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# A potential biomarker for fatigue: Oxidative stress and anti-oxidative activity

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ABSTRACT

We sought to determine whether oxidative stress and anti-oxidative activity could act as biomarkers that discriminate patients with chronic fatigue syndrome (CFS) from healthy volunteers at acute and sub-acute fatigue and resting conditions. We calculated the oxidative stress index (OSI) from reactive oxygen metabolites-derived compounds (d-ROMs) and the biological antioxidant potential (BAP). We determined changes in d-ROMs, BAP, and OSI in acute and sub-acute fatigue in two healthy groups, and compared their values at rest between patients with CFS (diagnosed by Fukuda 1994 criteria) and another group of healthy controls. Following acute fatigue in healthy controls, d-ROMs and OSI increased, and BAP decreased. Although d-ROMs and OSI were significantly higher after sub-acute fatigue, BAP did not decrease. Resting condition yielded higher d-ROMs, higher OSI, and lower BAP in patients with CFS than in healthy volunteers, but lower d-ROMs and OSI when compared with sub-acute controls. BAP values did not significantly differ between patients with CFS and controls in the sub-acute condition. However, values were significantly higher than in the resting condition for controls. Thus, measured of oxidative stress (d-ROMS) and anti-oxidative activity (BAP) might be useful for discriminating acute, sub-acute, and resting fatigue in healthy people from patients with CFS, or for evaluating fatigue levels in healthy people.



## 1. Introduction

Chronic fatigue syndrome (CFS) is debilitating fatigue with unknown etiology that includes a wide spectrum of symptoms such as pain, depression, and neurocognitive dysfunction. Although its specific precipitating factors have not been identified, inflammatory and oxidative and nitrosative stress (IO&NS) pathways can generate fatigue and somatic symptoms including fatigue (Maes & Twisk, 2010). Thus, the underlying mechanisms linking fatigue may include generation of IO&NS pathways (Morris & Maes, 2014). CFS symptoms among those who are normotensive and non-obese were shown to correlate with oxidative stress, as measured by isoprostane levels (Kennedy et al., 2005). Indeed, lowered levels of anti-oxidative activity, coenzyme Q10, vitamin E, and zinc have been reported in CFS (Castro-Marrero et al., 2013; Maes et al., 2009, 2011; Miwa & Fujita, 2010).

Fatigue is a frequent condition experienced by healthy individuals. The prevalence of subjective fatigue ranges from 14.3% to as high as 60% in the healthy Japanese population (Chen, 1986; Watanabe, 2008). The impact of oxidative stress at different levels of fatigue in healthy people has been reported. Total anti-oxidative activity was shown to increase after a bout of exercise (Wadley, Veldhuijzen van Zanten, Panie, Drayson, & Aldred, 2014). Isoprostanes and glutathinone/oxidized glutathione were shown to be correlated with overtraining exhaustion levels (Margonis et al., 2007), and increased 8-hydroxydeoxyguanosine (8-OH-dG) was

*Abbreviations:* CFS, chronic fatigue syndrome; d-ROMs, reactive oxygen metabolites-derived compounds; BAP, biological antioxidant potential; OSI, oxidative stress index; VAS, visual analogue scale; HVs, healthy volunteers; IO&NS, oxidative and nitrosative stress.

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reported to continue for two to three days after sleep deprivation (Ikegami et al., 2010). Job stress and examinations are psychological conditions that lead to physical fatigue. 8-OHdG and malondialdehyde (MDA) levels were reported to increase with job stress and subjective stress levels (Casado, De Lucas, López-Fernández, Sánchez, & Jimenez, 2006; Irie, Asamo, Nagata, Miyata, & Kasai, 2002; Ishihara et al., 2008; Takaki, 2013). In addition, levels of protein carbonyl, MDA, and 8-OH-dG were all shown to increase during and after exam stress (Nakhaee, Shahabizadeh, & Erfani, 2013; Sivonová, Zinanová, Hlincíková, & Duracková, 2004; Eskiocak et al., 2005).

