

Changes in oxidative stress levels during two weeks of smoking cessation treatment and their association with nutritional characteristics in Japanese smokers

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Abstract. Although several experimental studies have reported that oxidative stress levels decrease during smoking cessation, how they change among general smokers has yet to be completely elucidated. In the present study, a total of 23 smokers who underwent smoking cessation treatment were observed for two-week changes in their levels of 8-OHdG and 8-isoprostane. Physical and nutritional characteristics were measured at the initial patient visit, and casual urine samples were collected at the initial visit and at a follow-up visit two weeks later. Oxidative stress was measured by a high performance liquid chromatography electrochemical detector, and the two-week difference in the levels of oxidative stress was assessed according to demographic and nutrient factors. Neither the urinary level of 8-OHdG nor that of 8-isoprostane decreased, although the cotinine level was decreased at two weeks. A Two-way repeated ANOVA revealed a significant interaction for fat intake by time for the change in the 8-OHdG level ($P=0.03$) and significant interactions for α -tocopherol intake ($P=0.03$), iron intake, and carbohydrate intake ($P=0.03$), all of which were time-dependent for the change in the 8-isoprostane level. The 8-OHdG level decreased among smokers with a high fat intake and was increased with a low fat intake. The 8-isoprostane levels were decreased among smokers with a high carbohydrate intake and increased with a low carbohydrate intake, decreased with a low iron intake and

increased with a high iron intake and decreased with a low α -tocopherol intake and increased with a high α -tocopherol intake. Although the present study failed to observe a decrease in oxidative stress levels during the two-week smoking cessation period, we hypothesize that the intake levels of specific nutrients when initiating smoking cessation treatment may predict any subsequent changes in the oxidative stress levels.

Introduction

Smoking cessation alleviates diseases caused by smoking (1,2), and the reduction of oxidative damage is one expected benefit (3). Past intervention studies reported that smoking cessation yielded reduced levels of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin F₂ α (8-isoprostane) (3,4). 8-OHdG reflects the oxidative damage and repair of DNA (5), and 8-isoprostane is a marker of oxidative stress and lipid peroxidation (6,7). Unlike selected subjects in experimental studies, general smokers have varying characteristics; thus, the change in the levels of oxidative stress in conjunction with the decreasing cotinine level may be different. In addition, studying interactions between physical and nutrient factors and smoking cessation against oxidative stress levels would be of interest to researchers in the field of cigarette smoking. For instance, an interaction between cigarette smoking and nutrient supplementation against cancer and hemorrhagic stroke was previously reported (8). Yet information on the interaction, including differences in the changes in oxidative stress levels according to dietary food or nutrient intake during smoking cessation, is limited. Likewise, not much has been reported about whether the changes in oxidative stress levels during smoking cessation differ according to the differences in physical factors such as sex, age, and BMI. The cross-sectional associations of food or nutrient intake and physical factors with oxidative stress levels have been

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reported (9-12). However, whether these factors interact with smoking cessation and influence the changes in oxidative stress levels is unknown. The aim of the current study was to prospectively record the two-week change in the levels of oxidative stress for general individuals who received smoking cessation treatment. We further analyzed whether physical and nutritional factors influenced the two-week changes in the levels of oxidative stress.

Patients and methods

Study participants. This was a two-week study that observed patients starting smoking cessation treatment with varenicline, which has been approved for use in Japan since 2008 (13). Individuals who met the criteria of the smoking cessation treatment guidelines were included in the current study (13), namely, smokers who were willing to stop smoking immediately, who were diagnosed as tobacco/nicotine-dependent based on the Tobacco Dependence Screener (14), whose Brinkman index was 200 or higher, and who agreed to receive smoking cessation treatment. Individuals who had a history of cancer or cardiovascular disease were not included in the study. This study was approved by the ethics committee of the National Institute of Public Health of Japan (Saitama, Japan). Patients were informed of the purpose and procedure of the current study, and only those who agreed to participate signed the consent form. The sample size was determined based on a previous intervention study (3). Twenty-three individuals whose assays were available for both the initial visit and a visit two weeks later and who provided information on their age, sex, and nutrient intake were analyzed in the current study.

