Low grade inflammation and arterial wave reflection in patients with chronic fatigue syndrome

Vance A Spence, Gwen Kennedy, Jill Jf Belch, Alexander Hill, Faisel Khan

LOW GRADE INFLAMMATION AND ARTERIAL WAVE REFLECTION IN PATIENTS WITH CHRONIC FATIGUE SYNDROME

Vance A Spence, Gwen Kennedy, Jill J.F. Belch, Alexander Hill, Faisel Khan

Vascular and Inflammatory Diseases Research Unit. The Institute for Cardiovascular Research, Ninewells Hospital and Medical School, University of Dundee, DD1 9SY

Short title: Arterial stiffness and inflammation in CFS

Key words: Arterial stiffness, chronic fatigue syndrome, inflammation, pulse wave analysis

Address for correspondence:

Dr Faisel Khan, Vascular and Inflammatory Diseases Research Unit, The Institute for Cardiovascular Research, Division of Medicine and Therapeutics, Ninewells Hospital and Medical School, Dundee DD1 9SY

Tel: +44 (0) 1382 660111 ext 35574

Fax: +44 (0) 1382 63233

E-mail: f.khan@dundee.ac.uk

Abstract

Some of the symptoms reported by people with chronic fatigue syndrome (CFS) are associated with various cardiovascular phenomena. Markers of cardiovascular risk, including inflammation and oxidative stress, have been demonstrated in some CFS patients but little is known about the relationship of these and prognostic indicators of cardiovascular risk in this patient group. We sought to investigate the relationship between inflammation and oxidative stress and augmentation index, a measure of arterial stiffness, in 41 well characterised CFS patients and in 30 healthy subjects. The augmentation index, normalised for a heart rate of 75 beats per minute (AIx@75), was significantly greater in CFS patients than in control subjects (22.5 ± 1.7 versus 13.3 ± 2.3%, P=0.002). CFS patients also had significantly increased levels of C-reactive protein (2.58 ± 2.91 versus 1.07 ± 2.16 μg/mL, P<0.01) and 8-iso-prostaglandin $F_{2\alpha}$ isoprostanes (470.7 ± 250.9 versus 331.1 ± 97.6 pg/mL, P<0.005). In CFS patients, AIx@75 significantly correlated with log C-reactive protein (r=0.507, P=0.001), isoprostanes (r=0.366, P=0.026), oxidised LDL (r=0.333, P=0.039) and systolic blood pressure (r=0.371, P=0.017). In a stepwise multiple regression model, (including systolic and diastolic blood pressure, body mass index, C-reactive protein, TNFα, IL-1, oxidised LDL, HDL cholesterol levels, isoprostanes, age and gender), AIx@75 was independently associated with log CRP (β=0.385, P=0.006), age (β=0.363, P=0.022), and female gender (β=0.302, P=0.03) in CFS patients. The combination of increased arterial wave reflection, inflammation and oxidative stress may result in an increased risk of future cardiovascular events. Assessment of arterial wave reflection might be useful for determining cardiovascular risk in this patient group.

Introduction

Chronic fatigue syndrome (CFS) is a heterogeneous disorder of unknown etiology affecting neurological, immunological and cardiovascular systems. The syndrome, which is poorly understood, is characterized by disabling exercise intolerance with significantly reduced activity levels that have been linked to intracellular immune deregulation [1]. There is also accumulating evidence that the cardiovascular system is compromised in many CFS patients with reports of autonomic dysfunction [2], attenuated heart rate and blood pressure regulation [3] with increased vasomotor tone and loss of beat-to-beat heart rate control [4]; such abnormalities contribute to destabilization of blood pressure and orthostatic intolerance [5] and, in the more severe CFS cases, a reduced cardiac output [6].

Oxidative processes are increasingly being linked to symptoms in CFS [7-9] and we have recently demonstrated that CFS patients have increased in-vivo lipid peroxidation with elevated levels of F_2-isoprostanes that correlate with post-exertional myalgia, the dominating symptom that characterizes most patients [10]. We also showed that our patients have raised concentrations of active transforming growth factor β1 [11] and we postulated that CFS was pro-inflammatory with many patients in a pro-oxidant state consistent with significant cardiovascular risk [10].