Evaluating biomarkers for oxidative stress and anti-oxidant activity simultaneously and accurately is important because it allows several fatigue conditions in healthy people to be differentiated from CFS. Some oxidative stress biomarkers are not accurately measured in vivo because reactive oxygen species normally have a short biological half-life and high reactivity. In contrast, Diacron-Reactive Oxygen Metabolites (d-ROMs) might be a good biomarker because they indicate the level hydroperoxides, which are relatively stable reactive oxygen metabolites (Martinović et al., 2011; Takahashi et al., 2013). The aim of this study was to determine whether dROMs and BAP are biomarkers that discriminate patients with CFS from healthy volunteers experiencing acute and sub-acute fatigue, and at rest.

## 2. Methods

This study was approved by the University of Kansai Welfare Sciences ethics committee (Approval No.09-06) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent to participate in the study before enrolment.

### 2.1. CFS patients

One hundred and twenty-one patients (76 female and 45 male; mean age 37.3 ± 8.36 years) who visited Osaka City University Hospital and who were diagnosed with CFS by specialists based on the 1994 Center for Disease Control clinical criteria (Fukuda et al., 1994) were eligible to participate in the study. This portion of the study was also approved by the ethics committee of Osaka City University (Approval No. 1498).

Exclusion criteria were as follows: (1) neuro-inflammatory or immune disorders diagnosed by clinical laboratory tests and magnetic resonance imaging; (2) any active medical condition that could explain the presence of chronic fatigue; (3) presence of any diagnosable illness that relapsed or was not completely resolved, such as some types of malignancies or chronic cases of hepatitis B or C virus infection; (4) alcohol or other substance abuse; (5) sever obesity as defined by a body mass index ≥30; (6) pregnancy; or (7) lactation.

The presence of major depressive disorder, fibromyalgia, or somatoform disorder was not a criterion for exclusion. Abdominal discomfort syndrome was not assessed. Eighty-eight patients were taking antioxidants, such as vitamin C or coenzyme Q10. Seventy-one patients were taking psychotropic or sleeping medication. Psychiatric disorders associated with CFS symptoms were diagnosed by psychiatrists at Osaka University or Osaka City University Hospitals. Sixty-one patients were comorbid with psychiatric disorders that were categorized as F3 of ICD-10. Fifty-three patients were comorbid with fibromyalgia (FM) and 83 patients showed post-malaise fatigue.

### 2.2. Healthy volunteers

Three groups of healthy volunteers (HVs) participated in the experiment. The first group (HV1) underwent the acute stress condition, and comprised 12 healthy female students (mean age 20.4 ± 0.5 years). They were not taking any medications or supplements.

The second group (HV2) participated in the sub-acute fatigue condition, and comprised 24 computer programmers (12 male and 12 female, mean age 36.7 ± 8.8 years).

The third group (HV3) participated in the at-rest condition and comprised 656 volunteers (274 male and 382 female, mean age 40.8 ± 12.4 years) who were confirmed not to have abnormal results on any major clinical laboratory test (hemoglobin, c-reactive protein, albumin, triglycerides, glucose, AST, ALT, X-ray exam, or cholesterol) or have a BMI ≥28. From these 656 volunteers, we selected a group of 121 that were age and sex matched to the CFS patients (age-sex matched HV3; 76 female and 45 male; mean age 37.3 ± 8.43 years). This part of the study was also approved by the Ethical Review Board, Faculty of Health Sciences, Yamaguchi University Graduate School of Medicine. Data from this group were compared with those from the CFS patients.

### 2.3. Study design

We determined oxidative stress and anti-oxidative activity biomarkers in patients with CFS and in the three groups of healthy volunteers. Measurements were made from CFS patients at rest, and in healthy volunteers during the acute experimental task, the sub-acute fatigue condition, and at rest. We defined the sub-acute fatigue condition as fatigue that accompanied a fixed-duration of severe overwork.

### 2.4. The acute condition

In this condition, the participants (HV1) performed a computer-based mental arithmetic stress task for three hours. The task consisted of a series of addition questions with single-digit figures that were displayed on a computer monitor (e.g., 1 + 3 = "4" and 5 + 7 = "12"). Participants selected numbers on a numerical keypad that corresponded to their answers. Blood was collected before and after the task and serum levels of d-ROMs and biological antioxidant potential (BAP) were measured (see below). Fatigue was assessed by a visual analogue scale (VAS) both pre- and post-task.

