

# Biochemical and Vascular Aspects of Pediatric Chronic Fatigue Syndrome

Gwen Kennedy, PhD; Faisal Khan, PhD; Alexander Hill, PhD; Christine Underwood, MBBS; Jill J. F. Belch, MD

**Objective:** To evaluate the biochemical and vascular aspects of pediatric chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME).

**Design:** Cross-sectional clinical study.

**Setting:** Tayside, Scotland, United Kingdom.

**Participants:** Twenty-five children with CFS/ME and 23 healthy children recruited from throughout the United Kingdom.

**Interventions:** Participants underwent a full clinical examination to establish a diagnosis of CFS/ME and were asked to describe and score their CFS/ME symptoms. Biochemical markers were measured. Arterial wave reflection was estimated to assess systemic arterial stiffness.

**Main Outcome Measures:** Markers of oxidative stress and free radicals, C-reactive protein level, white blood cell apoptosis, and arterial wave reflection.

**Results:** Children with CFS/ME had increased oxidative stress compared with control individuals (isoprostanes: 252.30 vs 215.60 pg/mL,  $P = .007$ ; vitamin C, mean [SD]: 0.84 [0.26] vs 1.15 [0.28] mg/dL,  $P < .001$ ; vitamin E, 8.72 [2.39] vs 10.94 [3.46]  $\mu\text{g/mL}$ ,  $P = .01$ ) and increased white blood cell apoptosis (neutrophils: 53.7% vs 35.7%,  $P = .005$ ; lymphocytes: 40.1% vs 24.6%,  $P = .009$ ). Arterial stiffness variables did not differ significantly between groups (mean augmentation index,  $-0.57\%$  vs  $-0.47\%$ ,  $P = .09$ ); however, the derived variables significantly correlated with total ( $r = 0.543$ ,  $P = .02$ ) and low-density lipoprotein ( $r = 0.631$ ,  $P = .004$ ) cholesterol in patients with CFS/ME but not in controls.

**Conclusions:** Biomedical anomalies seen in adults with CFS/ME—increased oxidative stress and increased white blood cell apoptosis—can also be observed in children with clinically diagnosed CFS/ME compared with matched controls. Unlike in their adult counterparts, however, arterial stiffness remained within the reference range in these pediatric patients.

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**C**HRONIC FATIGUE SYNDROME/myalgic encephalomyelitis (CFS/ME) is a relatively common condition characterized by profound disabling fatigue in conjunction with a range of other symptoms.<sup>1,2</sup> It affects 0.2% to 0.4% of the population in developed countries, including 400 000 with CFS and 900 000 people with ME in

*See also pages 803, 810, and 880*

the United States according to population-based data.<sup>3,4</sup> Significant disability can result; specific physical symptoms can be as disabling as multiple sclerosis, rheumatoid arthritis, and other chronic conditions, placing a substantial burden on people affected by the illness, their families, and their caregivers.<sup>5</sup> Indeed, 1 study<sup>6</sup> estimated that the annual total value of lost productivity in the United States is \$9.1 billion (2004 figures), representing approximately \$20 000 per person with CFS/ME.

A variety of case definitions exist, often with varying nomenclatures. The historical literature on ME<sup>7,8</sup> was superseded by the Centers for Disease Control and Prevention (CDC) definition of CFS in 1988, subsequently revised in 1994.<sup>9</sup> Alternative criteria have since been proposed, including those for ME/CFS devised by the Expert Medical Consensus Panel in Canada,<sup>1</sup> which are gaining popularity, and the clinical definition of CFS/ME recently proposed by the National Institute for Health and Clinical Excellence in the United Kingdom.<sup>5</sup> Although there remains no clear consensus on definition or nomenclature, the most widely accepted for research purposes remains the 1994 CDC definition of CFS.<sup>2,9</sup> This definition relies on the presence of persistent or relapsing chronic fatigue with a duration of longer than 6 months and is of a new or definite onset that is not substantially alleviated by rest, is not the result of ongoing exertion, and results in a substantial reduction in occupational, social, or personal activities. In addition, there must be the simultaneous occurrence of 4 of 8 “minor criteria” symptoms.

**Author Affiliations:** Vascular and Inflammatory Diseases Research Unit, The Institute of Cardiovascular Research, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom.

