Yonago Acta Medica 2017;60:86-93

Original Article

# Relationship between Oxidative Stress, Physical Activity, and Vitamin Intake in Patients with Asthma

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#### **ABSTRACT**

**Background** Oxidative stress plays an important role in the pathogenesis of bronchial asthma. Antioxidant nutrition and supplementation have been used to reduce oxidative stress. However, a clinical trial with antioxidant supplementation showed no beneficial effects in patients with asthma. On the other hand, physical activity is related to the prognosis of chronic obstructive pulmonary disease (COPD) and is also related to oxidant status. We investigated the relationships between oxidative stress, serum levels of vitamins, dietary vitamin intake, daily activities, and pulmonary functions in patients with asthma.

**Methods** Eighteen patients with bronchial asthma were enrolled in this study. Reactive oxidative stress was assessed by measuring organic hydroperoxides (diacron reactive oxygen metabolites: dROM) in sera and by measuring H<sub>2</sub>O<sub>2</sub> levels in exhaled breath condensates. The biological antioxidant capacity in serum was evaluated by measuring antioxidant potential capacity against ferric ion. We also assessed pulmonary functions, fraction of exhaled nitric oxide, serum levels of vitamins, dietary vitamin intake, and physical activities.

**Results** There were no relationships between the index of oxidative stress (dROM and  $H_2O_2$  in exhaled breathe condensates) and pulmonary functions, serum levels of vitamins, daily vitamin intakes, and activity levels in patients with asthma.

**Conclusion** The status of transient oxidative stress may not be related to daily activities, vitamin levels, and pulmonary functions in patients with asthma in a real-life setting. However, our results were obtained in

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Received 2017 January 30

Accepted 2017 March 22

Abbreviations: BAP, biological antioxidant potential; BDHQ, brief self-administered diet history questionnaire; COPD, chronic obstructive pulmonary disease; dROM, diacron reactive oxygen metabolite; EBC, exhaled breath condensate; EIB, exercise induced bronchoconstriction; FeNO, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; VC, vital capacity

the short-term period from a small number of subjects, so a large longitudinal study is required to ascertain the relationships between oxidative stress, physical activity and vitamin intake in patients with asthma.

**Key words** asthma; exhaled breath condensate; oxidative stress; vitamin

Oxidative stress is one of the risk factors for developing and for exacerbating atherosclerosis, cardiovascular disease, and respiratory diseases, such as chronic obstructive disease (COPD) and bronchial asthma.<sup>1-4</sup> In the pathogenesis of asthma, eosinophilic airway inflammation induces asthmatic symptoms and oxidative stress. Since eosinophils contain nicotinamide adenine dinucleotide phosphate oxidase, eosinophilic peroxidase, and nitric oxide synthesis, reactive oxygen species can be generated in the asthmatic airway.<sup>5, 6</sup> A recent study in a mouse model showed that house dust mites increase oxidative stress.<sup>7</sup> Furthermore, environmental stimuli, such as diesel exhaust particles, cigarette smoke, ozone, and viral infections, can result in increased reactive oxygen species.<sup>8-11</sup> Oxidative stress generated in the airway of patients with asthma causes epithelial cell injury. Furthermore, reactive oxygen induces transforming growth factor-β1 secretion from epithelial cells, 12 which may induce airway remodeling in asthma. Therefore, antioxidants may be effective in the treatment of asthma.<sup>13</sup>

The therapeutic strategies for protecting against oxidative stress are intake of antioxidants, such as dietary and vitamin supplementation (nutritional antioxidants), and chemical compounds or minerals, including N-acetyl cysteine, <sup>14, 15</sup> selenium, magnesium, and zinc. <sup>16</sup> However, there are no clinical trial data suggesting that antioxidants prevent asthma development. Furthermore, a few small clinical trials showed either improved asthma symptoms or pulmonary function. <sup>16–18</sup>