### 2.5. The sub-acute condition

For this condition, the computer programmers (HV2) constructed new computer systems at a department within the hospital laboratory for two weeks. They worked from 8:00 AM to 6:00 PM checking the new system and then programming the system from 6:00 PM to overnight. They did not show symptoms of fatigue and did not exhibit abnormal results from the major clinical laboratory tests (hemoglobin, c-reactive protein, albumin, triglyceride, glucose, AST, ALT, X-ray, and cholesterol) or show a BMI ≥28. Blood was collected before and after the overwork period, and serum levels of d-ROMs and BAP were evaluated.

### 2.6. The at rest condition

Blood was collected from the CFS patients and the HV3 control group at rest, and serum levels of d-ROMs and BAP were evaluated.

### 2.7. Measurement of d-ROMs and BAP

We measured both oxidation and anti-oxidation activities simultaneously in serum. Oxidative activity was assessed by measuring d-ROMs (Diacron International, Grosseto, Italy) and anti-oxidative activity by measuring the Biological Antioxidant Potential (BAP; Diacron International) using a AU480 automated analyzer (Beckman Coulter, Tokyo, Japan). For the d-ROMs test (Cesarone et al., 1999; Trotti, Carratelli, & Barbieri, 2002), according to the Fenton reaction, the ROMs (primarily hydroperoxides) of a serum sample are able to generate alkoxyl and peroxyl radicals in the presence of iron released from serum proteins with the help of an acidic buffer. Such radicals are in turn able to oxidize an alkyl-substituted aromatic amine (N,N-diethylparaphenylendiamine), thus producing a pink-colored derivative, which is photometrically quantified at 505 nm. The ROMs concentration runs directly parallel with color intensity and is expressed in Carratelli Units (1 CARR U = 0.08 mg hydrogen peroxide/dl) (Trotti et al., 2002).

For the BAP test (Kakita et al., 2006), the addition of a serum sample to a colored solution, obtained by mixing a ferric chloride solution with a thiocyanate derivative solution, causes a decoloration, the intensity of which is measured photometrically at 505 nm and is proportional to the anti-oxidant capacity of a serum sample. Inter-assay variability in d-ROMs and BAP levels were 0.6%–0.9% (the mean values from 20 different serum samples ranged from 159.7–463.9 CARR U) and 0.5%–1.6% (the mean values from 20 samples ranged from 1649 to 2677 μmol/L), respectively. Intra-assay variability in d-ROMs was 1.4% (mean value, 332.8 CARR U, n = 10 days) and in BAP levels was 2.2% (the mean value was 2462 μmol/L).

We determined the normal reference range of d-ROMs and BAP in 312 healthy controls (164 female, 148 male; mean age 36.7 ± 8.8 years) who failed to pass the first or second criteria as assessed by a self-administered questionnaire, and who exhibited no abnormalities in the clinical laboratory tests from a total of 2053 medical staff samples. The first set of exclusion criteria included smoking, heavy drinking, metabolic syndrome, being within one year of having delivered a baby, currently taking medication, or being overworked. The second exclusion criteria included no abnormal values in the clinical laboratory test and lifestyles, which included sleep and dietary status, and no fatigue symptoms evaluated by the questionnaire. The normal reference values for the d-ROMs and BAP tests, which were determined as being within two standard deviations from the mean, were 286.9 ± 100.2 CARR U and 2541.3 ± 122.0 μmol/L, respectively.

To obtain a parameter representing an overall shift toward oxidative stress, we used an oxidative stress index (OSI), which was derived by the following formula: OSI = C × (d-ROMs/BMP), where C denotes a coefficient for standardization to set the mean of OSI in the healthy controls to 1.0 (C = 8.85 in this study). We measured the two control specimens (C1 and C2) in twenty repeated measurements for each day during the basic assessment of the two assays for d-ROMs and BAP. We accumulated test results for the two controls and computed CV-intra and CV-inter using a one-way ANOVA. The calculated CV-intra and CV-inter for the d-ROMs test were 0.57% and 1.6%, respectively, using C1 (mean = 159.7 U) and 0.85% and 1.4%, respectively, using C2 (mean = 463.6 U). Similarly, CV-intra and CV-inter for the BAP test were 0.50% and 2.2%, respectively, using C1 (mean = 2518.4 μmol/L) and 1.60% and 2.8%, respectively, using C2 (mean = 1649.1 μmol/L)