It is widely recognized that CFS/ME also affects children and adolescents, although their prognosis is considered to be better than that for adults with this diagnosis.<sup>10,11</sup> Estimates of the numbers affected vary depending on methods used and populations sampled, as a recent review illustrates,<sup>12</sup> but a community-based study in the United States<sup>13</sup> found the overall prevalence to be 60 cases per 100 000, or 0.06%, in line with other estimates. Recent studies<sup>12,14,15</sup> have concluded that CFS/ME represents a substantial problem in the young, yet it remains unclear whether the illness seen in children and adolescents is the same as that in adults in terms of disease mechanism and manifestations; little research exists to inform the debate.

We reported significant differences between adults with CFS/ME and matched control individuals in the early death of white blood cells (WBCs) (neutrophil apoptosis)<sup>16</sup> and increased cardiovascular risk markers (lipids, oxidative stress markers, and inflammatory C-reactive protein).<sup>17</sup> Apoptosis plays a crucial role in developing and maintaining health by eliminating unhealthy cells, old cells, and unnecessary cells; accelerated apoptosis of neutrophils (which are recruited to the site of injury within minutes of trauma and are the hallmark of acute inflammation)<sup>18</sup> is observed in patients with infection.<sup>19</sup> Oxidative stress describes cell damage caused by an overabundance of oxidants, including reactive oxygen species (eg, oxygen ions, free radicals, and peroxide), which can interact with other molecules in cells and can cause oxidative damage to proteins, membranes, and genes. We also recently reported that other independent determinants of cardiovascular risk and outcome, such as arterial wave reflection, are significantly raised in people with CFS/ME and are related to levels of inflammation.<sup>20</sup> The augmentation index (AIx) is a measure of arterial wave reflection and is determined by the speed of wave travel, the amplitude of the reflected waves, and the elastic properties of the aorta. It has been shown to be an important determinant of cardiovascular risk and outcome<sup>21,22</sup> and is an independent marker for severity of coronary obstruction.<sup>23</sup> In addition, the AIx is a significant predictor of major adverse cardiovascular events in patients with established coronary artery disease.<sup>24</sup> From these findings we have provisionally inferred that in adults, CFS/ME might be a chronic inflammatory disorder resulting in a significant risk of future cardiovascular events.

The primary objective of the present study, therefore, was to investigate a group of children with well-defined CFS/ME, in whom there is the possibility of long-term ill health, for the presence of early abnormalities in cellular behavior related to cardiovascular risks already observed in adults with the illness. In addition, we wanted to determine whether the alteration in arterial wave reflection found in adults with CFS/ME was also seen in children with the illness.

## METHODS

Children with CFS/ME were recruited from throughout the United Kingdom; the study was approved by the regional ethics committee (Tayside Committee on Medical Research, East of Scotland Research Ethics Service, Ninewells Hospital and Medical School, Dundee). All the participants and their parents or guardians (if the child was <16 years of age) gave written informed consent. Potential participants were identified initially from ad-

vertisements in the newsletters and magazines of local CFS/ME self-help groups in the United Kingdom. The advertisements encouraged potential participants aged 9 to 18 years or their parents or guardians to contact the Unit to request further information and to receive an initial screening form. The initial screening form asked about length of illness, current illnesses, and school or work attendance, as well as whether and from whom they had been previously given a formal diagnosis of CFS/ME. Eighty-eight screening questionnaires were requested and sent out, and 65 were returned for assessment. From the screening questionnaires, the research physician assessed the initial suitability of applicants to be invited to take part in the study. Thirty-five children and adolescents with CFS/ME were invited to attend the Unit (Vascular and Inflammatory Diseases Research Unit, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, University of Dundee) for initial assessment for suitability for the study; of these, 3 were excluded (2 because of apparent cardiac problems and 1 who tested positive for Lyme disease). After invitations had been issued, 7 children reported that they were too unwell to take part.

Accordingly, 25 individuals were eligible for and willing to take part in the study. All the participants had previously been given a formal diagnosis of CFS/ME by their local consultant pediatrician or general practitioner, but the diagnosis was confirmed at their first attendance by the research physician from a detailed clinical examination, including completion of a pro forma clinical examination sheet. All eligible participants fulfilled the 1994 CDC case definition for CFS.<sup>2,9</sup> However, we added the additional specification that the requirement for debilitating fatigue and reduction of activity as less than 50% of the patient's premorbid activity for at least 6 months be reduced to 3 months for pediatric patients. This stipulation follows recommendations in key guidelines<sup>14,15</sup> that earlier diagnosis is preferred.