C-reactive protein (CRP) is an acute phase protein and a sensitive but non-specific biochemical marker of chronic inflammation. Highly sensitive assays have consistently shown CRP to be a robust and independent predictor of cardiovascular risk [12] including in healthy individuals without any evidence of cardiovascular disease [13]. Markers of inflammation, including raised CRP levels, have already been demonstrated in some CFS patients [14] but little is known about the relationship between chronic inflammation and prognostic indicators of cardiovascular risk in this patient group. In

other populations, elevated CRP has been consistently linked to increased pulse wave velocity [15,16], and in some, but not all, studies with augmentation index [15-18], which is a combined measure of elastic and muscular arterial stiffness and wave reflection.

Van de Putte, et al [19] recently reported increased arterial stiffness and hypotension in a cohort of 32 adolescent CFS patients, age range 12 to 18 years, which could not be explained by changes in arterial wall characteristics or lifestyle changes. Vascular stiffness, however, impacts on resting and exercise-induced haemodynamics and may contribute to orthostatic hypotension by blunting cardiovagal baroreflex sensitivity in response to changes of arterial pressure in barosensitive regions such as the aorta and carotid arteries [19], and may also the worsen cardiac performance under conditions of augmented preload in CFS patients [6].

Given the association between inflammation and increased arterial stiffness in other populations, and the recent emerging evidence that increased arterial stiffness is an independent predictor of adverse cardiovascular outcome [20], we sought to investigate the relationship between CRP levels and augmentation index in well characterised CFS patients. Augmentation index has been shown to be a useful marker of cardiovascular risk both in healthy subjects and in patients with cardiovascular disease [21,22], and is independently associated with adverse cardiovascular events [23-27].

Subjects and Methods

Forty one subjects (19-63 years old) were recruited from a local register of CFS/ME patients and underwent a medical examination by the same physician; all were found to fulfil the Centres for Disease Control (CDC) classification for CFS [28]. Any patient with either an inflammatory or systemic disease history was excluded. The mean length of illness was 9.2 years (SD 5.7 years). Seventeen patients were on more than one medication. Ten patients were on low dose amitriptyline taken at night for sleeping problems, 8 were on thyroxine, 13 patients were on paracetamol based drugs, 3 were on β-blockers, and 5 were on benzodiazepine derivatives. Thirty healthy volunteers (19-63 years old) were also recruited from the local community and served as the control group. Details of the CFS patients and control subjects are given in Table 1. The local medical ethics committee approved the study and all volunteers gave written informed consent.

Blood samples were obtained from the antecubital fossa and collected into tubes containing EDTA for plasma or clotting beads for serum preparation. All blood samples were taken at the same time of day. The blood was centrifuged immediately for EDTA plasma preparation for 15 minutes at 3500rpm at 4°C. The serum tube was placed in a water bath at 37°C for 1 hour before it was centrifuged for 15 minutes at 3500rpm at 4°C. The plasma or serum was removed, aliquoted and stored at –70°C until assayed for 8-iso-prostaglandin $F_{2\alpha}$ isoprostanes, CRP, tumor necrosis factor α (TNFα), interleukin 1 (IL-1), total and high density lipoprotein (HDL) cholesterol, and oxidised low density lipoprotein (LDL) cholesterol. Plasma isoprostanes were measured by gas chromatography-mass spectrometry following the method described by Roberts and Morrow [29]. Total cholesterol and HDL levels were measured on a Cobas Bio centrifugal analyser using products from Roche. Plasma oxidised LDL levels were measured by ELISA (Mercodia, Sweden), serum levels of CRP was measured using a

high sensitivity ELISA (Kalon Biological Ltd, UK), and serum levels of TNFα and IL-1 were measured by high sensitivity ELISAs (R&D Systems, UK).

Assessment of Augmentation Index - Pulse Waveform Analysis

Measurements were conducted in a temperature-controlled room (23 ± 1°C). Subjects were rested in a supine position for at least 10 minutes after which blood pressure was measured in triplicate using an automated blood pressure monitor (Omron 705 CPII). An index of arterial stiffness was assessed non-invasively by measuring the augmentation index (AIx), which is an estimate of systemic arterial and muscular stiffness, using the validated SphygmoCor pulse waveform analysis system (Scanmed Medical Instruments, Moreton-in-Marsh, UK) [30,31]. Peripheral pressure waveforms were recorded at the radial artery by applanantion tonometry using a high fidelity micromanometer (Miller Instruments). At least 15 high quality pressure waveform recordings were obtained from which the central aortic pressure waveform was calculated using a validated generalised transfer function. From the averaged aortic pulse wave, the following parameters were calculated:

- Augmentation index (AIx), defined as the augmented pressure divided by the pulse pressure and expressed as a percentage
- Augmentation index normalised for a heart rate of 75 beats per minute (AIx@75). This parameter was calculated to take into account the effect of heart rate on AIx [31,32].
- Time to return of the reflected wave (Tr). This was used as a marker of pulse wave velocity [31].