### 2.8. Statistics

The mean changes in d-ROMs, BAP, and OSI after the acute and sub-acute fatigue conditions (HV groups 1 and 2, respectively) were examined by a multivariate repeated-measures generalized linear mixed-model (GLM) analysis with sex as a second factor. We examined the main effects of time and the interaction between time and sex (time × sex) using the oxidative stress biomarkers or VAS from the acute fatigue condition as the dependent variable. Because participants in the acute condition were all female, we did not perform a sex interaction analysis.

The correlations between age and the d-ROM, BAP, and OSI values were tested with a Pearson's correlation in the 656 HVs. A *t*-test was used to determine whether the mean differences in these values depended on sex. Other *t*-tests were used to determine whether the values obtained from HV2 significantly differed from those obtained from HV3 and whether those obtained from the age-matched HV3 groups significantly differed from the CFS patients. The mean differences in these values between X and CFS patients taking antioxidants, medications, or who were comorbid with FMs or psychiatric diseases were tested by *t*-test. Statistical analyses were performed using IBM SPSS version 22.0J software for Windows (IBM Japan Corp., Tokyo, Japan).

**Table 1**
Levels of d-ROMs, BAP and OSI in control group HV1 before and after the experimental acute fatigue task.

| Markers | Pre Mean ± SD | Post Mean ± SD | *F* | *P* |
|---|---|---|---|---|
| d-ROMs (CARR Unit) | 303.4 ± 24.4 | 327.9 ± 37.2 | 19.6 | 0.001 |
| BAP (μmol/L) | 2371.8 ± 77.6 | 2475.3 ± 68.9 | 11.0 | 0.007 |
| OSI | 1.13 ± 0.07 | 1.17 ± 0.13 | 2.47 | 0.15 |
| Fatigue score | 53.5 ± 30.4 | 73.3 ± 25.5 | 9.99 | 0.009 |

N = 12.
The mean d-ROMs, BAP, and OSI before and after the acute experimental task were examined multivariate repeated-measures generalized linear mixed-model (GLM) analysis with sex as a second factor. We examined the main effects of time and the interaction between time and sex (time × sex) using the oxidative stress biomarkers or VAS from the acute fatigue condition as the dependent variable. Because participants in the acute condition were all female, we did not perform a sex interaction analysis.
Fatigue score was evaluated by visual analogue scale.
OSI: oxidative stress index.

## 3. Results

Table 1 shows the levels of d-ROMs, BAP, and OSI before and after the acute fatigue task in the HV1 group. Analysis showed that after considering the effect of age, d-ROMs and BAP levels were significantly higher after the task, and that the OSI value trended to be higher.

The levels of d-ROMs, BAP, and OSI before and after sub-acute fatigue in the HV2 group are shown in Fig. 1A, B, and C, respectively. Analysis showed that after considering the effects of sex and age, d-ROMs and OSI were significantly higher after sub-acute fatigue. No significant changes in BAP were observed between before and after the 2-week working period.

The d-ROMs, BAP, and OSI values at rest in the 656 HVs were 305.4 ± 109.2 CARR U, 2696.3 ± 398.8 μmol/L, and 1.00 ± 0.34, respectively. d-ROMs and OSI levels in female volunteers were significantly greater than those in males (d-ROMs: 318.3 ± 52.6 CARR U vs. 287.4 ± 52.3 CARR U, $P < 0.001$; OSI: 1.04 ± 0.16 vs. 0.95 ± 0.18, $P < 0.001$). Levels of d-ROMs and OSI increased significantly with age ($P < 0.001$ and $P < 0.001$, respectively), while BAP levels decreased with age ($P = 0.06$). These age-related differences were not significantly affected by sex.