Study design. Participants were treated based on standard treatment guidelines by a collaboration of the Japanese Circulation Society, the Japan Lung Cancer Society, the Japanese Cancer Association, and the Japanese Respiratory Society (13). We used data collected at the initial visit and a visit two weeks into the course of treatment. At both visits, casual urine samples were collected and stored at -70°C until assayed. 8-OHdG levels of the samples were measured using high performance liquid chromatography with an electrochemical detector (15). The effect of the smoking treatment, which was smoking cessation or a reduction of smoking, was confirmed by a decrease in the level of cotinine. For the analysis of cotinine, samples were extracted on an Oasis MCX cartridge and ENVI-Carb™ cartridge. The extracts were analyzed by a gas chromatography mass spectrometry system (16). For the analysis of 8-isoprostane, urinary samples were extracted on a C18 solid phase extraction cartridge and ENVI-Carb™ cartridge. The extracts were analyzed using a Micromass Quattro LC in MRM mode with an 'electrospray' source in negative mode and MassLynx software. For 8-isoprostane, the limit of detection and limit of quantification were 0.02 and 0.05 ng/ml, respectively, and reproducibility was 2.9%. The reagents for cotinine (1.0 mg/ml in methanol), 8-OHdG (>98%) and ENVI-Carb™ cartridge were purchased from Sigma-Aldrich Inc. (Merck KGaA, Darmstadt, Germany). Cotinine-d3 (99.9%) was purchased from CDN isotopes Inc. (Pointe-Claire, Quebec, Canada). 8-isoprostane (>99%),

8-isoprostane-d4 (>98%) and OASIS MCX were purchased from Cayman Chemical (Ann Arbor, MI, USA) and Waters Corporation (Milford, MA, USA), respectively. All other chemicals and solvents used were of an analytical grade.

Each subject's height and weight were measured during the initial visit to the clinic, and BMI was calculated. Participants also answered a self-administered questionnaire at this visit. The amount of regular physical activity was estimated from the validated questionnaire, and this was used to determine the weekly metabolic equivalents (METs) (17). The intake of food and nutrients in usual diet was measured with brief-type self-administered diet history questionnaire (BDHQ) (18).

Statistical analysis. Values for the levels of cotinine, 8-OHdG, and 8-isoprostane were adjusted for creatinine. These values, as well as BMI and METs, were logarithmically transformed to approximate a normal distribution for the analysis. Nutrient and food intakes were also logarithmically transformed and adjusted for total energy intake using a residual model for statistical analyses (19). To assess changes in the levels of cotinine, 8-OHdG, and 8-isoprostane, geometric means with 95% confidence intervals (CIs) and standard errors were obtained, and paired t-tests were conducted. To assess the influences of physical and nutritional factors on changes in the levels of oxidative stress, age, BMI, physical activity, and the intakes of alcohol, coffee, and nutrients (total calories, vitamin C, α tocopherol, vitamin A, iron, carbohydrate, fat, and protein) were categorized into two groups according to their median value. These traits were selected from previous studies that reported their association with oxidative stress levels (11,20-25). Vitamin A is expressed in retinol equivalents, calculated as a sum of retinol, β -carotene/12, α -carotene/24, and cryptoxanthin/24. To investigate the time course of each oxidative stress level, we performed a two-way repeated ANOVA with the factor of each group and time. A factor-by-time interaction was assessed for statistical significance to test whether changes in value over time differ between the groups of each factor. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The characteristics of participants at the initial visit are summarized in Table I. The participants ranged between 29 and 82 years of age and 52% of them were male. Table II summarizes the geometric means of cotinine, 8-OHdG, and 8-isoprostane levels at the initial visit and the visit two weeks later. Compared with the level at the initial visit, the cotinine level was largely and significantly decreased after two weeks. Levels of 8-OHdG and 8-isoprostane had not changed two weeks after the initiation of smoking cessation treatment.