Statistical analysis

Values are expressed as means ± 1SD unless stated otherwise. Differences in values between CFS patients and control subjects were tested using unpaired t-tests or non-

parametric tests if the data were not normally distributed. The independent effect of potential determinants (systolic and diastolic blood pressure, body mass index, CRP, TNFα, IL-1, oxidised LDL, HDL cholesterol levels, and isoprostanes, age and gender), on augmentation index were assessed using stepwise multiple regression models. For non-normally distributed data, values were log transformed for regression analysis. A P value of < 0.05 was considered significant. All analyses were performed using SPSS statistical package (version 13).

Results

Patients with CFS and control subjects were matched for age, gender distribution, smoking status, height and weight (Table 1). Systolic and diastolic blood pressure, and heart rate were significantly greater in CFS patients than in control subjects ($P<0.005$, $P<0.005$ and $P<0.05$, respectively).

Total cholesterol was similar in the two groups, but HDL cholesterol was significantly lower in CFS patients than in control subjects ($P<0.005$). CFS patients had significantly increased levels of CRP ($P<0.01$, Mann-Whitney test) and 8-iso-prostaglandin $F_{2\alpha}$ isoprostanes ($P<0.005$). There were no significant differences between the two groups for IL-1 and TNFα.

Figure 1 shows that the augmentation index (AIx) was significantly greater in CFS patients than in control subjects ($P=0.017$). AIx was 52% higher in CFS patients than in control subjects. However, as AIx is influenced by heart rate, which was significantly higher in CFS patients, an index normalised for 75 beats per minute was used to provide a better comparison of arterial wave reflection between CFS patients and control subjects (Figure 1). This index, AIx@75, was also significantly greater in CFS patients than in control subjects ($P=0.002$), but the difference (69%) was greater than for the unadjusted AIx (52%). The time to return of the reflected wave (Tr) was significantly shorter in CFS patients (137.6±21.4 ms) compared with control subjects (146.5±12.3 ms), indicating a faster pulse wave velocity ($P=0.039$).

Table 2 shows the significant univariate associations between markers of arterial stiffness and physical and biochemical variables for CFS patients. AIx@75 showed the strongest correlations with systolic blood pressure, logCRP, isoprostanes and oxLDL. Tr was significantly correlated with logCRP and isoprostanes. There were borderline

associations between AIx@75 and age and gender (r=0.265, P=0.099 and r=0.297, P=0.087, respectively). In a stepwise regression model for the CFS patients, the independent associations with AIx@75 were logCRP (β=0.385, P=0.006), age (β=0.363, P=0.022), and female gender (β=0.302, P=0.03). For Tr, the only independently associated parameter was logCRP (β=-0.420, P=0.012).

In the control group and the independent associations with AIx@75 were female gender (β=0.516, P<0.001) and brachial diastolic pressure (β=0.301, P=0.026). There were no independent associations with Tr.

Discussion

This study found that CFS patients have higher serum hs-CRP levels, elevated levels of isoprostanes and oxidized LDL and significantly increased AIx@75, a composite measure of arterial stiffness and wave reflection. AIx@75 significantly correlated with CRP and isoprostanes. CFS patients also had higher blood pressure and lower HDL cholesterol levels than control subjects, which could potentially affect AIx@75. However, the independent determinants of AIx@75 in CFS patients were hs-CRP, age and female gender. The combination of increased arterial wave reflection, inflammation and oxidative stress may result in unfavorable haemodynamics and an increased risk of a future cardiovascular event in these patients.

Very few long term follow-up studies have been carried out in CFS patients and none on the occurrence of other health conditions, so it is not possible to estimate cardiovascular risk in this patient group. However, Lerner [33] documented repetitively abnormal T–wave oscillations, indicative of cardiomyopathy, in many of his CFS patients and, using radionuclide ventriculography, he demonstrated post-exertional abnormal cardiac wall motion in approximately one quarter of CFS patients indicative of cardiac dysfunction [34]. Furthermore, in a recent report, heart failure was seen as a major cause of death in a sample of CFS mortalities, and to occur at a considerably younger age than would have been expected from the general population [35].