For comparison with the CFS/ME group, healthy sex- and age-matched children served as controls; these children were recruited via the recommendations of family and friends of study participants (n=12) or hospital staff (n=11). They were also matched to the CFS/ME group regarding puberty status (self-assessed on the Tanner scale).<sup>25</sup>

Children with CFS/ME were asked about the background of their illness, ie, precipitating factors, the status of their illness (improving, static, or worsening), and attendance at school and sporting activities (before having become unwell). They were also asked how each of the CDC's 1994 minor criteria symptoms<sup>2,9</sup> (ie, short-term memory, sore throat, tender lymph nodes, muscle pain, multijoint pain, headaches of new type, unrefreshing sleep, and postexertional malaise) affected their lives. They were asked to score each symptom as "no symptom," "slight impact," "moderate impact," or "severe impact." Each child's height, weight, blood pressure (BP) measurements taken supine and standing, and heart rate were recorded.

## ASSESSMENT OF BLOOD MARKERS

For blood sample variables, local anesthetic cream (lidocaine-prilocaine [EMLA cream, 5%; AstraZeneca UK Ltd, Luton, England, United Kingdom]) was applied to the children's antecubital fossa and was left there for 20 minutes before it was removed; a blood sample (50 mL) was obtained using a 23-gauge butterfly needle. From this, the following measurements were performed: (1) full blood cell count (using a hematology analyzer [Humacount; Human GmbH, Weisbaden, Germany]); (2) oxidative stress and free radical measurements (oxidized low-density lipoprotein [LDL] [using enzyme-linked immunosorbent assay (ELISA) (Mercodia AB, Uppsala, Sweden)], 8-iso-prostaglandin F<sub>2</sub>α isoprostanes [using gas chromatography-mass spectrometry (Thermoquest GCQ plus Ion

Trap GCMS system, supplied by Thermo Electron Corporation, Hemel, Hampstead, England), plasma vitamin C and vitamin E [using high-performance liquid chromatography (Lab Alliance pumps/Agilent 1200 VW Detector/Clarity software; Speck & Burke Analytical, Alva, Scotland)], red blood cell glutathione [using spectrophotometry (Kalon Biological Ltd, Guildford, England), and myeloperoxidase [using ELISA (Hycult biotechnology bv, Uden, the Netherlands)]]; (3) a full lipid profile and glucose levels (using a Cobas Bio centrifugal analyzer [Randox Laboratories Ltd, Antrim, Northern Ireland, United Kingdom]); (4) high-sensitivity C-reactive protein (Kalon Biological Ltd) and nitrotyrosine (Hycult biotechnology bv) (using ELISA); (5) the apoptosis-extrinsic pathway (expression of Annexin V [R&D Systems Europe Ltd, Abingdon, England] on neutrophils and lymphocytes [using a flow cytometer (Coulter Epics XL-MCL, Beckman Coulter, High Wycombe, England)]; propidium iodide was used to aid in the differentiation of apoptotic, necrotic, and viable cells; and levels of tumor necrosis factor receptor 1 and the Fas receptor were also measured [using fluorescent-labeled antibodies] [Serotec Ltd, Oxford, England]); and (6) the apoptosis-intrinsic pathway (caspase 1 and bcl-2 [using ELISAs] [Bender MedSystems GmbH, Vienna, Austria]).

### ASSESSMENT OF ARTERIAL WAVE REFLECTION—PULSE WAVEFORM ANALYSIS

Measurements were conducted in a temperature-controlled room (mean [SD], 23°C [1°C]). Participants rested in a supine position for at least 10 minutes, after which BP was measured in triplicate using an automated BP monitor (Omron 705 CPII; Omron Healthcare Europe BV, Hoofddorp, the Netherlands). An index of arterial wave reflection was assessed noninvasively by measuring the AIx using the validated SphygmoCor pulse waveform analysis system (AltCor Medical Phy Ltd, WestRidge).<sup>26,27</sup> Peripheral pressure waveforms were recorded at the radial artery by applanation tonometry using a high-fidelity micromanometer (Millar Instruments Inc, Houston, Texas). At least 15 high-quality pressure waveform recordings were obtained from which the central aortic pressure waveform was calculated using a validated generalized transfer function. From the averaged aortic pulse wave, the following variables were calculated: (1) the AIx, defined as the augmented pressure divided by the pulse pressure and expressed as a percentage; (2) the AIx normalized for a heart rate of 75 bpm, which was calculated to take into account the effect of heart rate on the AIx<sup>28</sup>; and (3) time to return of the reflected wave, which was used as a marker of pulse wave velocity.<sup>27</sup>