Table 2 compares the d-ROMs, BAP, and OSI results from the CFS patients with the age-matched HV3 group, and with the values from the HV2 group taken at rest after experiencing sub-acute fatigue for 2 weeks. d-ROMs and OSI levels were significantly higher in patients with CFS compared with the age-sex matched HV3 group. However, d-ROMs and OSI levels observed in workers who experi-

**Table 2**
Comparison in levels of d-ROMs, BAP, and OSI between patients with CFS and age-sex matched HV3 and workers (HV2), respectively.

| Variables | CFS patients ($n = 121$) Mean ± SD | Age-sex matched HVs (HV3; $n = 121$) Mean ± SD | Workers in post sub acute fatigue (HV2; $n = 24$) Mean ± SD | *P* value CFS vs. HV3 | *P* value CFS vs. HV2 |
|---|---|---|---|---|---|
| d-ROMs (CARR Unit) | 335.0 ± 86.1 | 301.8 ± 59.5 | 409.9 ± 67.0 | 0.001 | <0.001 |
| BAP (μmol/L) | 2538.1 ± 111.4 | 2712.1 ± 199.6 | 2527.8 ± 115.4 | <0.001 | 0.68 |
| OSI | 1.17 ± 0.29 | 0.98 ± 0.17 | 1.43 ± 0.23 | <0.001 | <0.001 |

HVs: healthy volunteers; OSI: oxidative stress index.

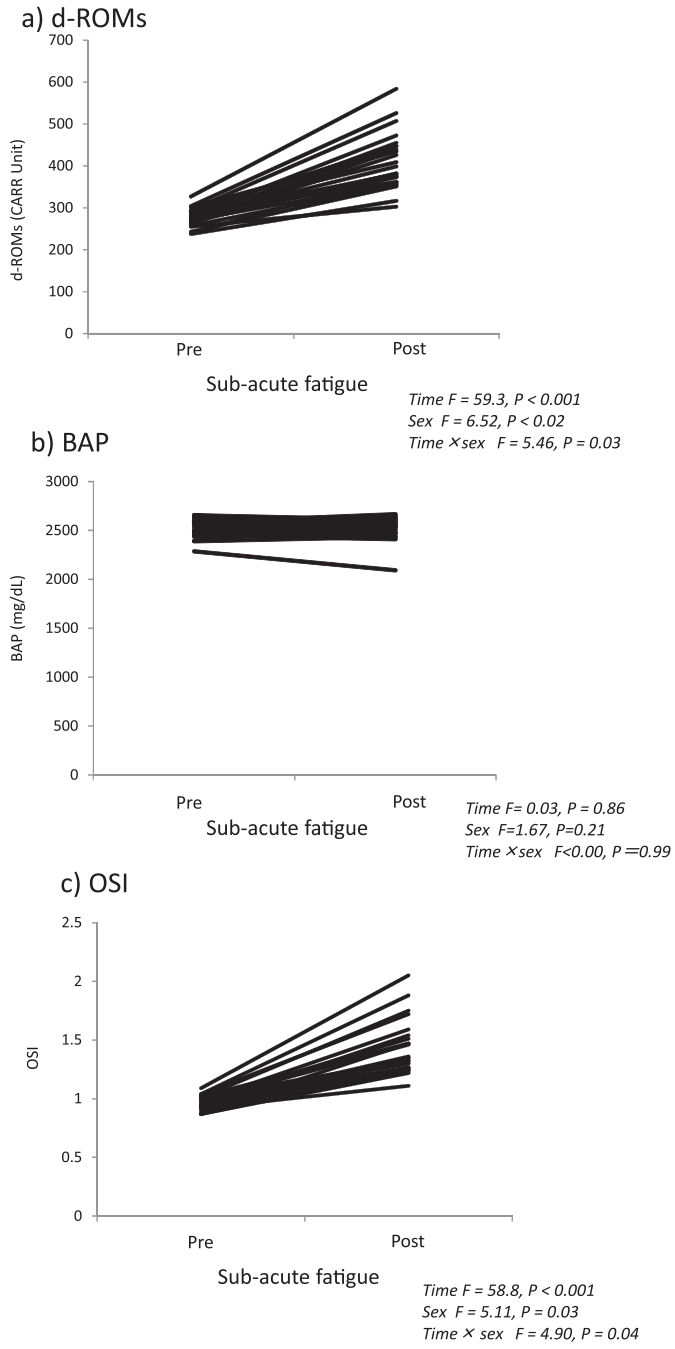


**Fig. 1.** Changes in levels of d-ROMs, BAP and OSI before and after the sub-acute fatigue condition. Sub-acute fatigue was generated by 2 weeks of overwork. *OSI*, oxidative stress index. n = 24.

enced sub-acute fatigue (HV2 group) were even higher than those from the CFS patients at rest. BAP values in CFS patients at rest were not significantly different from those taken from the HV2 group at rest after experiencing sub-acute fatigue, but were significantly less that those taken at rest from the age-sex matched HV3 group.