While increased levels of CRP have limited diagnostic value in CFS [14], hsCRP levels are indicative of chronic, low-grade, sub-clinical inflammation acting as a potential adjunct in the global prediction of cardiovascular risk [16,18]. Prospective epidemiological studies covering 30 years found that a single hsCRP measurement strongly predicted a cardiovascular event in a dose response manner, i.e. continuous across the full range of hsCRP levels, and that the prediction was independent of

traditional risk factors such age, smoking, hypertension, dyslipidaemia, and diabetes [36]. Others have recently reported that markers of inflammation in post-infective fatigue syndromes are not sustained into the chronic phase of illness and that they play no role in the development of persistent symptoms in an Australian population [37], however, CFS is heterogeneous and there is considerable diversity within and between patient cohorts used in research [38].

Our study is the first report of hsCRP levels in CFS patients but others have reported increased arterial wave reflection in CFS, albeit in adolescents with the illness [19]. Whether chronic, sub-clinical inflammation is the cause of increased arterial wave reflection in CFS patients remains to be determined, although it has been positively correlated to indices of stiffness in apparently healthy subjects in the general population [16], patients with systemic vasculitis [15], children with Kawasaki disease [39], although not in women with active rheumatoid arthritis [40]. Particular lifestyle characteristics such as smoking, obesity and physical fitness also play a role in the development of arterial stiffness [41], but only a small percentage of our patients were smokers, and body weight and BMI were similar in CFS patients and control subjects. Lower levels of physical conditioning are associated with increased arterial stiffness [42] and, by definition, most CFS patients perform very little physical activity, even when compared to sedentary, healthy individuals. Experimentally and epidemiologically, it is evident that long term physical activity has an anti-inflammatory effect [43]. In a recent study, markers of inflammation were shown to be inversely related to physical activity and fitness in sedentary men with well managed hypertension [44], and while prescriptive walking regimens improve fitness and reduce cardiovascular risk in sedentary civil servants, they failed to induce any significant reduction in CRP [45]. Furthermore, while physical activity might be effective in modulating pro-inflammatory activity in atherosclerotic disorders [43], many CFS patients are exercise-

Clinical Science Immediate Publication. Published on 21 Nov 2007 as manuscript CS20070274

intolerant, possibly because of oxidative damage within exercising muscle [46,47], or due to increased plasma levels of oxidation such as isoprostanes [10] that are associated with post-exertional myalgia in other disorders [48].

Oxidative stress may also be pro-inflammatory, and in adults free of clinical cardiovascular disease, markers of oxidation correlate significantly with hsCRP levels, independently of other confounders such as body mass index [49]. While there are significant univariate correlations between measures of oxidation, such as increased levels of isoprostanes and oxLDL and decreased HDL, and augmentation index in our study, none of these proved to independent determinants of arterial wave reflection within the multiple regression model. Patients in our study group have significantly increased neutrophil apoptosis [11], and there is recent evidence that CRP degradation products, generated by neutrophil elastase, serve to promote neutrophil apoptosis [50]. In circumstances where CRP is degraded by neutrophil elastase, the CRP acquires an anti-inflammatory capacity that functions to promote neutrophil clearance [50] and may offer protection against inflammatory injury.

Development of arterial stiffness results from a complex interaction between structural and cellular elements of the vessel wall involving scaffolding proteins such as collagen and elastin [51], the latter being influenced by serum elastase activity. A significant, positive relationship exists between the level of disability in CFS and elasatse activity [52], and it is also a key determinant of sub-maximal and peak exercise intensity in these patients [1]. Pulse wave velocity, but not augmentation index, correlates with serum elastase activity in apparently healthy individuals and in those with systolic hypertension, giving rise to the possibility that inflammation and increased elastase activity contribute to arterial stiffness [53]. However, not all studies show that elastases are positively correlated with arterial stiffness [54]. Although we did not measure

arterial stiffness directly, we cannot exclude the possibility that our measurement of augmentation index, which is a composite measure of arterial stiffness and wave reflections, might be associated with elastase activity. We did, however, measure the time for return of the reflected wave, which is an indirect measure of pulse wave velocity, and found that this was significantly shorter in CFS patients, suggestive of an increase in pulse wave transmission, and that CRP was independently associated with Tr.