Table III summarizes the geometric means of 8-OHdG levels at the initial visit and two-week visit according to each factor, as well as changes in values over time. The two-way repeated ANOVA showed a significant factor \times time interaction for fat intake, indicating a decrease in the 8-OHdG level in the group with lower fat intake, whereas there was an increase in the 8-OHdG level in the group with higher fat intake (Fig. 1).

Table I. Baseline characteristics of 23 smokers who underwent smoking cessation treatment.

Characteristics	Mean (n)	SD (%)	Minimum	Maximum
Age (years)	46.8	15.0	29.0	82.0
BMI (kg/m ²) ^a	23.6	5.7	16.6	42.9
Physical activity (metabolic equivalents-h/week)	8.5	11.1	0.0	46.5
Smoking pack-year	22.9	12.8	5.0	50.0
The Tobacco Dependence Screener	8.0	1.6	5.0	10.0
Alcohol/day (g)	14.9	20.8	0.0	69.7
coffee/day (g)	296.7	177.5	10.0	600.0
Vitamin C/day (mg)	92.7	43.9	17.9	203.1
α -tocopherol/day (mg)	6.4	2.4	2.0	11.3
Vitamin A (retinol equivalent) (μ g/day)	556.5	359.7	66.3	1553.7
Carbohydrate/day (g)	198.8	73.0	66.0	376.5
Fat/day (g)	44.4	17.9	19.2	84.8
Protein/day (g)	56.4	25.0	27.2	134.0
Iron/day (mg)	6.5	2.8	2.5	12.7
Sex (male, %)	12	52		

^aExcluding 3 individuals with missing value. SD, standard deviation.

Table II. The geometric means with 95% CI for urinary cotinine, 8-OHdG and 8-isoprostane before and after the treatment.

Biomarkers	Initial visit n=23		After 2 weeks n=23		
	Mean ^a	95% CI	Mean ^a	95% CI	P ^b
Cotinine (ng/ml creatinine)	1780.60	(1373.5-2308.4)	24.79	(5.3-117.0)	<.0001
8-OHdG (ng/mg creatinine)	3.02	(2.24-4.07)	2.77	(1.82-4.22)	0.65
8-isoprostane (ng/mg creatinine)	0.29	(0.22-0.39)	0.34	(0.27-0.43)	0.32

^aGeometric mean. ^bPaired t-test. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CI, confidence interval.

Other factor x time interaction terms did not reach statistical significance.

Table IV summarizes the geometric means of 8-isoprostane levels at the initial visit and two-week visit according to each factor, as well as the change in values over time. A significant factor x time interaction was observed for α -tocopherol, iron, and carbohydrate intakes. The level of 8-isoprostane increased in the group with low carbohydrate intake, whereas it decreased in the group with high carbohydrate intake (Fig. 2). The 8-isoprostane level decreased in the group with low iron intake, whereas it increased in the group with high iron intake (Fig. 3). The 8-isoprostane level decreased slightly in the group with low α -tocopherol intake but increased in the group with high α -tocopherol intake (Fig. 4). We performed an ad hoc analysis of the intake of α -tocopherol and vitamin A combined. The methods of statistical analysis was the same as those for each nutrient intake. The 8-isoprostane level increased in the group with high intakes of both α -tocopherol and vitamin A, whereas it slightly decreased in the group with low intakes of either or both nutrients (Fig. 5).