### STATISTICAL ANALYSES

When data were normally distributed, the *t* test statistic was used, but when variables were not normally distributed, the Mann-Whitney test (the nonparametric alternative to the *t* test) was used. The association between variables was performed using Pearson correlation; linear regression was used to correct for age, sex, body mass index (calculated as weight in kilograms divided by height in meters squared), and Tanner stage. *P* < .05 was considered significant. All analyses were performed using a statistical software package (SPSS, version 14; SPSS Inc, Chicago, Illinois).

## RESULTS

Twenty-five children with CFS/ME and 23 controls matched for sex, age, and puberty status participated in the study (**Table 1**). There were no significant differ-

**Table 1. Characteristics of the Study Participants<sup>a</sup>**

Characteristic	Patients With CFS/ME (n=25)	Controls (n=23)	P Value, Unpaired <i>t</i> Test
Sex, M/F, No.	7/18	7/16	NA
Age, y	15.1 (10.0-18.0)	15.0 (10.0-18.0)	.83
Systolic BP supine, mm Hg	109 (80-132)	116 (92-144)	.06
Diastolic BP supine, mm Hg	66 (40-86)	66 (51-82)	.87
Systolic BP standing, mm Hg	110 (90-140)	115 (90-142)	.14
Diastolic BP standing, mm Hg	68 (44-90)	67 (52-90)	.80
Pulse, bpm	81 (60-108)	69 (52-90)	.001
BMI	20.7 (16.7-25.0)	21.7 (15.8-25.5)	.13
BMI percentile	55.3 (10.9-90.8)	66.3 (30.4-90.2)	.11
Female: Tanner scale [I-V]			
Breast development	4.0 [II-V]	3.6 [II-V]	.20
Pubic hair	3.8 [I-V]	3.4 [I-V]	.31
Male: Tanner scale [I-V]			
Genital development	4.4 [II-V]	4.4 [II-V]	.89
Pubic hair	4.3 [I-V]	4.7 [I-V]	.17

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; NA, not applicable.

<sup>a</sup>Values are given as mean (range) except where indicated otherwise.

ences between groups in body mass index (*P* = .13) or in body mass index percentile adjusted for the height, sex, and age of the child (*P* = .11). Neither were there any significant differences in Tanner scale ratings between the groups. Heart rate was significantly higher in the CFS/ME group than in controls (*P* = .001, unpaired *t* test). There were no major differences in BP for either group between supine and standing, although the CFS/ME group had a lower systolic supine BP that was close to significant (*P* = .056, unpaired *t* test).

The major diagnostic criterion, debilitating fatigue for more than 3 months, was described by 13 of 25 children (52%) as severely affecting their lives and by 12 (48%) as affecting them to a moderate degree. With respect to the CDC's 1994 minor criteria symptoms,<sup>2,9</sup> postexertional malaise was reported to have a moderate or severe effect on everyday life by 19 of 25 children (76%); the equivalent figures for the other symptoms were as follows: unrefreshing sleep, 19 (76%); tender lymph nodes, 12 (48%); muscle pain, 17 (68%); headaches of new type, pattern, or severity, 14 (56%); multijoint pain, 11 (44%); sore throat, 11 (44%), and short-term memory, 10 (40%).

Seventeen of the 25 children (68%) said that their illness developed quickly, that is, over days or weeks, and 22 children (88%) reported that the illness had an infectious onset. Children with CFS/ME had been unwell for 6 months to 10 years; the median illness duration was 3 years. The median age of the group at illness onset was 12 years. Twelve children had been unwell for 3 years or longer, and 13 had been unwell for less than 3 years. When the group was split into 2 based on duration of illness (length of illness ≤ 3 years or ≥ 4 years), there were no significant differences in any of their blood variables

