

Nitric Oxide Concentrations Are Normal and Unrelated to Activity Level in Chronic Fatigue Syndrome: A Case-Control Study

MIRA MEEUS^{1,2}, INGE VAN EUPEN¹, JASMIEN HONDEQUIN¹,
LIEVE DE HAUWERE², DAPHNE KOS^{1,3} and JO NIJS^{1,2}

¹*Division of Musculoskeletal Physiotherapy, Department of Health Sciences,
Artesis University College, Antwerp (AHA), Belgium;*
Departments of ²*Human Physiology and* ³*Physiotherapy, Faculty of Physical Education and Physiotherapy,
Vrije University Brussels, Belgium*

Abstract. *Aim: Since patients with chronic fatigue syndrome (CFS) often present elevated levels of nitric oxide (NO) and low levels of physical activity, this study aimed at revealing possible correlations between NO concentration and physical activity. Patients and Methods: Thirty CFS patients and 29 age- and gender-matched sedentary controls wore an accelerometer for one week and underwent venous blood sampling at the beginning and the end of the week. Results: CFS patients were significantly less active ($p=0.001$), but no significant differences in the amounts of NO ($p=0.464$ and 0.569) or interaction between NO levels and activity levels in either the CFS patients or controls were revealed. Conclusion: These results provide further evidence for reduced activity levels in CFS patients, but refute there being any interaction between the amount of blood NO and activity level in both groups. The blood NO was neither predictive of, nor dependent on the activity level in CFS.*

Chronic fatigue syndrome (CFS) is known to be a debilitating and complex disorder, characterised by extreme fatigue. The fatigue does not improve with bed rest and may be aggravated by physical or mental activity (1). Malaise or an exacerbation of symptoms persisting more than 24 hours after exercise is one of the eight accompanying symptoms following the 1994 criteria of the Centre of Disease Control and Prevention (CDCP) (1). Another characteristic mentioned in the CDCP definition is a substantial reduction

of the premorbid activity level (1), evidenced by clinical research (2, 3). In addition to the reduced activity level compared to the premorbid level or to healthy controls, people with CFS display an abnormal activity pattern: their lifestyle appears to be characterised by activity peaks and longer bouts of rest after activity (3). The latter is in line with a physiological study showing a delayed recovery from exercise in CFS patients (4). Clinical studies revealed that overly vigorous exercise (5, 6) or even a 30% increase in activity (7), frequently triggers a relapse. However, the cause of this post-exertional malaise and the altered or reduced activity level remains unclear.

It is hypothesised that nitric oxide (NO) is involved in these phenomena of altered and reduced activity level and of exercise intolerance. It is known that patients with CFS present elevated levels of blood NO (8). Excessive NO concentrations are detrimental for physiological functions *via* the derivative peroxynitrite (9). Peroxynitrite is not a free radical, but leaves the hallmarks of oxidation typical of free radicals (9). Furthermore, NO as a mediator of vasodilatation, is critical for basal blood flow across many organs. In consequence, elevated amounts of NO in CFS can cause hypotension (10). As previously suggested (11), this may explain part of the abnormal exercise response in CFS. NO-induced vasodilatation may limit the capacity of the human body to increase blood flow during physical activity, limiting activity performance in CFS-patients. In addition, physical activity further increases NO amounts and vasodilatation and thus hypotension (12-14). In CFS patients this effect could be aggravated by the already elevated amounts of NO, explaining the malaise and the delayed recovery after physical activity (4). Secondly, pathological overproduction of NO will decrease oxygen consumption (15) and increase anaerobic glycolysis (lactate production) by modulating mitochondrial respiration (16) and iron metabolism (17). Finally, NO could alter muscular morphology and function by oxidative damage of cell

Correspondence to: Jo Nijs, Division of Musculoskeletal Physiotherapy, Artesis University College (AHA), Department of Health Sciences, Van Aertselaerstraat 31, 2170 Merksem, Belgium. Tel: +32 36418205, Fax: +32 36418270, e-mail: jo.nijs@vub.ac.be or jo.nijs@artesis.be

Key Words: Physical activity, accelerometry, ambulatory monitoring, nitric oxide, chronic fatigue syndrome.

membranes (18), structural proteins such as actin (9), and DNA (19), causing muscle weakness, soreness and fatigue. All these mechanisms compromise exercise capacity and worsen physical activity responses.

Therefore, it was hypothesised that NO plays an aetiological role in the reduced activity level and fluctuating symptom pattern in people with CFS. Either people with CFS are not capable of being physically active due to elevated NO amounts, or an activity peak could trigger NO release accompanied by post-exertional malaise.

In summary, the goal of this study was to investigate whether NO concentration in the serum was related to physical activity level in people with CFS and healthy sedentary controls.

Patients and Methods

Subjects. CFS patients were randomly selected from the medical files available at the host university-based chronic fatigue clinic. All patients fulfilled the CDCP criteria for CFS (1). Therefore, all subjects underwent an extensive medical evaluation by the same physician prior to study participation. The patients were contacted by telephone to verify study requirements and to invite them for participation. An age- and gender-matched healthy sedentary control group was recruited from the staff of the University Hospital of the Vrije University Brussel and from acquaintances of the researchers. Sedentary was defined as having a sedentary job and <3 h moderate physical activity/week (activity demanding at least threefold the energy spent passively) (20). Both patients and controls were Dutch speaking and aged between 18 and 65 years old. A total of 30 CFS patients and 29 healthy sedentary controls fulfilling all study requirements were recruited.

