Biological Psychology 94 (2013) 527–533

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Oxidative stress is involved in fatigue induced by overnight deskwork as assessed by increase in plasma tocopherylhydroqinone and hydroxycholesterol

Mototada Shichiri[a,*,1], Nobuyoshi Harada[a,b,1], Noriko Ishida[a], Lilian Kaede Komaba[a], Sunao Iwaki[a], Yoshihisa Hagihara[a], Etsuo Niki[a], Yasukazu Yoshida[a]

[a] Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Osaka, Japan
[b] Flicker Health Management Corp., Osaka, Japan

ARTICLE INFO

Article history:
Received 1 February 2013
Received in revised form 24 June 2013
Accepted 2 October 2013
Available online 10 October 2013

Keywords:
Lipid peroxidation
Fatigue
Overnight deskwork
Critical flicker frequency
α-Tocopherylquinone

ABSTRACT

In this study, we examined the relationship between fatigue and plasma concentrations of antioxidants and lipid peroxidation products. Fourteen healthy volunteers performed overnight desk work for 18 h then took a nap for 4 h. Participants answered questionnaires of subjective symptoms of fatigue (QSSF) and completed a self-assessment of fatigue using a visual analog scale (VAS). At each test time, they underwent a critical flicker frequency (CFF) test and blood samples were collected. Plasma levels of α-tocopherol (αT) decreased and α-tocopherylquinone (αTQ), the oxidation product of αT, increased. The ratio of 7β-hydroxycholesterol (7β-OHCh), the oxidation product of cholesterol, against total cholesterol increased until the end of experiment. αTQ levels correlated with VAS and QSSF scores. The ratio of 7β-OHCh to total cholesterol and the value of CFF showed a significant correlation. From these results, plasma levels of αTQ and 7β-OHCh are useful and objective indicators of fatigue induced by overnight deskwork.

1. Introduction

It is considered that fatigue is one of the most important feelings in daily life because it is closely related to the quality of life. However, fatigue is usually assessed only subjectively and no gold standard for its objective measurement has been established.

In previous reports, subjective evaluation of fatigue was usually assessed using visual analog scale (VAS) score (Kos, Nagels, D'Hooghe, Duportail, & Kerckhofs, 2006) and self report questionnaires (Yoshihara, Yamanaka, & Kawakami, 2009). Subjective evaluations of fatigue have many advantages as their simple operation and low costs but they have disadvantages such as difficulty in standardization.

Heart function (Hartley, Arnold, Smythe, & Hansen, 1994) and brain function (Kaseda, Jiang, Kurokawa, Mimori, & Nakamura, 1998; Suda et al., 2009) have been studied as correlates of fatigue in the search for objective biological indices of fatigue. Another objective index has been proposed; the critical flicker frequency (CFF) which is the level of individual sensitivity at the beginning of light flickering, caused by changes in the frequency of light flashes (Simonson & Brozek, 1952). The descending threshold is measured as the highest frequency of flashing light when the flicker is perceived. This is an accepted indicator of fatigue caused by the workload in many different types of occupations (Luczak & Sobolewski, 2005). Some reports used CFF as objective indicator of mental fatigue (Marziale & Rozestraten, 1995).

Nozaki et al. reported fatigue-related biochemical alterations after fatigue-inducing mental and physical sessions (Nozaki et al., 2009). They evaluated 40 biomarkers. But, none of these proved a key indicator for the evaluation of fatigue.

On the other hand, it is reported that strong exercise and various stresses that cause fatigue increase oxidative stress (Maes, Kubera, Obuchowiczwa, Goehler, & Brzeszcz, 2011; Siktar et al., 2011). We have proposed that the levels of hydroxyoctadecadienoic acid (HODE) and 7β-hydroxycholesterol (7β-OHCh) are potential biomarkers of the oxidative stress status (Kitano, Yoshida, Kawano, Hibi, & Niki, 2007; Shichiri et al., 2011; Yoshida, Yoshikawa, Kinumi, Imai, & Niki, 2009; Yoshida et al., 2010). HODE and 7β-OHCh are generated from linoleic acid and cholesterol, respectively.

The antioxidant α-tocopherol (αT) and coenzyme Q_{10} (CoQ_{10}) are able to prevent lipid peroxidation and we have previously

Abbreviations: CFF, critical flicker frequency; QSSF, questionnaires of subjective symptoms of fatigue; VAS, visual analog scale; αTα, -tocopherol; αTQ, α-tocopherylquinone; UQ_{10}, ubiquinone-10; $UQ_{10}H_2$, ubiquinol-10; CoQ_{10}, coenzyme Q_{10}; 7β-OHCh, 7β-hydroxycholesterol; HODE, hydroxyoctadecadienoic acid.

* Corresponding author. Tel.: +81 72 751 8734; fax: +81 72 751 9517.
E-mail address: mototada-shichiri@aist.go.jp (M. Shichiri).

[1] These authors are contributed equally to this work.