**Table 2. Full Blood Cell Counts<sup>a</sup>**

Factor	Patients With CFS/ME (n=25)	Controls (n=23)	P Value for Group Comparison
Total WBC count, / $\mu$ L	6500 (1800)	6100 (1800)	.41
Lymphocyte count, / $\mu$ L	2020 (720)	1950 (510)	.74
Monocyte count, / $\mu$ L	240 (250)	200 (100)	.53
Granulocyte count, / $\mu$ L	4290 (1740)	3960 (1660)	.51
WBCs that are lymphocytes, %	32.5 (11.4)	33.4 (8.7)	.78
WBCs that are monocytes, %	3.5 (2.8)	3.4 (1.8)	.88
WBCs that are granulocytes, %	63.9 (11.0)	63.2 (8.6)	.80
RBC count, $\times 10^9$ / $\mu$ L	4.7 (0.44)	4.8 (0.50)	.60
Hemoglobin, g/dL	13.2 (1.14)	13.7 (1.22)	.14
Hematocrit, %	39.9 (3.3)	40.1 (4.0)	.81

Abbreviations: CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; RBC, red blood cell; WBC, white blood cell.

SI conversion factors: To convert granulocyte, lymphocyte, monocyte, and WBC counts to  $\times 10^9$  per liter, multiply by 0.001; hematocrit to proportion of 1.0, multiply by 0.01; hemoglobin to grams per liter, multiply by 10.0; and RBC count to  $\times 10^{12}$  per liter, multiply by 1.0.

<sup>a</sup>Values are given as mean (SD).

or arterial wave reflection measurements ( $P > .05$ ). The patients were, therefore, treated as one group and were compared with their matched control group. Only 1 child with CFS was attending school full-time, and 12 had part-time attendance (1-8 h/wk).

There were no significant differences between the groups for any full blood cell count variables (**Table 2**). Although lipid and inflammatory markers did not differ between groups, the markers of oxidative stress were significantly increased. Levels of 8-iso-prostaglandin F<sub>2</sub> $\alpha$  isoprostane were significantly higher in the CFS/ME group (252.3 vs 252.30 pg/mL,  $P = .007$ ), and plasma vitamin E and vitamin C levels were significantly reduced (mean [SD] 8.72 [2.39] vs 10.94 [3.46]  $\mu$ g/mL,  $P = .01$ ; and 0.84 [0.26] vs 1.15 [0.28] mg/dL,  $P < .001$ , respectively; to convert vitamin E to micromoles per liter, multiply by 23.22; to convert vitamin C to micromoles per liter, multiply by 56.78) (**Table 3**). Mean levels of oxidized LDL, myeloperoxidase, and C-reactive protein were higher and mean levels of high-density lipoprotein and glutathione were lower in the CFS/ME group, although these differences did not reach statistical significance.

There was a significantly higher percentage of neutrophils undergoing apoptosis in the CFS/ME group than in the control group (53.7% vs 35.7%,  $P = .005$ ) (**Figure and Table 4**). In addition, lymphocytes from patients with CFS/ME showed significantly higher levels of apoptosis and a reduced percentage of viable healthy WBCs. Yet no differences were found between the 2 groups in levels of tumor necrosis factor receptor 1 and CD95 (Fas) or in intrinsic apoptotic markers (bcl-2 or caspase 1).

**Table 5** gives the derived variables from assessment of arterial wave reflection. No significant differences were found between the groups for brachial and central BP, AIx, and reflected wave. However, there was a trend for a greater AIx normalized for a heart rate of 75 bpm in the CFS/ME

**Table 3. Measures of Oxidative Stress, Lipids, and Inflammation<sup>a</sup>**

Measure	Patients With CFS/ME (n=25)	Controls (n=23)	P Value for Group Comparison
<b>Oxidative stress</b>			
Oxidized LDL, U/L	41.10 (10.90)	39.70 (11.90)	.68
8-Iso-prostaglandin F <sub>2</sub> $\alpha$ isoprostanes, pg/mL	252.30 (44.20)	215.60 (44.50)	.007
Plasma vitamin C, mg/dL	.84 (.26)	1.15 (.28)	<.001
Plasma vitamin E, $\mu$ g/mL	8.72 (2.39)	10.94 (3.46)	.01
Glutathione, $\mu$ mol/L	1654.00 (323.00)	1769.00 (390.00)	.27
Myeloperoxidase, ng/mL	66.58 (42.56)	62.30 (27.55)	.68
<b>Lipids</b>			
Total cholesterol, mg/dL	141.00 (31.00)	151.00 (29.00)	.26
HDL, mg/dL	51.00 (12.00)	54.00 (12.00)	.47
LDL, mg/dL	76.00 (24.00)	81.00 (23.00)	.45
Triglycerides, mg/dL	70.00 (27.00)	81.00 (39.00)	.26
<b>Inflammation</b>			
hs-CRP, mg/L	1.60 (3.49)	0.61 (1.00)	.30
Nitrotyrosine, nM	936.00 (2832.00)	1021.00 (2637.00)	.92
Neopterin, nmol/L	6.76 (5.13)	5.85 (1.81)	.57
Glucose, mg/dL	73.00 (7.00)	70.00 (8.00)	.22

Abbreviations: CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.