Design. At the meeting with the subjects, a leaflet explaining the purpose of the research was provided. In case of agreement, subjects were asked to sign an informed consent. The protocol and the information leaflet were approved by the local Ethics Committee (University Hospital Vrije University Brussels; O.G. 016). After an experienced nurse collected a venous blood sample (9.5 ml), subjects received an accelerometer for activity monitoring. Height, weight and gender were entered in the accelerometer before attaching it on the non-dominant wrist. Subjects were instructed to wear the accelerometer 24 hours a day until the second appointment, one week later. At the second appointment, a new venous blood sample was taken and the accelerometer data were recorded.

Accelerometry. The Actical accelerometer (Mini Mitter, Bend, Ore, USA) has an omnidirectional sensor, which functions via a cantilevered rectangular piezoelectric bimorph plate and seismic mass, and it is capable of detecting movements in the 0.5- to 3-Hz range. Voltage generated by the sensor is amplified and filtered via analogue circuitry. The amplified and filtered voltage is passed into an analogue to a digital converter, and the process is repeated 32 times per second (32 Hz). The resulting 1-s value is divided by four, then added to an accumulated activity value (activity counts) for the epoch. The Actical is the smallest accelerometer available (28×27×10 mm, 17 g) and is water resistant. The Actical has previously been used in studies and has shown to be valid in

different populations (e.g. 21, 22). For the present study, the monitors were initialised to save data at 1-minute intervals (epochs). The Actical is able to subdivide the daily activity into 4 activity levels: sedentary activity (≤ 1 METs), light activity (< 3 METs), moderate activity (3–6 METs), and vigorous activity (> 6 METs).

Nitric oxide assay. Venous blood was collected in heparinised vacuum tubes. NO concentrations in the serum were analysed with a Nitric Oxide Quantitation Kit (Active Motif Inc., Carlsbad, CA, USA). NO measurements were based on total nitrite and nitrate levels in the serum. Preparation and analysis of the samples were performed following the manufacturer's protocol. All the blood analyses were performed at RED Laboratories (Zellik, Belgium).

Statistics. All data were analysed using SPSS 14.0 © for Windows (Chicago, IL, USA). Given the normality of the variables (one-sample Kolmogorov-Smirnov goodness-of-fit test), parametric statistics were used. Appropriate descriptive statistics were used: mean, range, and standard deviation for age, serum NO levels and activity counts. Differences in NO concentrations between CFS patients and healthy controls were analysed with the independent Student's *t*-test. The Pearson correlation coefficient was used to evaluate the relationship between NO and activity levels. NO concentration in the first blood sample (NO 1) was correlated with activity counts of the following two hours, and NO levels in the second blood sample (NO 2) were correlated with activity counts of the preceding two hours, the preceding day and the preceding six days. The significance level was set at 0.05.

Results

Descriptive statistics for demographical data, activity levels and NO concentrations are presented in Table I. Two CFS patients did not complete the study (1st: illness and 2nd: no specific reason). In the control group, one subject left the study after 4 days and another subject detached the accelerometer a few hours before blood sampling. The groups were well matched in gender and age. Twenty-five of the subjects in each group were women.

As shown in Table I, there was a significant difference between the average activity counts per minute for the whole week between the healthy controls and the CFS patients ($p=0.001$). Day by day, CFS patients spent significantly more time on sedentary activities ($p<0.05$) and in general significantly less on light and moderate activities (p ranging between 0.110 and 0.001) compared to the controls. For vigorous activities, there were no significant differences.

NO concentrations in CFS patients tended to be higher as seen in Table I, but the difference was not significant (NO 1: $p=0.464$ and NO 2: $p=0.569$).

Average activity counts two hours before and after blood sampling, presented in Table I, were significantly different between the two groups (AC after NO 1: $p=0.008$ and AC before NO 2: $p=0.017$). Also the average activity counts on the day before NO 2 were significantly different ($p=0.003$).

As shown in Table II, there were no significant correlations between the NO amounts in the serum and the

Table I. *Descriptive statistics and differences.*

	CFS patients				Healthy controls				<i>t</i> -Test
	N	Mean	SD	Range	N	Mean	SD	Range	<i>p</i> -Value
Age (years)	30	42.00	8.21	25.00-58.00	29	41.34	9.04	23.00-57.00	0.838
NO 1 (μmol/l)	30	9.98	8.71	0.50-35.00	28	8.60	4.82	1.10-18.40	0.464
NO 2 (μmol/l)	28	10.67	9.75	0.50-38.70	28	9.35	7.24	0.50-29.80	0.569
AAC whole week	29	215.09	49.21	127.58-325.56	28	278.71	81.27	143.56-457.41	0.001
AAC 2 h after NO 1	29	292.09	118.91	118.43-631.32	27	410.92	195.96	169.99-1092.48	0.008
AAC 2 h before NO 2	28	331.87	165.48	108.20-814.30	27	478.01	262.07	155.60-1255.50	0.017
AAC day before NO 2	29	196.24	53.12	95.90-306.60	28	270.05	113.04	112.80-575.90	0.003

AAC: Average activity counts; NO 1/2: NO concentration in serum on the first/second appointment.