It has been reported that the level of physical activity is the one of the predictors of mortality in COPD patients. <sup>19, 20</sup> Waschki et al reported that the level of physical activity is the strongest predictor compared to other established predictors, such as the ADO (age, dyspnea,

airflow obstruction) index, the BODE (BMI, airway obstruction, dyspnea, and exercise capacity) index, steps per day, forced expiratory volume in 1 second (FEV<sub>1</sub>)% predicted, and six-minute walk distance. 19 Although physical activity has not been reported to improve asthmatic symptoms, exercise improved asthmatic symptoms in adults and children.<sup>21-23</sup> Furthermore, exercise reduced the antioxidant status in asthmatic children.<sup>24</sup> However, it has been reported that exercise induces oxidative stress in humans.<sup>25,26</sup> A few clinical trials on exercise-induced bronchoconstriction (EIB) found that some natural antioxidants, such as ascorbic acid,  $\alpha$ -tocopherol, and lycopene, improved both asthma symptoms and pulmonary function.<sup>27–29</sup> In summary, these studies on the relationship between physical activity, asthma, and oxidative stress are inconclusive.

In this study, we studied the correlations among oxidative stress, serum antioxidant levels, dietary intake of antioxidants, pulmonary function, and daily activities in patients with asthma in a real-life setting.

#### **SUBJECTS AND METHODS**

Eighteen patients with bronchial asthma were enrolled in this study. Written informed consent was obtained from all of the patients. The diagnosis of asthma was made by respiratory physicians according to the American Society Criteria.<sup>30</sup> No patient experienced deterioration of asthma symptoms from four weeks prior to enrollment of this study.

### Assessment of oxidative stress and antioxidant potential in peripheral blood

Peripheral blood samples were collected from all patients on the first day of the enrollment of this study and stored at -20 °C until further analysis. Oxidative stress was assessed by a radical analyzer system (FREE Carpe Diem; Wismerll, Tokyo, Japan) according to the manufacturer's instructions. In this study, we assessed oxidative stress by measuring the diacron reactive oxygen metabolite (dROM) which reflects organic hydroperoxides formed by oxidative stress. The results are expressed as U.Carr. One unit of U.Carr equals 0.8 mg/L of hydrogen peroxide in the serum.<sup>31</sup> We also assessed the biological antioxidant potential (BAP). The BAP test reflects the antioxidant capacity in the serum by measuring the potential capacity against ferric ions.

### Assessment of oxidative stress in exhaled breath condensates

All subjects breathed into an EcoScreen (Jaeger, Wurzburg, Germany) for 10 to 15 minutes to collect exhaled breath condensate (EBC) on the first day of the enroll-

ment of this study. The EBCs were stored at -20 °C until analyzed. The  $H_2O_2$  level in the EBCs was measured by a radical analyzer system (FREE Carpe Diem; Wismerll).

#### Measurement of respiratory functions

On the first day of the enrollment in this study, all patients were asked to perform a pulmonary function test with a Chestac 33 spirometer (Chest, Tokyo, Japan). Forced vital capacity (FVC) and  $FEV_1$  were recorded from a minimum of three pulmonary function tests. Percent (%) of vital capacity (VC) is the VC expressed as a percentage of the predicted values  $^{32}$  and  $\%FEV_1$  is the  $FEV_1$  expressed as a percentage of the predicted values. $^{33}$ 

# Measurement of fractional exhaled nitric oxide (FeNO)

The FeNO levels were measured using the Aerocrine NIOX MINO, a portable, hand-held NO analyzer (Aerocrine AB, Solna, Sweden). In each measurement, participants were tested in a seated position without a nose clip with a constant flow rate of 50 mL/s. The measurement was performed once with each subject on the first day of enrollment in this study.

#### Measurement of serum vitamin levels

Serum vitamin levels were measured in each patient on the first day of enrollment in this study. Fractions of vitamin E ( $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol), and the levels of retinol, and vitamin C were measured by high performance liquid chromatography and 1,25 (OH)<sub>2</sub> vitamin D was measured by radioimmunoassay. All vitamin levels in the serum were measured at SRL Inc. (Hachioji, Tokyo, Japan).