We appreciate that the gold standard measure of arterial stiffness is pulse wave velocity. Augmentation index is mainly affected by the speed of wave travel, the amplitude of the reflected waves, and the elastic properties of the aorta, in addition to the duration and pattern of ventricular ejection. Nevertheless, augmentation index has been also been shown to be an important determinant of cardiovascular risk and outcome [21,25,26]. It is an independent predictor of all-cause mortality in patients with end stage renal disease [27] and an independent marker for severity of coronary obstruction [22]. Additionally, augmentation index is independently associated with an increased risk for short and long term cardiovascular events in patients undergoing percutaneous coronary interventions [23], and is a significant predictor of major adverse cardiovascular events in patients with established coronary artery disease [24].

A limitation of the study is that we cannot be certain of the extent to which arterial wave reflections were affected in CFS patients by drug therapy such as thyroxine and beta-blockers. We did however, correct for any possible effect on heart rate by using the augmentation index normalised for a heart rate of 75 beats/min.

In conclusion, we have demonstrated an independent association between increased arterial wave reflection and low grade inflammation in patients with CFS. The results of

this study are hypothesis generating and the clinical impact of this association cannot be determined at this point. Nevertheless, since measures of arterial stiffness are predictive of cardiovascular outcome they might be useful for assessing cardiovascular risk in this patient group. There is, however, uncertainty surrounding both the diagnosis and prognosis of CFS so further studies will be required to address the relationship between inflammation and vascular stiffness in a wider population of CFS patients. It also remains to be determined if suppression of the chronic inflammatory process in carefully selected CFS patients leads to improvement of arterial stiffness and cardiovascular outcome in the longer term.

Acknowledgements

The authors wish to thank Dr Christine Underwood for carrying out the medical examinations. This work was supported by a project grant from ME Research UK.

References

1. Nijs, J., Meeus, M., McGregor, N.R., Meeusen, R., de Schutter, G., van Hoof, E. and De Meirleir, K. (2005) Chronic fatigue syndrome: exercise performance related to immune dysfunction. Med. Sci. Sports. Exer. 37, 1647-1654

2. Yoshiuchi, K., Quigley, K.S., Ohashi, K., Yamamoto, Y. and Natelson, B.H. (2004) Use of time-frequency analysis to investigate temporal patterns of cardiac autonomic response during head-up tilt in chronic fatigue syndrome. Auton. Neurosci-Basic. 113, 55–62

3. DeBecker, P., Roeykens, J., Reynders, M., McGregor, N. and De Meirleir, K. (2000) Exercise capacity in chronic fatigue syndrome. Arch. Intern. Med. 160, 3270-3277

4. Stewart, J.M. (2000) Autonomic nervous system dysfunction in adolescents with postural orthostatic tachycardia syndrome and chronic fatigue syndrome is characterized by attenuated vagal baroreflex and potentiated sympathetic vasomotion. Pediatr. Res. 48, 218-226

5. Spence, V.A. and Stewart, J.M. (2004) Standing up for ME: Cardiovascular mechanisms of orthostatic intolerance. The Biologist. 51, 65-70

6. Peckerman, A., Lamanca, J.J., Dahl, K.A., Chemitiganti, R., Qureishi, B. and Natelson, B.H. (2003) Abnormal impedance cardiography predicts symptom severity in chronic fatigue syndrome. Am. J. Med. Sci. 326, 55–60

7. Richards, R.S., Roberts, T.K., McGregor, N.R., Dunstan, R.H. and Butt, H.L. (2000) Blood parameters indicative of oxidative stress are associated with symptom expression in chronic fatigue syndrome. Redox. Rep. 5, 35–41

8. Manuel-Y-Keenoy, B., Moorkens, G., Vertommen, J. and De Leeuw, I. (2001) Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. Life. Sci. 68, 2037–2049.

9. Vecchiet, J., Cipollone, F., Falasca, K., Mezzetti, A., Pizzigallo, E., Bucciarelli, T., De Laurentis, S., Affaitati, G., De Cesare, D. and Giamberardino, M.A. (2003) Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. Neurosci. Lett. 335, 151–154

10. Kennedy, G., Spence, V.A., McLaren, M., Hill, A., Underwood, C. and Belch, J.J.F. (2005) Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. Free. Radical. Bio. Med. 39, 584–589

11. Kennedy, G., Spence, V.A., Underwood, C. and Belch, J.J.F. (2004) Increased neutrophil apoptosis in chronic fatigue syndrome. J. Clin. Pathol. 57, 891-893