0301-0511/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.biopsycho.2013.10.002

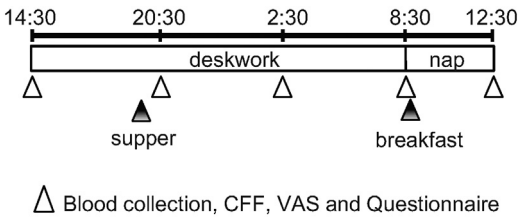

Fig. 1. Investigation protocol.

reported that negative correlations were observed between the level of total HODE (tHODE) and the concentration of αT and CoQ_9 in mouse liver (Yoshida, Hayakawa, Habuchi, & Niki, 2006). Miwa et al. reported that plasma αT level of chronic fatigue syndrome patients was lower than control subjects (Miwa & Fujita, 2010). Maes et al. reported that plasma CoQ_{10} was significantly lower in chronic fatigue syndrome patients (Maes et al., 2009a) and in depressed patients (Maes et al., 2009b). There is a possibility that reduction of plasma levels of αT and CoQ_{10} increases oxidative stress.

In the present study, we examined the relationship between fatigue and oxidative stress. Healthy volunteers performed overnight deskwork and the change of fatigue level was evaluated by VAS score, questionnaire of subjective symptoms of fatigue (QSSF) and CFF. We measured the plasma levels of HODE and 7β-OHCh. We also measured plasma concentrations of the reduced form and oxidized forms of antioxidant, including αT; α-tocopherylquinone (αTQ), the oxidation product of αT; ubiquinone-10 (UQ_{10}) the oxidized form of CoQ_{10}; and ubiquinol-10 ($UQ_{10}H_2$), the reduced form of UQ_{10}. The correlation between the above indicators of fatigue and plasma HODE, 7β-OHCh, αT and CoQ levels were analyzed.

2. Methods

2.1. Participants

A total of 14 males, aged 32.9 ± 10.8 years old were invited to participate in the present study. Written informed consent was obtained from all subjects after the procedures had been fully explained. All subjects were non-smokers, in good physical health taking no medication, and had no history of psychiatric or somatic diseases. The present protocol was approved by the committee for the ethics on the experiments with human derivative samples and the committee for ergonomic experiments of AIST, Japan.

2.2. Study design

In the present study, participants did deskwork by staying up all night. The experiment started at 14:30, and deskwork was done until 8:30 of the following day. From 8:30 to 12:30, they took naps up to 4-h in individual rooms. Most of their time was spent in desk working and they engaged in no hard physical activities. Supper was taken at 19:30 and breakfast was taken at 8:30.

Fig. 1 shows the schedule of this experiment. At 14:30, 20:30, 2:30, 8:30 and 12:30, participants were requested to answer a questionnaire concerning their subjective feelings regarding fatigue and instructed to note their self-assessment of fatigue using a visual analog scale. At the same time, they underwent a critical flicker frequency (CFF) test, and blood samples were collected.

2.3. Measurements

To evaluate the subjective symptoms of fatigue, a Questionnaire of Subjective Symptoms of Fatigue (QSSF) "Jikaku-sho shirabe" was used, based on the Industrial Fatigue Research Committee of the Japan Occupational Health in 2002 (Takeyama et al., 2009). This questionnaire consists of 25 subjective fatigue symptom items that are categorized into 5 factors: feeling of drowsiness (Factor I), feeling of instability (Factor II), feeling of uneasiness (Factor III), feeling of local pain or dullness (Factor IV), and feeling of eyestrain (Factor V). For each item, respondents are requested to estimate the intensity of the feelings as "Disagree completely," "Agree scarcely," "Agree slightly," "Agree considerably," and "Agree strongly." These five intensities were assigned scores of 1–5 points, respectively.

Self-assessment of fatigue was assessed using a visual analog scale (VAS). VAS scores was indicated from 0 to 10, with 0 = no fatigue and 10 = strong fatigue.

The critical flicker frequency (CFF) was successively measured five times in descending order using a Roken Digital Flicker (Model RDF-1: Shibata Co., Ltd., Tokyo) and the mean value was obtained.

2.4. Materials

13-hydroxy-9Z,11E-octadecadienoic acid (13-(Z,E)-HODE), 9-hydroxy-10E,12Z-octadecadienoic acid (9-(E,Z)-HODE), and 13S-hydroxy-10E,12Z-octadecadienoic-9,10,12,13-d4 acid (13-HODE-d4) were obtained from Cayman Chemical (Ann Arbor, MI, USA). 9-Hydroxy-10E,12E-octadecadienoic acid (9-(E,E)-HODE), 13-hydroxy-9E,11E-octadecadienoic acid (13-(E,E)-HODE), 10-hydroxy-8E,12Z-octadecadienoic acid (10-(Z,E) -HODE), and 12-hydroxy-9Z,13E-octadecadienoic acid (12-(Z,E)-HODE) were obtained from Larodan Fine Chemicals (Malmö, Sweden). 7β-OHCh were obtained from Steroids (New Port, RI, USA), and its isotope 7β-hydroxycholesterol-25,26,26,26,27,27,27-d7 (7β-OHCh-d7) were obtained from Medical Isotopes (Pelham, NH, USA). The standard of α-tocopherol was kindly supplied by Eisai Co., Ltd. (Tokyo, Japan). The standard of α-tocopherylquinone was prepared by oxidation of α-tocopherol with copper sulfate. Other materials were of the highest grade available commercially.