# Estimation of vitamin intake by a brief self-administered diet history questionnaire (BDHQ)

The intake of retinol,  $\beta$ -carotene, vitamin C, vitamin D,  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol, and cryptoxanthin was assessed based on results of a brief self-administered diet history questionnaire (BDHQ) version 1-1. The BDHQ used in this study was written in Japanese and supplied from EBNJAPAN (Tokyo, Japan). The questions in the BDHQ included 58 on both food and beverage intake, and the frequency of food and beverage intake for the previous month was also included.

#### Assessing activity by actigraphy

The actigraph accelerometer is worn like a light-weight wrist watch (Actiwatch 2; Philips Respironics, Murrysville, PA). Actiwatch records a digitally-integrated mea-

Table 1. Characteristics of the patients Number of patients, n 18 Age, years, mean ± SD  $63.3 \pm 11.2$ Sex, n (%) Male 8 (44.4) Female 10 (55.6) Smoking status, n (%) Current 0(0)Ex-smoker 10 (55.6) Non-smoker 8 (44.4)  $FEV_1/FVC$ , %, mean  $\pm SD$  $70.7 \pm 9.8$ %FEV<sub>1</sub> predicted, %, mean  $\pm$  SD  $103.6 \pm 19.7$ %VC predicted, %, mean ± SD  $111.7 \pm 16.8$ FeNO (ppb), mean ± SD  $32.4 \pm 18.3$ 

FeNO, fractional exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; VC vital capacity.

Table 2. Actigraphy data	
Activity counts (24 h total), $\times$ 10 <sup>4</sup> , mean $\pm$ SD	$260 \pm 124$
Activity counts per minutes, mean ± SD	$283.3 \pm 81.1$

sure of gross motor activity, which can be used to quantitate rest and activity counts. The patients were an actigraph for seven consecutive days, and the activity counts (AC) were collected. We defined 60 seconds as 1 epoch. Total activity counts were calculated by the following formula: total activity counts =  $0.04E_{-2} + 0.020E_{-1} + E + 0.2E_{+1} + 0.04E_{+2}$ . E = activity counts during a scored epoch. En = activity counts during previous or successive epochs. We assessed total activity counts and total activity per minute according to the Actiwatch 5 software.

#### Statistical analysis

All the data were expressed as mean  $\pm$  SD. The correlations in the selected data sets were analyzed by Spearman's correlation coefficients. GraphPad Prism 5 (GraphPad Software, San Diego, CA) was used for all statistical analyses.

This study was approved by the Ethics Committee of Tottori University (#1974) and was conducted according to the principles of the Declaration of Helsinki.

#### **RESULTS**

#### Characteristics of the patients

In total, 18 patients were enrolled in this study. The average age of the patients was 63.3 years, and included eight

Table 3. Oxidative and antioxidative markers in patients with asthma

Oxidative and antioxidative markers	Patients $(n = 18)$	Normal range
dROM (U.Carr), mean ± SD	$333.9 \pm 49.7$	< 300
BAP ( $\mu$ mol/L), mean $\pm$ SD	2363.2 ± 290.7	> 2200
$H_2O_2$ in EBC ( $\mu$ mol/L), mean $\pm$ SD	$0.6 \pm 0.3$	$0.23 \pm 0.03*$

<sup>\*</sup>Adapted from reference 43.

BAP, biological antioxidant potential; dROM, diacron reactive oxygen metabolite; EBC, exhaled breath condensate.

Table 4. Serum vitamin levels in patients with asthma

Serum vitamin levels	Patients $(n = 18)$	Normal range
Retinol (IU/dL)	$156.1 \pm 44.4$	97–316
Vitamin C (μg/mL)	$9.8 \pm 3.6$	5.5-16.8
1, 25 (OH) <sub>2</sub> Vitamin D (pg/mL)	$59.1 \pm 15.6$	20.0-60.0
Vitamin E fraction		
α-tocopherol (mg/dL)	$1.3 \pm 0.3$	0.49-1.09
β-tocopherol (mg/dL)	$0.02 \pm 0.006$	< 0.02
γ-tocopherol (mg/dL)	$0.09 \pm 0.05$	0.05-0.17
δ-tocopherol (mg/dL)	Below detection limit	< 0.01

males and 10 females of which 10 were ex-smokers and eight were non-smokers. The mean percentage of  $FEV_1/FVC$  was 70.7%, and the mean %FEV<sub>1</sub> was 103.6%. The mean FeNO level was 32.4 ppb. (Table 1).