12. Rifai, N. and Ridker, P.M. (2001) High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin. Chem. 47, 403-411

13. Bassuk, S.S., Rifai, N., and Ridker, P.M. (2004) High-sensitivity C-reactive protein: clinical importance. Curr. Prob. Cardiology. 29, 439-493

14. Buchwald, D., Wener, M.H., Pearlman, T. and Kith, P. (1997) Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J. Rheumatol. 24, 372-376

15. Booth, A.D., Wallace, S., McEniery, C.M., Yasmin, Brown, J., Jayne, D.R. and Wilkinson, I.B. (2004) Inflammation and arterial stiffness in systemic vasculitis: a model of vascular inflammation. Arthritis. Rheum. 50, 581–588

16. Yasmin, McEniery, C.M., Wallace, S., Mackenzie, I.S., Cockcroft, J.R. and Wilkinson, I.B. (2004) C-reactive protein is associated with arterial stiffness in apparently healthy individuals. Arterioscler. Thromb. Vasc. Biol. 24, 969–974

17. Kullo, I.J., Seward, J.B., Bailey, K.R., Bielak, L.F., Grossardt, B.R., Sheedy , P.F., Peyser, P.A. and Turner, S.T. (2005) C-reactive protein is related to arterial wave

18. Vlachopoulos, C., Dima, I., Aznaouridis, K., Vasiliadou, C., Ioakeimidis, N., Aggeli, C., Toutouza, M. and Stefanadis, C. (2005) Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. Circulation. 112, 2193-2200

19. Van de Putte, E.M., Uiterwaal, C.S.P.M., Bots, M.L., Kuis, W., Kimpen, J.L.L. and Engelbert, R.H.H. (2005) Is chronic fatigue syndrome a connective tissue disorder? A cross-sectional study in adolescents. Pediatrics. 115, 415-422

20. Mattace-Raso, F.U.S., Van der Cammen, T.J.M., Hofman, A., van Popele, NM., Bos, M.L., Schalekamp, M.A.D.H., Asmar, R., Reneman, R.S., Hoeks, A.P.G., Bretler, M.M.B. and Witteman, J.C.M. (2006) Arterial stiffness and risk of coronary heart disease and stroke. The Rotterdam study. Circulation. 113, 657-663

21. Nürnberger, J., Keflioglu-Scheiber, A., Saez, A.M.O., Wenzel, R.R., Philipp, T. and Schäfers, R.F. (2002) Augmentation index is associated with cardiovascular risk. J Hypertens. 20, 2407-2414

22. Coviv, A., Haydar, A.A., Bhamra Ariza, P., Taomir-Gusbeth, P. and Goldsmith, D.J. (2005) Aortic pulse wave velocity and arterial wave reflection predict the extent and severity of coronary artery disease in chronic kidney disease patients. J Nephrol. 18(4), 388-396

23. Weber, T., Auer, J., O'Rourke, M.F., Kvas, E., Lassnig, E., Lamm, G., Stark, N., Rammer, M. and Eber, B. (2005) Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. Eur Heart J. 26, 2657-2663

24. Chirinos, J.A., Zambrano, J.P., Chakko, S., Veerani, A., Schob, A., Willens, H.J., Perez, G. and Mendez, A.J. (2005) Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease. Hypertension. 45, 980-985

25. Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I. and Struijker-Boudier, H. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 27, 2588-2605

26. Agabiti-Rosei, E., Mancia, G., O'Rourke, M.F., Roman, M.J., Safar, M.E., Smulyan, H., Wang, J.G., Wilkinson, I.B., Williams, B. and Vlachopoulos, C. (2007) Central blood pressure measurements and antihypertensive therapy: a consensus document. Hypertension. 50, 154-160

27. London, G.M., Blacher, J., Pannier, B., Guerin, A.P., Marchais, S.J. and Safar, M.E. (2001) Arterial wave reflections and survival in end-stage renal failure. Hypertension. 38, 434-438

28. Fukuda, K., Straus, S., Hickie, I., et al. and the International Chronic Fatigue Syndrome Study Group. (1994) Chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann. Intern. Med. 121, 953-959

29. Roberts, L.J.and Morrow, J.D. (2000) Measurement of F_2 isoprosotanes as an index of oxidative stress in vivo. Free Radical Biol. Med. 28(14), 505-513

30. Chen, C.H., Nevo, E., Fetics, B., Pak, P.H., Yin, F.C., Maughan, W.L.and Kass, D.A. (1997) Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. Circulation. 95(7), 1827-1836