## 4. Discussion

After taking age into account, d-ROMs and OSI increased and BAP decreased after acute fatigue. After experiencing sub-acute fatigue (two weeks of overworking), d-ROM values increased, but BAP only increased in one case (taking age and sex into account). Patients with CFS showed higher d-ROMs and OSI values and lower BAP values at rest compared with age-sex matched controls. In contrast, when patients were compared with the workers who experienced sub-acute fatigue, d-ROMs and OSI values were higher in the workers. BAP values did not differ between CFS patients and those who experienced sub-acute fatigue, but compared with the patients, were significantly higher in age-sex matched controls. Taking antioxidants, medication, or being comorbid with FM or psychiatric diseases did not effect the values of oxidative markers in patients with CFS.

After the acute fatigue task, d-ROMs increased and BAP decreased compared with pre-task levels. The decrease of d-ROMs was shown two hours after the experiment (data were shown in Japanese paper). Under the acute fatigue condition, both oxidative stress and anti-oxidative activity simultaneously increased, then immediately recovered to prior levels within two hours. Changes in d-ROMs and BAP have been reported following a physical exercise load (Martarelli & Pompei, 2009; Martinović et al., 2011; Parker, McGuckin, & Leicht, 2014; Takahashi et al., 2013), and in animal models of fatigue (Piccione, Fazio, Casella, Pennisi, & Caola, 2011). Experimentally induced transient inflammation had no impact on mental arithmetic stress changes in plasma oxidative stress (Wadley et al., 2014). Therefore, our results might not be directly related to inflammation or abnormalities in immune function.

After the 2-week period of overwork that constituted the sub-acute fatigue condition, d-ROM values increased, while BAP values only decreased in one case. Under acute fatigue conditions, the increase of d-ROMs and BAP occurred at the same time and recovered to the original values within two hours as described above. The sub-acute fatigue condition lasted two weeks. Therefore, increased levels of BAP were shown in the workers. However, BAP levels in almost all workers returned to normal within 2 weeks. Only one worker showed decreased BAP levels after 2 weeks. Though we did not collect the recovery data, we speculate that BAP levels in this volunteer could not recover to prior levels because of an inhibited ability in BAP. Job stress or subjective stress levels have been frequently reported to increase various measures of oxidative stress (Casado et al., 2011; Irie et al., 2002; Ishihara et al., 2008; Takaki, 2013). Undergraduate students showed increased oxidative stress during and after examination periods (Eskiocak et al., 2005; Nakhaee et al., 2013; Sivonová et al., 2004). We identified the same tendency in d-ROMs and BAP, although fatigue loading and fatigue evaluation were achieved through different means.

**Table 3**
Summary of d-ROMs, BAP, and OSI values in HVs from the post-acute fatigue condition, post sub-acute fatigue condition, and at rest, and in patients with CFS at rest.

| Variables | CFS patients vs. Post acute fatigue condition in HV1 ($n = 12$) | CFS patients vs. Post sub-acute fatigue condition in HV2 ($n = 24$) | CFS patients vs. resting condition in age, sex-matched HV3 ($n = 121$) |
|---|---|---|---|
| d-ROMs | Equal but recovery within two hours | Lower | Higher |
| BAP | Equal but recovery within two hours | No change | Lower |
| OSI | Equal but recovery within two hours | Lower | Higher |

HVs: healthy volunteers; OSI: oxidative stress index.