#### **Actigraphy**

The average of total activity counts in 24 hours was 260  $\times$  10<sup>4</sup> counts. The activity counts per minutes were 283.3 counts (Table 2).

### Oxidative and antioxidative markers in patients with asthma

The average dROM level, an oxidant marker, was 333.9 U.Carr. The average BAP level, another antioxidant marker, was 2363.2 mmol/L. The average  $H_2O_2$  in the exhaled breath condensates was 0.6 mmol/L (Table 3).

#### Serum vitamin levels in patients with asthma

Several vitamins with antioxidant capacity were measured. The average level of retinol was 156.1 IU/L, vitamin C was 9.8 µg/mL, 1,25 (OH)<sub>2</sub> was 59.1 pg/mL. Among the fraction of vitamin E, the average level of  $\alpha$ -tocopherol was 1.3 mg/dL,  $\beta$ -tocopherol was 0.02 mg/dL, and  $\gamma$ -tocopherol was 0.09 mg/dL. The level of  $\delta$ -tocopherol was below the detection limit (Table 4).

Table 5. Daily vitamin and provitamin intake estimated by BDHQ

Retinol (µg)	$349.6 \pm 290.0$
$\beta$ -carotene ( $\mu g$ )	$5336.8 \pm 3416.4$
Vitamin C (mg)	$144.8 \pm 81.1$
Vitamin D (µg)	$15.5 \pm 11.8$
Vitamin E fraction	
α-tocopherol (mg)	$7.8 \pm 3.0$
β-tocopherol (mg)	$0.4 \pm 0.1$
γ-tocopherol (mg)	$12.1 \pm 3.9$
δ-tocopherol (mg)	$3.3 \pm 1.3$
Cryptoxanthin (µg)	$381.9 \pm 289.8$

BDHQ, brief self-administered diet history questionnaire.

Table 6. Relationships between dROM, BAP and H<sub>2</sub>O<sub>2</sub> in EBC

Spearman	r	P-value
BAP and dROM	-0.2666	0.2848
H <sub>2</sub> O <sub>2</sub> and dROM	-0.3245	0.1888
H <sub>2</sub> O <sub>2</sub> and BAP	-0.4564	0.0569

BAP, biological antioxidant potential; dROM, diacron reactive oxygen metabolite; EBC, exhaled breath condensate.

#### Daily vitamin intake estimated by BDHQ

The daily vitamin intake was estimated from the BDHQ. The average daily intake of retinol was 349.6  $\mu$ g,  $\beta$ -carotene was 5336.8  $\mu$ g, vitamin C was 144.8 mg, vitamin D was 15.5  $\mu$ g,  $\alpha$ -tocopherol was 7.8 mg,  $\beta$ -tocopherol was 0.4 mg,  $\gamma$ -tocopherol was 12.1 mg,  $\delta$ -tocopherol was 3.3 mg, and cryptoxanthin was 381.9  $\mu$ g (Table 5).

### Relationships between dROM, BAP, and H<sub>2</sub>O<sub>2</sub> in EBC

The correlations of dROM, BAP, and H<sub>2</sub>O<sub>2</sub> in EBC were analyzed. There were no significant correlations between either dROM and BAP, dROM and H<sub>2</sub>O<sub>2</sub> in EBC, or BAP and H<sub>2</sub>O<sub>2</sub> (Table 6).

### Relationships between dROM and pulmonary function, vitamin levels, and actigraphy data

We examined the relationships between dROM and other parameters tested in this study. We could not find any significant correlation between dROM and either pulmonary function, serum levels of vitamins, daily dietary intake of vitamins, or activity counts (Table 7).