Discussion

We did not observe a decrease in the levels of oxidative DNA and lipid peroxidation, which were estimated from urinary 8-OHdG and 8-isoprostane, respectively, two weeks after starting smoking cessation treatment. This was despite the fact that subjects quit smoking or largely reduced the number of cigarettes smoked, which was estimated from the cotinine levels of the same urine samples. This study was first to assess changes in oxidative stress levels among smokers with greater demographic variability in an actual clinical setting. The result contradicts a previous finding from an intervention study, which reported a reduction of 8-OHdG and 8-isoprostane levels after two weeks of smoking cessation (3). We did not find this surprising; our data were collected in a clinical setting, and study participants had wide demographic variability, whereas the previous study was conducted among less varied subjects-young healthy male medical students (3).

In the current study, the comparison by dietary intake implied that changes in oxidative stress levels were modified

Table III. Geometric means of 8-OHdG levels at the initial visit and 2-week visit according to the physical and nutritional factors.

Characteristics	< Median value						≥ Median value			P-value for group by time interaction ^a			
	Initial visit			2-week visit			Initial visit				2-week visit		
	n	Geometric mean	95% CI	Geometric mean	95% CI	n	Geometric mean	95% CI	Geometric mean		95% CI	Geometric mean	95% CI
Age (years)	12	2.95	(1.79-4.84)	2.66	(1.33-5.30)	11	3.10	(2.05-4.68)	2.90	(1.60-5.26)	0.92		
BMI	10	3.41	(2.16-5.38)	2.47	(0.94-6.50)	10	3.22	(2.01-5.15)	3.05	(1.88-4.95)	0.51		
Alcohol (g/day)	12	2.34	(1.44-3.80)	1.90	(1.04-3.47)	11	3.98	(2.83-5.58)	4.18	(2.35-7.46)	0.49		
Coffee (g/day)	12	3.12	(2.03-4.78)	3.37	(2.35-4.85)	11	2.91	(1.76-4.80)	2.23	(0.94-5.27)	0.36		
Total calories (kcal/day)	12	3.07	(1.86-5.07)	3.31	(2.17-5.06)	11	2.96	(1.96-4.45)	2.28	(0.99-5.21)	0.38		
Vitamin C (mg/day)	12	2.20	(1.44-3.36)	2.19	(1.11-4.36)	11	4.25	(2.90-6.22)	3.57	(2.05-6.20)	0.66		
α-tocopherol (mg/day)	12	3.17	(2.17-4.64)	3.34	(2.13-5.26)	11	2.85	(1.01-5.05)	2.25	(1.01-5.05)	0.45		
Vitamin A (retinol equivalent) (μg/day)	12	2.55	(1.63-3.99)	2.64	(1.41-4.93)	11	3.62	(2.32-5.66)	2.92	(1.48-5.75)	0.51		
Iron (mg/day)	12	2.76	(1.74-4.39)	2.87	(1.75-4.72)	11	3.32	(2.12-5.21)	2.66	(1.19-5.92)	0.49		
Carbohydrate (g/day)	12	3.17	(2.31-4.36)	2.10	(0.97-4.54)	11	2.86	(1.58-5.16)	3.75	(2.64-5.31)	0.06		
Fat (g/day)	12	3.04	(1.84-5.01)	4.04	(2.98-5.47)	11	3.00	(1.99-4.51)	1.83	(0.81-4.16)	0.03		
Protein (g/day)	12	3.05	(1.94-4.81)	3.73	(2.61-5.32)	11	2.98	(1.86-4.77)	2.00	(0.87-4.59)	0.11		
α-tocopherol and vitamin A													
	16	2.94	(2.05-4.22)	2.66	(1.59-4.47)	7	3.20	(1.56-6.56)	3.03	(1.14-8.02)	0.92		
Sex													
	12	3.05	(2.01-4.63)	2.09	(1.01-4.36)	11	2.98	(1.79-4.97)	3.75	(2.42-5.83)	0.10		
			Male					Female					

^a2-way ANOVA. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; CI, confidence interval.

Table IV. Geometric means of 8-isoprostane levels at the initial visit and 2-week visit according to the demographic, physical and nutritional factors.