31. Weber, T., Auer, J., O'Rourke, M.F., Kvas, E., Lassnig, E., Berent, R.and Eber, B. (2004) Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation. 109, 184-189

32. Wilkinson, I.B., MacCallum, H., Flint, L., Cockcroft, J.R., Newby, D.E. and Webb, D.J. (2000) The influence of heart rate on augmentation index and central arterial pressure in humans. J. Physiol. 525, 263-270

33. Lerner, A.M., Goldstein, J., Change, C.H. et al. (1997) Cardiac involvement in patients with chronic fatigue syndrome as documented with Holter and biopsy data in Birmingham. Michigan. 1991-1993. Infect Dis Clin Prac. 6, 327-33

34. Lerner, A.M., Dworkin, H.J., Sayyed, T., Chang, C.H., Fitzgerald, J.T., Beqaj, S., Deeter, R.G., Goldstein, J., Gottipolu, P. and O'Neill W. (2004) Prevalence of abnormal cardiac wall motion in the cardiomyopathy associated with incomplete multiplication of Epstein-barr Virus and/or cytomegalovirus in patients with chronic fatigue syndrome. In Vivo. 18, 417-24

35. Jason, L.A., Corradi, K., Gress, S., Williams, S. and Torres-Harding, S. (2006) Causes of death among patients with chronic fatigue syndrome. Health Care Women Int. 27, 615-626

36. Bassuk, S.S., Rifai, N., and Ridker, P.M. (2004) High-sensitivity C-reactive protein: clinical importance. Curr. Prob. Cardiol. 29, 439-493

37. Hickie, I., Davenport, T., Wakefield, D. et al. (2006).Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ, doi:10.1136/bmj.38933.585764.AE

38. Jason, L.A., Corradi, K., Torres-Harding, S., Taylor, R.R. and King, C. (2005) Chronic fatigue syndrome: the need for subtypes. Neuropsychol. Rev. 15, 29-58

39. Cheung, Y.F., Ho, M.H.K., Tam, S.C.F., and Yung, T.C. (2004) Increased high sensitivity C reactive protein concentrations and increased arterial stiffness in children with a history of Kawasaki disease. Heart. 90, 1281-1285.

40. Turesson, C., Jacobsson, L., Ryden Ahlgren, A., Sturfelt, G., Wollmer, P., and Lanne, T. (2005) Increased stiffness of the abdominal aorta in women with rheumatoid arthritis. Rheumatology. 44, 896–901

41. Vlachopoulos, C., Aznaouridis, K. and Stefanadis, C. (2006) Clinical appraisal of arterial stiffness: the Argonauts in front of the Golden Fleece. Heart. 92, 1544-1550

42. Bruunsgaard, H. (2005) Physical activity and modulation of systemic low-level inflammation. J. Leukocyte Biol. 78, 819–835

43. Kasapis, C. and Thompson, P.D. (2005) The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J. Am. Coll. Cardiol. 45, 1563-1539.0

44. Hjelstuen, A., Anderssen, S.A., Holme, I., Seljeflot, I. and Klemsdal, T.O. (2006) Markers of inflammation are inversely related to physical activity and fitness in sedentary men with treated hypertension. Am. J. Hypertens. 19, 669-675

45. Murphy, M.H., Murtagh, E.M., Boreham, C.A., Hare, L.G.and Nevill, A.M. (2006) The effect of a worksite based walking programme on cardiovascular risk in previously sedentary civil servants. BMC Public Health. 22, 136

46. Fulle, S., Mecocci, P., Fano, G., Vecchiet, I., Vecchini, A., Racciotto, D., Cherubini, A., Pizzigallo, E., Vecchiet, L., Senin, U. and Beal, M.F. (2000) Specific oxidative alterations in vastus lateralis muscle of patients with the diagnosis of chronic fatigue syndrome. Free Radical Biol. Med. 29, 1252-1259

47. Jammes, Y., Steinberg, J.G., Mambrini, O., Bregeon, F. and Delliaux, S. (2005) Chronic fatigue syndrome: assessment of increased oxidative stress and altered muscle excitability in response to incremental exercise. J. Intern. Med. 257, 299-310

48. Sinzinger, H., Lupattelli, G., Chehne, F., Oguogho, A. and Furberg, C.D. (2001) Isoprostane 8-epi-PGF2α is frequently increased in patients with muscle pain and/or CK-elevation after HMG-Co-enzyme-A-reductase inhibitor therapy. J. Clin. Pharm. Ther. 26, 303-310