Table 7. Relationships between dROM and pulmonary function, vitamin levels and activity counts

		Spearman	
		r	P-value
Pulmonary	FEV <sub>1</sub> /FVC	0.08983	0.732
function	%FEV <sub>1</sub> predicted	0.3283	0.1834
	%VC predicted	0.253	0.3112
Serum level	Retinol	0.2024	0.4206
	Vitamin C	0.04134	0.8706
	1,25 (OH) <sub>2</sub>	-0.1033	0.6835
	Vitamin E	-0.1177	0.6418
	$\alpha$ -tocopherol	0.01809	0.9432
	β-tocopherol	0.006814	0.9786
	γ-tocopherol	0.06625	0.7939
Daily dietary intake	Retinol	0.2024	0.4206
	$\beta$ -carotene	-0.1972	0.4328
	Vitamin C	-0.1074	0.6715
	Vitamin D	-0.3191	0.1969
	α-tocopherol	-0.3418	0.1651
	β-tocopherol	-0.2425	0.3323
	γ-tocopherol	-0.3717	0.1288
	δ-tocopherol	-0.3573	0.1456
	Cryptoxanthin	0.06505	0.7976
Actigraphy	Total activity counts	0.07537	0.7663
date	Activity counts/min	0.3748	0.1254

dROM, diacron reactive oxygen metabolite; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; VC vital capacity.

# Relationships between BAP and pulmonary function, vitamin levels, and actigraphy data

We next examined the relationships between BAP and additional parameters. In these comparisons, we could not find any significant correlation between BAP and either pulmonary function, serum levels of vitamins, daily dietary intake of vitamins, or activity counts (Table 8).

## Relationships between H<sub>2</sub>O<sub>2</sub> in EBC and pulmonary function, vitamin levels, and actigraphy data

We also examined the relationships between  $H_2O_2$  in EBC and other parameters tested in this study. In these comparisons, we could not find any significant correlation between  $H_2O_2$  in EBC and either pulmonary function, serum levels of vitamins, daily dietary intake of vitamins, or activity counts (Table 9).

### Relationships between activity counts and pulmonary function

Lastly, we examined the relationships between activity

Table 8. Relationships between BAP and pulmonary function, vitamin levels and activity counts

		Spearman	
		r	P-value
Pulmonary	FEV <sub>1</sub> /FVC	-0.2941	0.2361
function	%FEV <sub>1</sub> predicted	-0.0774	0.7602
	%VC predicted	0.02993	0.9062
Serum level	Retinol	-0.08678	0.7321
	Vitamin C	0.2769	0.266
	1,25 (OH) <sub>2</sub>	0.08772	0.7293
	Vitamin E	-0.1476	0.559
	$\alpha$ -tocopherol	-0.1198	0.6358
	$\beta$ -tocopherol	0.05572	0.8262
	γ-tocopherol	0.02816	0.9117
Daily dietary intake	Retinol	0.06502	0.7977
	β-carotene	-0.1073	0.6717
	Vitamin C	-0.1084	0.6687
	Vitamin D	-0.2157	0.39
	α-tocopherol	-0.195	0.438
	β-tocopherol	0.05572	0.8262
	γ-tocopherol	-0.02222	0.9303
	δ-tocopherol	-0.1078	0.6703
	Cryptoxanthin	0.1199	0.6355
Actigraphy	Total activity counts	0.1269	0.6157
date	Activity counts/min	0.01342	0.9579

BAP, biological antioxidant potential;  $FEV_1$ , forced expiratory volume in 1 second; FVC, forced vital capacity; VC vital capacity.

counts and pulmonary function. Total activity counts were not correlated with pulmonary function. Activity counts per minute were significantly correlated with FEV<sub>1</sub>/FVC. However, activity counts per minute were not correlated with percent FEV<sub>1</sub> nor percent VC (Table 10).

#### DISCUSSION

In this study, we assessed the correlations among oxidative stress, antioxidant capacity, dietary intake of antioxidants, pulmonary functions, and daily activities in patients with asthma in a real-life setting. We found no significant relationships between the index of oxidative stress (dROM and H<sub>2</sub>O<sub>2</sub> in exhaled breathe condensates) and pulmonary functions, levels of vitamin in serum, daily vitamin intakes, and activity counts in asthmatic patients. Our data suggest that oxidative stress is not affected by daily activity, intake of antioxidants, and pulmonary function in patients with asthma in a real-life setting.