**Table 4**
Summary of d-ROMs, BAP, and OSI values from HVs in the acute and sub-acute conditions.

| pre vs. post fatigue conditions | Acute fatigue condition in HVs ($n = 12$) | Sub-acute fatigue condition in HVs ($n = 24$) |
|---|---|---|
| d-ROMs | Increase | Increase |
| BAP | Increase | No change |
| OSI | Equal | Increase |

HVs: healthy volunteers; OSI: oxidative stress index.

d-ROM and OSI levels in patients with CFS were higher and BAP levels were lower than in age-sex matched controls. However, lower d-ROM and OSI levels were observed in patients compared with workers who experienced sub-acute fatigue. BAP in patients with CFS did not significantly differ from that found in the workers. Muscle markers of oxidative stress have been linked to the pathological mechanism of CFS (Morris & Maes, 2014) and blood markers of oxidative stress contribute to CFS pathology and symptoms (Fulles et al., 2000; Kennedy et al., 2005), particularly in response to exercise in patients with CFS (Nijs et al., 2014). Further, lowered antioxidant levels were reported in CFS, such as CoQ10 (Castro-Marrero et al., 2013).

Tables 3 and 4 show the summary of d-ROMs, BAP, and OSI levels obtained in this study. Values of dROMs and BAP from the post-acute condition in HVs were equal to the levels of patients with CFS, however these levels recovered within two hours (data were shown in Japanese paper). dROM values from HVs in the post sub-acute fatigue condition were lower than those from CFS patients at rest, while BAP values between these two groups were comparable. Patients with CFS showed higher levels of d-ROMs and OSI that did age-sex matched controls. Patients with CFS showed lower BAP levels compared with HVs at rest. d-ROMs increased after acute and sub-acute fatigue condition in healthy people. These results suggest that when using a combination of d-ROMs, BAP, and OSI, we can evaluate the different levels of fatigue or discriminate patients with CFS from healthy individuals who are experiencing different levels of fatigue.

We have made the differences in both oxidative stress and anti-oxidative activity clear in a relatively large sample of patients with CFS. The pathological mechanisms involved in CFS might be linked to inflammation and abnormalities of immune and sympathetic functions, as suggested by a recent review (Morris & Maes, 2014). Increased sympathetic activity after the acute fatigue task has been examined (Kuratsune et al., 2012). Heart rate variability in the high frequency band was significantly reduced and alpha levels of tumor necrosis factor were higher during a long-lasting stress period (sub-acute) compared with those observed at rest (Visnovcova et al., 2015). Patients with CFS have been shown to have abnormalities in sympathetic activity (Yamaguti, Tajima, & Kuratsune, 2013) and inflammation (Nakatomi et al., 2014) compared with HVs. The link between induction of inflammation and immune function, as well as between sympathetic function in patients with CFS compared with HVs at acute, sub-acute, and resting conditions might be shown in the study. However, as we have not simultaneously examined sympathetic function and immune activity, further studies are needed to clarify any linkage.

There were several limitations to the study. First, smoking and BMI are two risk factors known to influence levels of oxidative stress (Del Pozo-Cruz et al., 2014; Irie et al., 2002; Møller, Wallin, & Knudse, 1996) and antioxidant supplements might influence these values as well (Braakhuis, Hopkins, & Lowe, 2014). We did not perform analyses that considered these factors. However, HVs in this study were not taking any medications or antioxidant supplements, and we excluded participants who showed abnormally high BMI. Further, the levels of oxidative biomarkers were not shown to differ based on antioxidant supplements. This, we minimized the potential effects of these confounding factors. Second, we did not measure other oxidative stress biomarkers, such as hydroperoxides, which have been shown to be related to CFS and chronic fatigue in previous studies (Maes et al., 2011). Third, we could not follow the sub-acute fatigue sample group during the recovery period because they changed their work environment after finishing the installation of the computer system. Forth, we did not examine changes in oxidative stress biomarkers in the acute and sub-acute conditions in patients with CFS. A final limitation is that the durations of acute, sub-acute, and chronic fatigue were not clearly identified.

In summary, oxidative stress and anti-oxidative activity might be useful for discriminating acute, sub-acute, and resting fatigue in healthy people from patients with CFS by combining measures of d-ROMs and BAP or by evaluating the levels of fatigue in healthy people. Study results indicate that evaluating levels of fatigue using d-ROMs and BAP, combined with OSI might be useful for discriminating CFS from fatigue status (such as being overworked) in healthy people.

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