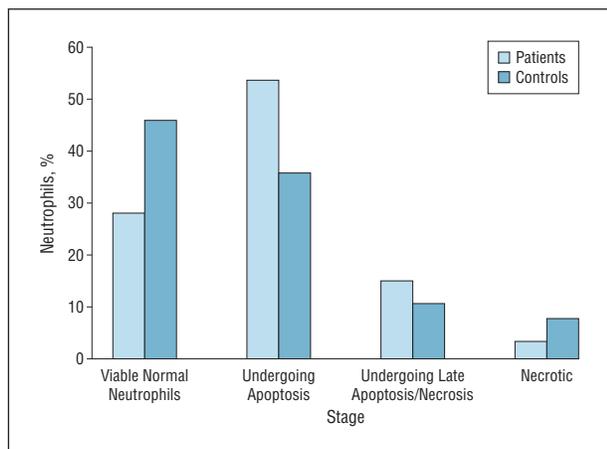
SI conversion factors: To convert HDL, LDL, and total cholesterol to millimoles per liter, multiply by 0.0259; hs-CRP to nanomoles per liter, multiply by 9.524; glucose to millimoles per liter, multiply by 0.0555; triglycerides to millimoles per liter, multiply by 0.0113; vitamin C to micromoles per liter, multiply by 56.78; and vitamin E to micromoles per liter, multiply by 2.32.

<sup>a</sup>Values are given as mean (SD).

group ( $P = .09$ ). In the CFS/ME group, AIx normalized for a heart rate of 75 bpm significantly correlated with total ( $r = 0.543$ ,  $P = .02$ ) and LDL ( $r = 0.631$ ,  $P = .004$ ) cholesterol levels. The reflected wave was negatively correlated with total ( $r = -0.489$ ,  $P = .03$ ) and LDL ( $r = -0.532$ ,  $P = .02$ ) cholesterol levels, suggesting a greater pulse wave velocity with increasing total and LDL cholesterol levels.

## COMMENT

This study shows that the biomedical anomalies seen in adults with CFS/ME—increased oxidative stress<sup>17</sup> and higher levels of WBC apoptosis<sup>18</sup>—can also be observed in a group of children with clinically diagnosed CFS/ME compared with age and sex-matched controls. Regarding oxidative stress, the pediatric CFS/ME group exhibited significantly elevated levels of F<sub>2</sub>-isoprostanes with reduced antioxidant plasma levels of vitamin C and vita-



**Figure.** Percentage of neutrophils at each stage of the apoptotic cycle in patients with chronic fatigue syndrome/myalgic encephalomyelitis and control individuals.

min E. This is an important finding given the sensitivity and reliability of isoprostanes as indicators of oxidative stress and their association with other measures of lipid peroxidation in vivo.<sup>29,30</sup> F<sub>2</sub>-isoprostanes are a series of prostaglandin F<sub>2</sub> $\alpha$  isomers described as products of noncyclooxygenase oxidative modifications of arachidonic acid or circulating LDL particles that have resulted from free radical attack of cell membrane phospholipids.<sup>31</sup> In this study, we measured plasma levels of 8-iso-prostaglandin F<sub>2</sub> $\alpha$  isoprostane because it is the most abundant of the family of isoprostanes, with plasma levels reflecting those in vivo. Isoprostanes not only reflect oxidative stress in integrated systems but also have potent biological effects associated with the peroxidation of membrane lipid, increased cell permeability, and a consequent increase in intracellular calcium.<sup>32</sup> They have also been shown to be powerfully vasoconstricting and are involved in endothelial injury.<sup>31,33</sup> It is not clear whether this increased oxidative stress is secondary to dietary deficiency of antioxidants or to persistent chronic WBC stimulation and release of free radicals. We believe that the latter mechanism may be implicated in light of the increased WBC apoptosis observed, but further studies, including dietary assessment, are required. Regardless of the mechanism, excessive oxidative stress is seen in this patient group.