We measured the dROM and BAP in sera and H<sub>2</sub>O<sub>2</sub>

Table 9. Relationships between H<sub>2</sub>O<sub>2</sub> in EBC and pulmonary function, vitamin levels and activity counts

		Spea	Spearman	
		r	P-value	
Pulmonary	FEV <sub>1</sub> /FVC	0.08983	0.732	
function	%FEV <sub>1</sub> predicted	0.3283	0.1834	
	%VC predicted	0.253	0.3112	
Serum level	Retinol	0.2024	0.4206	
	Vitamin C	0.04134	0.8706	
	1,25 (OH) <sub>2</sub>	-0.1033	0.6835	
	Vitamin E	-0.1177	0.6418	
	α-tocopherol	0.01809	0.9432	
	β-tocopherol	0.006814	0.9786	
	γ-tocopherol	0.06625	0.7939	
Daily dietary intake	Retinol	0.2024	0.4206	
	β-carotene	-0.1972	0.4328	
	Vitamin C	-0.1074	0.6715	
	Vitamin D	-0.3191	0.1969	
	α-tocopherol	-0.3418	0.1651	
	β-tocopherol	-0.2425	0.3323	
	γ-tocopherol	-0.3717	0.1288	
	δ-tocopherol	-0.3573	0.1456	
	Cryptoxanthin	0.06505	0.7976	
Actigraphy	Total activity counts	0.07537	0.7663	
date	Activity counts/min	0.3748	0.1254	

EBC, exhaled breath condensate;  $FEV_1$ , forced expiratory volume in 1 second; FVC, forced vital capacity; VC vital capacity.

Table 10. Relationships between activity counts and pulmonary function

Total activity counts		Spec	Spearman	
		r	P-value	
Pulmonary	FEV <sub>1</sub> /FVC	0.3829	0.1168	
function	%FEV <sub>1</sub> predicted	-0.08978	0.7231	
	%VC predicted	0.1620	0.5270	

Activity counts/min		Spe	Spearman	
Activity count	counts/min r		<i>P</i> –value	
Pulmonary	FEV <sub>1</sub> /FVC	0.6017	0.0083	
function	%FEV <sub>1</sub> predicted	0.4118	0.0895	
	%VC predicted	0.2963	0.2798	

FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; VC, vital capacity.

in exhaled breath condensates. These parameters can be measured from a small volume of either blood samples (dROM and BAP) or exhaled breath condensates (H<sub>2</sub>O<sub>2</sub>)

and can be evaluated for oxidative status. The level of dROM has been measured in various diseases.<sup>34–37</sup> The international observational oxidative stress classified dROM levels into six stress levels as follows: Normal: < 300 U.Carr, Borderline: 301-320 U.Carr, Low oxidative stress: 321-340 U.Carr, Middle oxidative stress: 341-400 U.Carr, High oxidative stress: 401-500 U.Carr, and Very high oxidative stress: > 501 U.Carr.<sup>38</sup> In our study, the average level of dROM was 333.9 U.Carr, which is classified as low oxidative stress. Suzuki et al. reported that the dROM level was constant in the stable condition and increased during acute exacerbation of asthma.<sup>39</sup> They reported that the dROM level was 268.2 U.Carr in eleven stable asthmatic patients, while Nakamoto et al. reported the dROM level was 345 U.Carr in 110 stable asthmatic patients. 40 Therefore, our result is almost consistent with the previous results. BAP level in patients with asthma is not yet well defined, the average BAP level in glaucoma patients is  $1955.8 \pm 276.7 \,\mu\text{mol/L}$  and about 2100 to 2200 µmol/L in normal subjects. 26, 41, 42 In our study, the average level of BAP was 2363 µmol/L, which is slight compared to previous results.<sup>26, 41, 42</sup> It has been reported that a BAP level above 2200 µmol/L is considered to have a normal biological antioxidant potential<sup>42</sup> and an increased BAP level possibly counteracts increased levels of exercise-induced reactive oxygen species.<sup>26</sup> Therefore, elevated levels of BAP might be related to elevated levels of dROM. Further study is necessary to ascertain the relationships between levels of dROM and BAP in the same patients with repeated measurements. The H<sub>2</sub>O<sub>2</sub> levels in EBC from patients with asthma have been reported in previous studies, and the levels of H<sub>2</sub>O<sub>2</sub> ranged from 0.238 to 0.91.43-45 These levels are consistent with our study.