Characteristics	< Median value						≥ Median value			P-value for variable by visit interaction ^a			
	Initial visit			2-week visit			Initial visit				2-week visit		
	n	Geometric mean	95% CI	Geometric mean	95% CI	n	Geometric mean	95% CI	Geometric mean		95% CI	Geometric mean	95% CI
Age (years)	12	0.35	(0.23-0.55)	0.41	(0.32-0.52)	11	0.24	(0.17-0.34)	0.27	(0.18-0.42)	0.93		
BMI	10	0.25	(0.14-0.46)	0.40	(0.28-0.56)	10	0.36	(0.26-0.50)	0.33	(0.21-0.51)	0.06		
Alcohol (g/day)	12	0.27	(0.18-0.41)	0.28	(0.19-0.41)	11	0.32	(0.21-0.49)	0.41	(0.31-0.54)	0.45		
Coffee (g/day)	12	0.31	(0.20-0.48)	0.37	(0.28-0.49)	11	0.28	(0.18-0.42)	0.30	(0.19-0.46)	0.69		
Total calories (kcal/day)	12	0.26	(0.18-0.37)	0.29	(0.21-0.38)	11	0.33	(0.20-0.54)	0.40	(0.27-0.60)	0.71		
Vitamin C (mg/day)	12	0.33	(0.21-0.52)	0.34	(0.24-0.47)	11	0.26	(0.18-0.37)	0.34	(0.22-0.50)	0.35		
α-tocopherol (mg/day)	12	0.37	(0.25-0.55)	0.33	(0.23-0.46)	11	0.23	(0.15-0.34)	0.35	(0.23-0.51)	0.03		
Vitamin A (retinol equivalent) (μg/day)	12	0.29	(0.18-0.47)	0.29	(0.20-0.43)	11	0.30	(0.22-0.42)	0.39	(0.29-0.53)	0.33		
Iron (mg/day)	12	0.34	(0.23-0.50)	0.29	(0.20-0.43)	11	0.25	(0.16-0.40)	0.40	(0.29-0.53)	0.02		
Carbohydrate (g/day)	12	0.22	(0.15-0.33)	0.33	(0.23-0.47)	11	0.41	(0.29-0.56)	0.35	(0.24-0.50)	0.03		
Fat (g/day)	12	0.36	(0.24-0.54)	0.34	(0.25-0.48)	11	0.24	(0.16-0.35)	0.33	(0.22-0.49)	0.14		
Protein (g/day)	12	0.35	(0.23-0.51)	0.37	(0.27-0.51)	11	0.25	(0.16-0.38)	0.31	(0.21-0.46)	0.56		
α-tocopherol and vitamin A													
	16	0.32	(0.22-0.46)	0.30	(0.23-0.40)	7	0.24	(0.16-0.38)	0.42	(0.25-0.70)	0.03		
Sex													
	12	0.29	(0.19-0.46)	0.33	(0.26-0.43)	11	0.30	(1.79-4.97)	0.34	(0.21-0.54)	0.996		
			Male					Female					

^a2-way ANOVA. CI, confidence interval.

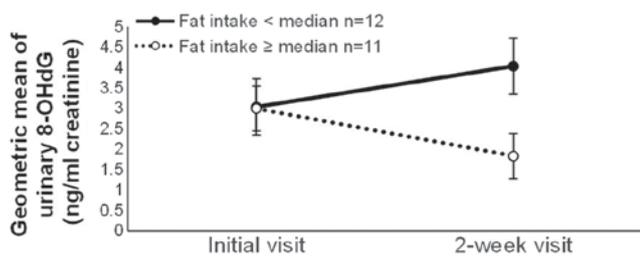


Figure 1. The geometric means with standard errors of 8-OHdG levels at the initial visit and the visit 2 weeks after the start of smoking cessation treatment according to fat intake group. Two-way repeated ANOVA analysis showed a significant group x time interaction for the log transformed 8-OHdG level ($P=0.03$). 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