Excessive free radical generation in patients with CFS/ME involving the oxidation of lipids and proteins<sup>34</sup> might arise from a variety of altered biological processes. For example, exercising muscle is a prime contender for excessive free radical generation, and recent evidence has shown correlations between various blood markers of oxidative injury in patients with CFS/ME<sup>35</sup> and muscle pain thresholds and fatigue. In a recent pilot study investigating patients with CFS/ME and matched controls, for both groups we found that F<sub>2</sub>-isoprostane levels are increased immediately after a standardized submaximal exercise challenge and return to their respective baseline levels 24 hours later; however, at all time points, patients with CFS/ME had significantly higher F<sub>2</sub>-isoprostane levels than did controls.<sup>36</sup> This finding may have important implications for cardiovascular risk in the longer term. There is evidence of viral persistence in muscle tissue in at least some people

**Table 4. Extrinsic and Intrinsic Apoptotic Variables<sup>a</sup>**

Variable	Patients With CFS/ME (n=25)	Controls (n=23)	P Value for Group Comparison
<b>Extrinsic apoptosis, %</b>			
CD95 expression			
On neutrophils	57.2 (3.7)	58.9 (4.0)	.76
On lymphocytes	10.3 (4.5)	10.0 (2.9)	.78
On all white blood cells	21.8 (12.8)	22.0 (12.2)	.95
TNFR-1 expression			
On neutrophils	4.1 (0.6)	5.8 (1.3)	.25
On lymphocytes	0.4 (0.1)	0.7 (0.2)	.20
On all white blood cells	1.2 (0.1)	1.7 (0.4)	.22
<b>Neutrophils</b>			
Viable normal	28.0 (4.5)	45.9 (5.9)	.03
Undergoing apoptosis	53.7 (3.3)	35.7 (5.2)	.005
Undergoing late apoptosis/necrosis	15.0 (2.6)	10.6 (2.2)	.20
Necrotic	3.3 (0.4)	7.8 (1.9)	.02
<b>Lymphocytes</b>			
Viable normal	44.3 (5.3)	64.6 (5.4)	.01
Undergoing apoptosis	40.1 (4.0)	24.6 (4.1)	.009
Undergoing late apoptosis/necrosis	10.0 (2.4)	3.9 (1.0)	.02
Necrotic	5.6 (0.6)	6.9 (1.1)	.35
<b>Intrinsic apoptosis</b>			
Caspase 1, pg/mL	55.5 (19.4)	49.2 (13.1)	.42
bcl-2, ng/mL	2.3 (1.9)	10.0 (8.2)	.12

Abbreviations: CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis; TNFR-1, tumor necrosis factor receptor 1.  
<sup>a</sup>Values are given as mean (SD).

**Table 5. Hemodynamic Measures in Patients With CFS/ME and Control Subjects<sup>a</sup>**

Measure	Patients With CFS/ME	Controls
Brachial systolic BP, mm Hg	111.0 (10.9)	115.7 (11.3)
Brachial diastolic BP, mm Hg	65.3 (8.4)	65.0 (6.9)
Central systolic BP, mm Hg <sup>b</sup>	93.6 (9.5)	96.2 (7.7)
Central diastolic BP, mm Hg <sup>b</sup>	66.6 (8.8)	65.8 (7.0)
Alx, %	-0.57 (10.4)	-0.47 (8.1)
Alx normalized for a heart rate of 75 bpm, %	-0.1 (10.4)	-4.9 (9.0)
Time to return of the reflected wave, milliseconds	156.0 (22.5)	168.4 (34.5)

Abbreviations: Alx, augmentation index; BP, blood pressure; CFS/ME, chronic fatigue syndrome/myalgic encephalomyelitis.  
<sup>a</sup>Values are given as mean (SD); for all group comparisons,  $P > .05$ .  
<sup>b</sup>Derived from the SphygmoCor integrated software (AltCor Medical Pty Ltd, West Ryde, New South Wales, Australia).

with CFS/ME<sup>37</sup> and also of oxidative damage to DNA and lipids<sup>38</sup> in muscle biopsy samples of patients with CFS consistent with metabolic abnormalities to mitochondria and phospholipids.