Although the levels of dROM, BAP, and H<sub>2</sub>O<sub>2</sub> are consistent with previous studies, we could not find any relationships among pulmonary function tests and these parameters. Suzuki et al. reported that the dROM level is increased during acute exacerbation of asthma and is correlated with serum inflammatory mediators.<sup>39</sup> Murata et al. reported that no relationships were observed in H<sub>2</sub>O<sub>2</sub> levels and pulmonary functions<sup>46</sup> while Al-Obadity reported an inverse correlation between H<sub>2</sub>O<sub>2</sub> and FEV<sub>1</sub> percent predicted.<sup>43</sup> Therefore, these oxidative stress markers may show a strong correlation with inflammatory markers, and not with pulmonary functions in patients with asthma.

A variety of antioxidant supplements and nutrients has been studied for the treatment of asthma.<sup>16, 47</sup> A few studies have shown the beneficial effect of antioxidants on pulmonary functions and on asthmatic symptoms.<sup>17, 18</sup> In our study, we studied the relationships between oxida-

tive stress markers and either vitamins in serum or the daily intake of vitamins over a very short period, and no relationships were found. Therefore, no clear and obvious relationships may exist in antioxidant vitamins or nutrition in a real-life setting. It should be noted that the number of subjects was small, and the duration of our study was very short.

We also studied the serum levels and intake of vitamin D. Vitamin D has no antioxidant capacity, but an effect of vitamin D on severe exacerbation of asthma has been observed. Brumpton et al. reported that low vitamin D is associated with lung function decline in adult patients with asthma. We could not find any correlation between pulmonary functions and either the serum levels or intake of vitamin D (data not shown). The protective effects with exacerbation were not observed because of the short period of this study.

In this study,  $\alpha$ -tocopherol was slightly above the normal range and  $\gamma$ -tocopherol was within normal range. Kodama et al. reported the concentration of  $\alpha$ -tocopherol in patients with asthma was not significantly different from control subjects. The reason for the inconsistency with our study is not known. On the other hand, Cook-Mills J et al. reported that the risk of asthma is increased in low levels of  $\alpha$ -tocopherol with high levels of  $\gamma$ -tocopherol. Again, further study is necessary to clarify the relationships between  $\alpha$ -tocopherol and  $\gamma$ -tocopherol in patients with established asthma.

It has been reported that transient exercise increases both oxidative stress and antioxidant capacity.<sup>25, 26</sup> Furthermore, exercise training increases antioxidant capacity.<sup>52</sup> In this study, we could not find any significant relationship between daily activity and oxidative stress level. Since our study evaluated average daily activity over a short period without exercise, and we did not compare the individual differences with activity level, further study is needed to show how either daily activity or exercise might influence oxidative stress on the same day in a single subject with asthma.

In this study, FEV<sub>1</sub>/FVC was positively correlated with activity counts per minute. Although FEV<sub>1</sub>/FVC was not correlated with total activity, activity counts per minutes were positively correlated with total activity (data not shown). Probably, patients who show better FEV<sub>1</sub>/FVC are likely to have more physical activity. Physical activity is reduced in patients with severe asthma.<sup>53</sup> Furthermore, Brumpton et al. reported that the decline in FEV<sub>1</sub> was less in physically active patients with asthma.<sup>54</sup> In their study, physical activity was measured by self-reporting. Therefore, it might be useful to study the relationship between physical activity and pulmonary function by using actigraphy.

In conclusion, we have found no relationships among oxidative stress, antioxidant capacity, vitamin levels, and physical activity in patients with asthma. In a real life setting with a short-term evaluation, oxidative stress may not be affected by daily activity and by antioxidant nutrition intake. A large and longitudinal prospective study is necessary to confirm our results.

Acknowledgments: This work was supported by JPPS KAKENHI 24500976.

The authors declare no conflict of interest.

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