Table II. *Pearson correlation coefficients between NO and average activity counts (AAC).*

	CFS patients				Healthy controls			
	NO 1 (N=29)		NO 2 (N=28)		NO 1 (N=27)		NO 2 (N=27)	
	R	<i>p</i> -Value	R	<i>p</i> -Value	R	<i>p</i> -Value	R	<i>p</i> -Value
AAC 2 h after NO 1	-0.150	0.437			-0.119	0.556		
AAC 2 h before NO 2			-0.025	0.899			-0.020	0.920
AAC day before NO 2			-0.040	0.839			-0.136	0.527
AAC week before NO 2			-0.100	0.614			-0.212	0.320

AAC: Average activity counts; NO 1/2: NO concentration in serum on the first/second appointment.

activity counts two hours before or after the blood sampling, one day before and the whole week before the second blood sampling. Analysis of the data of only the female subjects gave similar results (data not shown).

Discussion

The objective of the present investigation was to study the relation between NO in the serum and activity level in people with CFS. In addition, possible differences in NO concentrations and activity patterns between CFS patients and healthy sedentary controls were studied in order to find possible explanations for eventually abnormal NO levels or altered activity patterns.

Healthy sedentary controls were found to be significantly more active compared to the CFS patients. This observation confirms previous studies addressing activity levels in people with CFS (2, 3). Comparing the time spent undertaking sedentary, lightly active or moderately active activities, CFS patients spent significantly more time undertaking sedentary activities and less time active than healthy controls. This phenomenon may partly be caused by work status differences. Most CFS patients (20) were unemployed, while only seven healthy controls were unemployed. The fact that there were no

significant differences for vigorous activities can be explained by the lack of intensive activities in the daily life of both groups. One should however be careful with interpreting the time spent per activity category. This division is based on energy estimates calculated on activity counts, gender, age, body weight and height. To the Authors' knowledge, there is no evidence for the validity of these estimates.

Concerning the NO amounts there were no significant differences between the two groups. Although CFS patients tended to present higher concentrations, these differences were not significant in contrast to previous reported elevated NO amounts in CFS patients (8). Kurup and Kurup (8) assessed NO in the plasma following a different protocol, which might explain the difference with the present study. Furthermore, NO is a very volatile molecule affected by many external factors.

Regarding the correlations with NO, no significant associations were revealed in either group. There was no direct relation between the NO concentration and the level of activity before or after blood sampling. Although physical activity normally causes an increase in NO production (11), the absence of a relation between activity and NO may be explained by the fact that this relation is mostly evaluated in studies where participants are asked to perform exercise (11,

12). Imposed exercise may be strong enough to trigger NO release, while usual daily activity may be too light or the variation may be too small to cause real changes in NO production. Since the subjects in the present study were all quite passive during their daily living, more and imposed physical activity would be required to record a substantial rise in NO. Based on these results it can be said that CFS patients are unlikely to present fluctuating symptoms due to NO increases as a response to normal physical activities. Therefore, malaise accompanied by hypotension, lactate accumulation *etc.* in CFS patients are probably not the result of exercise-induced NO boosts. Conversely, the increased NO concentration in the serum may not contribute to impaired physical functioning. Finally, NO levels in (smooth) muscle cells are more determinant by modulating mitochondrial respiration, iron metabolism, *etc.*, rather than the NO measured in the serum.

Future research should focus on NO levels in other fluids and cells, and should determine whether physical activity promotes serum NO concentrations in CFS and whether elevated NO amounts may affect physical performance; serum NO should be assessed before and after a (sub)maximal exercise test.

One should not focus only on the detrimental role of NO. In normal concentrations, NO has an immunological function (9) and is also a major endogenous regulator of vascular tone, maintaining adequate tissue perfusion and effective cardiovascular function and homeostasis (23). The increase of NO may be one among more beneficial effects of exercise in healthy people, but usual daily activities do not seem strong enough to trigger a substantial rise. In CFS patients, more research is required to study the effect of exercise on NO production to elucidate the benefits or the dangers of exercise, since a subgroup of them present with elevated NO-amounts, according to the study of Kurup and Kurup (8).

In conclusion, the present study revealed no significant differences between serum NO amounts of CFS patients and healthy controls and no relation with daily activity levels.

Acknowledgements

The Authors are grateful to K. De Meirleir for diagnosing the study participants and to Annemie Wielant and Marc Frémont (RED Laboratories, Zellik, Belgium) for analysing the blood samples. The study was financially supported by the Research Council of the University College Antwerp, Belgium (project number PWO G822). Mira Meeus is a postdoctoral research fellow of the Flanders Research Foundation (FWO).

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Received May 11, 2010

Revised June 4, 2010

Accepted June 9, 2010