2.5. Plasma sample processing

Plasma was obtained by centrifugation at 830 × *g* for 5 min at 4 °C and immediately subjected to analysis. The plasma samples were processed for analysis by a reduction and saponification using the previously reported method (Yoshida, Kodai, Takemura, Minamiyama, & Niki, 2008). The plasma (200 μl) was mixed with 300 μl of saline. Subsequently, 500 μl of methanol containing internal standards, 13-HODE-d4 (50 ng), 7β-OHCh-d7 (19 ng), 16-hydroxyhexadecanoic acid (70 ng) and 100 μM butylated hydroxytoluene was added to the samples. This was followed by the reduction of hydroperoxides and ketones using 1 mM triphenylphosphine (Sigma–Aldrich) at room temperature for 30 min. The reduced sample was then mixed with 1 M KOH in methanol (500 μl) under nitrogen and incubated on a shaker for 30 min in the dark at 40 °C. The mixture was cooled on ice and acidified with 10% (v/v) acetic acid in water (2 ml), then extracted with chloroform/ethyl acetate (4:1, v/v, 5 ml). The sample was mixed using a vortex mixer for 1 min and centrifuged at 1500 × *g* for 5 min at 4 °C. The chloroform/ethyl acetate layer was concentrated to approximately 1 ml after the removal of the water layer and was divided equally into two portions, a sample for liquid chromatography–mass spectrometry (LC–MS/MS) and a sample for gas chromatography mass spectrometry (GC–MS).

2.6. Analysis of lipid peroxidation products by LC–MS/MS and GC–MS

The levels of t7-OHCh, total cholesterol (tCh), and total linoleic acid were measured by GC–MS and the levels of HODE were measured by LC–MS/MS using a previously reported method (Yoshida, Kodai, Takemura, Minamiyama, & Niki, 2008). The divided chloroform/ethyl acetate solution was evaporated to dryness under nitrogen.

A silylating agent, *N,O* bis(trimethylsilyl)-trifluoroacetamide (30 μl), was added to the dried residue. The solution was mixed vigorously by vortexing for 0.5 min and incubated for 60 min at 60 °C to obtain trimethylsilyl esters and ethers. An aliquot of this sample was injected into a gas chromatograph (GC 6890N, Agilent Technologies, Palo Alto, CA, USA) that was equipped with a quadrupole mass spectrometer (5973 Network, Agilent Technologies). A fused-silica capillary column (HP-5MS, 5% phenyl methyl siloxane, 30 mm × 0.25 mm, Agilent Technologies) was used. Helium was used as the carrier gas at a flow rate of 1.2 ml/min. Temperature programming was carried out from 60 °C to 280 °C at 10 °C/min. The injector temperature was set at 250 °C, and the temperatures of the transfer line to the mass detector and ion source were 250 °C and 230 °C, respectively. Electron energy was set at 70 eV. 7β-OHCh, Ch, and linoleic acid were identified on the basis of their retention times and mass patterns; ions having $m/z = 456$ for 7β-OHCh, $m/z = 458$ for Ch, and $m/z = 337$ for linoleic acid were selected for the quantification. 7β-OHCh and Ch were identified quantitatively using 7β-OHCh-d7 as internal standard and linoleic acid was quantified using 16-hydroxyhexadecanoic acid as an internal standard.

The other portion of the chloroform/ethyl acetate solution was also evaporated to dryness under nitrogen. The sample was reconstituted with methanol/water (70:30, v/v, 200 μl), and a portion of the sample (10 μl) was subjected to the LC-MS/MS analysis. LC was carried out on an octadecylsilane (ODS) column (Hypersil Gold, 3.0 μm, 100 × 2.1 mm, Thermo Fisher Scientific, Fremont, MA, USA) in a column oven (CTO-20A, Shimadzu, Kyoto, Japan) set at 40 °C. The LC apparatus consisted of an autosampler (SIL-20AC, Shimadzu, Kyoto, Japan), pump and mixer (LC-20AB, Shimadzu, Kyoto, Japan). The eluent condition was a gradient comprising solvent A (2 mM ammonium acetate in water) and solvent B (methanol/acetonitrile, 5:95) at a flow rate of 0.2 ml/min. The initial composition of the gradient was 80% A and 20% B. It was holded for 2 min, and the composition was changed to 50% A and 50% B after 45 min. MS was carried out using a Thermo Finnigan TSQ Quantum Discovery Max, a triple quadrupole mass spectrometer (Thermo Fisher Scientific, MA, USA) fitted with an electrospray ionization (ESI) source. ESI was carried out at a needle voltage of 4.2 kV. Nitrogen was used as the sheath gas (17 psi) and auxiliary gas (12 units). The capillary was heated to 280 °C, and the mass spectrometers were optimized to obtain the maximum sensitivity. A specific precursor-to-product ion transition was