49. Abramson, J.L., Hooper, W.C., Jones, D.P., Ashfaq, S., Rhodes, S.D., Weintraub, W.S., Harrison, D.G., Quyyumi, A.A. and Vaccarino, V. (2005) Association between novel oxidative stress markers and C-reactive protein among adults without clinical coronary heart disease. Atherosclerosis. 178, 115–121

50. Kakuta, Y., Aoshiba, K. and Nagai, A. (2006) C-Reactive protein products generated by neutrophil elastase promote neutrophil apoptosis. Arch. Med. Res. 37, 456–460

51. Zieman, S.J., Melenovsky, V. and Kass, D.A. (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. Arterioscler. Thromb. Vasc. Biol. 25, 932-943

52. Meeus, M., Nijs, J., McGregor, N., Meeusen, R., De Schutter, G., Truijen, S., Van Hoof, E. and De Meirleir, K. (2005) Unraveling intracellular immune dysfunctions in chronic fatigue syndrome: interactions between protein kinase R activity, RNase L cleavage, and elastase activity and their clinical significance. Acta. Clin. Belg. 60, 314

53. Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR and Wilkinson IB. (2005) Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol. 25, 372-378.

54. Vlachopoulos C, Aznaouridis K, Dima I, Ioakeimidis N, Vasiliadou C, Zervoudaki A, Gialernios T and Stefanadis C. (2007) Negative association between serum levels of matrix metalloproteinases-2 and -9 and aortic stiffness in healthy adults. Int J Cardiol. [Epub ahead of print], doi.org/10.1016/ j.ijcard. 2006.11.099

Clinical Science Immediate Publication. Published on 21 Nov 2007 as manuscript CS20070274

Table 1. Physical and clinical characteristics of CFS patients and control subjects.

	CFS **n = 41**	**Control** **n = 30**
Age (years)	47.9 ± 11.3	45.7 ± 10.6
Height (m)	1.68 ± 0.10	1.70 ± 0.12
Weight (kg)	74.6 ± 15.8	72.7 ± 11.0
BMI (m^2/kg)	26.2 ± 4.7	25.6 ± 2.6
No. males	16	12
Brachial SBP (mmHg)	124.0 ± 17.1	114.9 ± 11.8†
Brachial DBP (mmHg)	80.0 ± 10.6	73.6 ± 7.8†
Central SBP (mmHg)	114.8 ± 16.4	103.8 ± 12.0†
Central DBP (mmHg)	81.2 ± 10.9	74.4 ± 7.9†
Heart rate (beats/min)	71.4 ± 12.7	66.1 ± 10.0*
Smoking No.		
Never	35	25
Former	2	1
Current	4	4
Total cholesterol (mmol/l)	5.13 ± 1.03	5.15 ± 1.01
HDL (mmol/l)	1.22 ± 0.33	1.49 ± 0.38†
ox LDL (mU/mL)	35.4 ± 14.3	33.2 ± 11.2
CRP (μg/mL)	2.58 ± 2.91	1.07 ± 2.16**
Isoprostanes (pg/mL)	470.7 ± 250.9	331.1 ± 97.6†
IL-1 (pg/mL)	0.36 ± 0.31	0.43 ± 0.49
TNF α (pg/mL)	2.25 ± 1.06	2.60 ± 1.64

* P<0.05
** P<0.01, using non-parametric tests
† P<0.005

Table 2. Univariate associations between markers of arterial stiffness and physical and biochemical parameters in CFS patients.

CFS	**AIx**		**AIx@75**		**Tr**	
	Coefficient	P value	Coefficient	P value	Coefficient	P value
Systolic BP	0.369	0.018	0.371	0.017	-0.255	0.108
logCRP	0.438	0.007	0.507	0.001	-0.369	0.025
Isoprostanes	0.259	0.121	0.366	0.026	-0.385	0.019
oxLDL	0.274	0.094	0.333	0.039	-0.135	0.414

AIx Augmentation index (%)

AIx@75 Augmentation index normalised for a heart rate of 75 beats per minute (%).

Tr Time to return of the reflected wave

Figure legend

Figure 1. Measures of arterial wave reflection in CFS patients (n=41) and matched controls (n=30). AIx and AIx@75 were significantly greater in CFS patients than in control subjects.

* P=0.017, ** P=0.002.

Clinical Science Immediate Publication. Published on 21 Nov 2007 as manuscript CS20070274