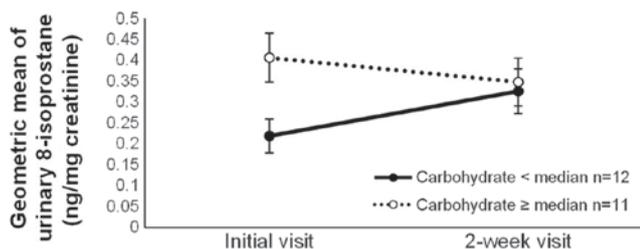


Figure 2. The geometric means with standard errors of 8-isoprostane levels at the initial visit and a visit two weeks after the start of smoking cessation treatment according to the carbohydrate intake group. Two-way repeated ANOVA analysis showed a significant group x time interaction for the log transformed 8-isoprostane level ($P=0.03$).

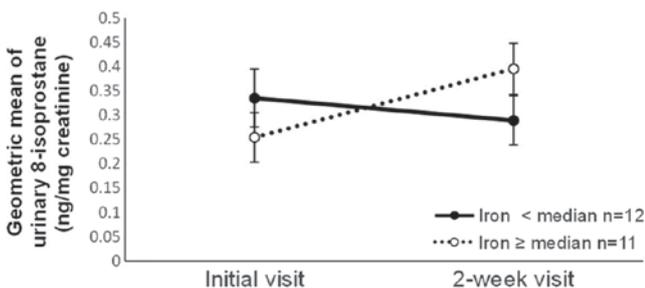


Figure 3. The geometric means with standard errors of 8-isoprostane levels at the initial visit and a visit two weeks after the start of smoking cessation treatment according to the iron intake group. Two-way repeated ANOVA analysis showed a significant group x time interaction for the log transformed 8-isoprostane level ($P=0.02$).

by the regular intake of certain nutrients. Although not many studies have reported changes in oxidative stress levels during smoking cessation, previous findings with different study designs may reasonably support our findings. Previous cross-sectional studies reported an inverse association between fat intake and the urinary 8-OHdG level, although statistical significance was not achieved (5,26). These studies correspond with our finding that the 8-OHdG level decreased during smoking cessation among smokers with high fat intake but increased among those with low fat intake, although no mechanism was suggested.

The 8-isoprostane level decreased after two weeks of smoking cessation treatment among smokers with high carbohydrate intake, whereas the level increased among those with low carbohydrate intake. Studies of the association between the

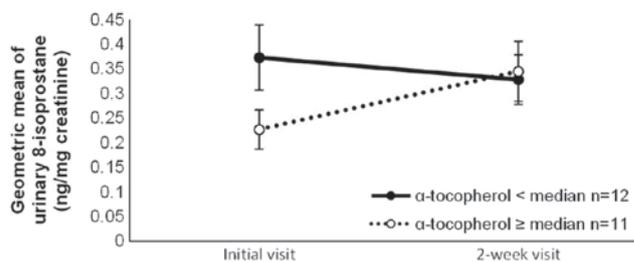


Figure 4. The geometric means with standard errors of 8-isoprostane levels at the initial visit and a visit two weeks after the start of smoking cessation treatment according to the α -tocopherol intake group. Two-way repeated ANOVA analysis showed a significant group x time interaction for the log transformed 8-isoprostane level ($P=0.03$).

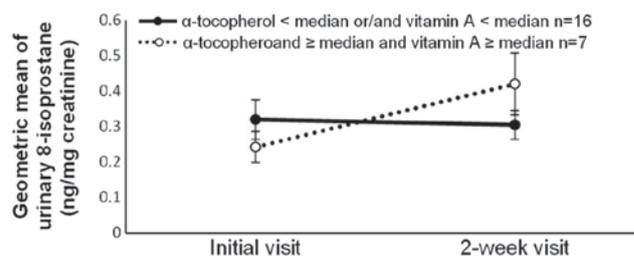


Figure 5. The geometric means with standard errors of 8-isoprostane levels at the initial visit and a visit two weeks after the start of smoking cessation treatment according to intakes of the α -tocopherol and retinol equivalent combined group. Two-way repeated ANOVA analysis showed a significant group x time interaction for the log transformed 8-isoprostane level ($P=0.03$).