Regarding apoptosis, we showed that neutrophils and lymphocytes of pediatric patients with CFS/ME are significantly less viable compared with those of healthy children; both WBC types showed an increased percentage of cells undergoing apoptosis. The characteristics that de-

termine apoptosis are complex, and the observed increased rate of apoptosis may be a consequence of single or multiple factors, including a persistent or reactivating viral infection or a toxic state, reprogramming of apoptotic pathways by an infectious or toxic agent, or quicker neutrophil and lymphocyte turnover secondary to an abnormal host response to noxious stimuli.<sup>39</sup>

The increased heart rate in the CFS/ME group compared with matched controls agrees with the findings of other researchers,<sup>40,41</sup> as does the suggestion that BP can be lower in children with CFS/ME.<sup>42</sup> This increased resting heart rate may be secondary to decreased physical fitness and the reduced BP to the decreased vascular tone that has been reported.

We found a borderline trend representing increased arterial stiffness (ie, the AIx) in patients with CFS/ME, although this did not reach statistical significance. This finding contrasts with that of a previous study<sup>20</sup> in adults in which the AIx was significantly increased in patients with CFS/ME; however, the duration of illness was much shorter in the children in the present study (mean, 3.7 vs 9.2 years in the adult study), and these changes might be expected to become greater with increasing duration of illness. It is also possible, given the more subtle changes in arterial stiffness observed herein, that the power of this study (based on an adult population) was too low to adequately detect significant changes. However, we also found associations among the AIx, total cholesterol, and LDL cholesterol and among reflected wave, total cholesterol, and LDL cholesterol, indicating that even at this relatively early stage of illness duration there is a clustering of markers indicative of future cardiovascular risk. Measures of arterial wave reflection are useful markers of cardiovascular risk in healthy individuals and in patients with cardiovascular disease<sup>22</sup> and are independently associated with adverse cardiovascular events.<sup>21,43</sup>

In similar research studies in our Unit, 2 of us investigated cardiovascular risk factors in healthy children and adolescents aged 11-14 years and showed that a clustering of cardiovascular risk factors already existed in some of these children despite their age.<sup>44</sup> In addition, we investigated oxidative stress in patients aged 9-22 years with type 1 diabetes mellitus and found an increase in free radical generation and reduced plasma vitamin C and vitamin E levels compared with controls.<sup>45</sup> These studies highlighted the importance of monitoring indices of vascular dysfunction and oxidative stress in people aged 9-22, given the association between these markers and an increased risk of cardiovascular disease.

We showed for the first time, to our knowledge, that oxidative stress (lower plasma antioxidant levels and increased plasma isoprostane levels) and increased WBC apoptosis occur in children with CFS/ME. We believe that the data presented herein are consistent with the finding that many patients with CFS/ME have an underlying detectable abnormality in the behavior of their immune cells consistent with an activated inflammatory process.<sup>46</sup> The data are also consistent with a reactivating or persistent viral infection triggering WBC apoptosis with an increased production of free radicals resulting from the consequent neutrophil respiratory burst. This is a particularly intriguing possibility given the recent re-

port<sup>47</sup> of a potential retroviral link to CFS/ME involving the novel xenotropic murine leukemia virus-related virus that could be detected in the peripheral blood mononuclear cells of 67% of US adult patients compared with 3.7% of controls. The ensuing oxidative stress will affect the vasculature in a time-dependent manner; the data showing significantly increased arterial stiffness in adults and a less significant increase in children is consistent with this suggestion/supposition. In adults, there is often increased arterial stiffness when increased oxidative stress is detected; however, in this younger age group, it has not yet reached a level at which it seems to compromise the vasculature.

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**Correspondence:** Gwen Kennedy, PhD, Vascular and Inflammatory Diseases Research Unit, The Institute of Cardiovascular Research, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Mail Box 1, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, United Kingdom (g.y.kennedy@dundee.ac.uk).

**Author Contributions:** Dr Kennedy had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Kennedy, Khan, Underwood, and Belch. *Acquisition of data:* Kennedy, Khan, Hill, and Underwood. *Analysis and interpretation of data:* Kennedy, Khan, Hill, and Belch. *Drafting of the manuscript:* Kennedy, Khan, and Belch. *Critical revision of the manuscript for important intellectual content:* Kennedy, Khan, Hill, Underwood, and Belch. *Statistical analysis:* Kennedy, Khan, and Belch. *Obtained funding:* Kennedy, Khan, Hill, and Belch. *Administrative, technical, and material support:* Kennedy. *Study supervision:* Kennedy and Belch.

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