic activity increases

following exercise, the greater the reduction in oxidative stress. In line with an increasing number of studies showing beneficial effects of vagal nerve stimulation,[33,73–75] we propose that vagal activity exerts regulatory actions on oxidative stress. Patients with ME/CFS and other chronic pain conditions lack this control, and that might account for the lack of exercise-induced hypoalgesia that is often found in these patients. Exploring these mechanisms could potentially lead to helpful and innovative treatments for chronic pain.

Authors' Statements

Research Funding

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Conflict of Interest

Authors declare no conflict of interests.

Informed Consent

All subjects were well informed about the aim of the study and the procedures and provided written informed consent before data collection was initiated.

Ethical Approval

blinded

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Figure 1. Associations between Pain symptoms (VAS) and Oxidative stress levels in healthy controls and people with Chronic Fatigue Syndrome / Myalgic encephalomyelitis (ME/CFS), before and after a sub-maximal aerobic exercise. Only patients showed statistically significant correlations (Fig. B and D). VAS: Visual Analogic Scale. MDA-TBARS: Malondialdehyde – Thiobarbituric Acid Reactive Substances

Figure 2. Associations between exercise-induced changes in para-sympathetic activity and changes in Oxidative stress levels in healthy subjects and patients with Chronic Fatigue Syndrome / Myalgic Encephalomyelitis (ME/CFS). HRV: heart rate variability; rmssd: Root Mean Square of Successive Differences; MDA-TBARS: Malondialdehyde – Thiobarbituric Acid Reactive Substances (this is a standardized method to measure lipid peroxidation, a measure thought to reflect Oxidative stress).