8-isoprostane level and carbohydrate intake have been limited; however, one study reported a significant inverse correlation between carbohydrate intake and serum total antioxidant capacity among breast cancer patients and controls (27). A study of cyclists reported that the increase in F_2 -isoprostanes was significantly lower after the ingestion of carbohydrate beverages as compared to placebo beverages (28). This study speculated that this was induced by the suppression of the cortisol and epinephrine levels after the intake of carbohydrate. The level of epinephrine also increases with smoking (29), which may explain a mechanism for the observed interaction between carbohydrate intake and smoking cessation.

We did not expect that a high intake of dietary α -tocopherol would be associated with an increase in the level of 8-isoprostane during the smoking cessation period. The antioxidant activity of α -tocopherol or vitamin E in diets or from supplements has been frequently reported (30-32). However, a study of patients with histories of colorectal adenoma reported an interesting result: A supplementary cocktail that included α -tocopherol and β -carotene increased F_2 -isoprostane levels among smokers, whereas it decreased them among non-smokers (33). Our additional analysis indicated that individuals who had high intakes of both α -tocopherol and vitamin A had an increasing change in their levels of 8-isoprostane. A study in rats may explain the mechanism, which is a pro-lipid peroxidation activity of β -carotene (34). However, no evidence has suggested that vitamin E by itself acted as a pro-oxidant among smokers. Another explanation may be that the sufficient intake of dietary antioxidant nutrients may have suppressed the oxidative stress

level before the initiation of smoking cessation treatment. There may be certain threshold levels of oxidative stress level to make smoking cessation be effective. Further analysis exploring the interaction between these nutrients is difficult with our data.

The current study has several limitations. The limited number of participants caused a lack of statistical power, which would have caused our failure to observe a decrease in oxidative stress during smoking cessation treatment. The accurate measures utilized in the current study may have diminished this limitation. Another major limitation is that there is no control group, and all participants underwent smoking cessation treatment. The main objective of this study was to observe general individuals who were willing to undergo smoking cessation treatment, and changes in oxidative stress levels were evaluated according to the levels of several key factors. The study period was limited to two weeks, and further follow-up was not available. Achievement of smoking cessation during the two weeks was confirmed by measuring cotinine level; however, the final outcome of the smoking cessation treatment was not evaluated. Despite these limitations, important information can be drawn from the current results, especially since limited data exist on the change in oxidative stress levels during a smoking cessation period.

In conclusion, we observed a decrease in cotinine levels but not in oxidative stress levels among Japanese participants who underwent smoking cessation treatment. The change in oxidative stress levels two weeks from the beginning of the smoking cessation treatment may vary based upon several factors. Levels of 8-OHdG decreased among smokers with high fat intake, whereas they increased among smokers with low fat intake. The level of 8-isoprostane increased among smokers with low carbohydrate intake but decreased among those with high carbohydrate intake, decreased among those with low iron intake but increased among those with high iron intake, and decreased among smokers with low α -tocopherol intake whereas it increased among those with high α -tocopherol intake. Further research on changes in oxidative stress levels and various factors among smokers undergoing smoking cessation treatment with a greater number of subjects, as well as research on estimating the changes among such smokers in general populations may confirm our current findings.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

SO and TI conceptualized and designed the study. YI and NK measured the oxidative stress levels. TS, JO, KS, TK, and TI acquired data. SO drafted the manuscript, and YI, TS, JO, KS, TK, NK, and TI revised it critically. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the National Institute of Public Health of Japan (Saitama, Japan). All participants were informed of the purpose and procedures of the current study and signed the consent form.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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