# **Original Article**

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**Background and Objectives:** Eggs, an important source of high-quality protein, contain a variety of key nutrients and antioxidants. Here we conducted an intervention study to evaluate whether the additional consumption of one egg per day would affect the daily nutritional intakes and blood antioxidant status in Japanese woman university students. **Methods and Study Design:** For 4 weeks, the 14 subjects were provided a nutritious breakfast including one boiled egg, and they were asked to keep a daily dietary record. **Results:** The subjects' daily energy intake during the intervention did not differ compared to the baseline, whereas the protein energy ratio and cholesterol intake were significantly increased. The subjects' consumption of confectionery during the intervention was significantly decreased compared to the pre-trial period. The total score of adherence to the food-based Japanese dietary guidelines for a healthy diet during the intervention was higher than that at baseline. The analysis of fasting blood samples showed that the subjects' serum lipids levels were not altered, whereas their malondialdehyde modified low-density lipoprotein (MDA-LDL) levels and the oxidative susceptibility of LDL were significantly reduced after the intervention. More importantly, serum folic acid levels were significantly increased. **Conclusions:** Our results suggest that regular egg consumption at breakfast can help improve the daily nutritional status and dietary habits and also ameliorate certain indices of antioxidant status in young women.

Key Words: egg, breakfast, nutritional balance, dietary habit, antioxidant

# **INTRODUCTION**

According to the International Egg Commission survey (2014), the reported per capita annual egg consumption in Japan was 329, the third highest in the world.<sup>1</sup> Eggs are one of the most nutritious foods, with significant protein, vitamins and minerals, and eggs can make a great contribution to a nutritious diet. It was reported that compared to nonconsumers, the daily nutrient intake of American egg consumers was significantly greater for all nutrients except dietary fiber and vitamin B-6.<sup>2</sup> Thus, eggs can be a nutritious inclusion in the diet for people of all ages and at different stages of life, especially for those at risk of low-nutrient intakes such as the elderly, pregnant women and children.<sup>3-5</sup>

Although the importance of regular dietary habits and not skipping breakfast is well appreciated, the percentage of breakfast skippers among college students in Japan is high. It was reported that managing the frequency of skipping breakfast and reducing it to <3 times per week may be beneficial for the maintenance of bone health in young women.<sup>6</sup> Even among breakfast eaters, younger adults — especially those living alone — tend to consume convenience breakfasts such as white rice or bread without side dishes, which comprise a nutritionally poor and unbalanced diet. Tani et al recently reported that a higher proportion of total energy intake during the morning might reduce the absolute intake of energy during the day in Japanese women.<sup>7</sup> It was also reported that increases in dietary protein at breakfast had greater effects on satiety compared to other mealtimes.<sup>8-10</sup> These findings suggest that the addition of a protein-rich breakfast might be an effective strategy to improve the nutritional balance and appetite control in young people. Considering its relatively low cost, ease of preparation and variety of dishes, the egg is an ideal source of high-quality protein, especially

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In addition to their macronutrients, eggs contain high amounts of antioxidants in both the egg white and yolk. Several aromatic amino acids in egg (tryptophan and tyrosine), egg lipids such as phospholipids, and micronutrients such as vitamin E, vitamin A, selenium, and carotenoids are reported to have antioxidant properties.<sup>11,12</sup> A number of egg-derived peptides have been shown to have antioxidant activities in vitro.<sup>13-15</sup> Carotenoids are lipophilic compounds responsible for the orange-yellow color of the egg yolk. The xanthophyll carotenoids lutein and zeaxanthin are known to be effective to protect the eyes from age-related macular degeneration,<sup>16</sup> and they also play an important role in preventing atherosclerosis development through their antioxidant activities.<sup>17,18</sup> An intervention trial with elderly subjects revealed that consuming only one egg per day significantly increased the serum concentrations of both lutein and zeaxanthin.<sup>19</sup> The health benefits of antioxidants are in large attributed to its protective effect against reactive oxygen species damage by quenching singlet oxygen and scavenging free radicals.<sup>20</sup>

Despite its essential nutritious aspects, eggs have sometimes been considered an adverse food for human health, mainly due to their cholesterol content. Nowadays it is known that the response of cholesterol in human serum to dietary cholesterol consumption depends on several factors, such as an individual's genetic makeup, hormonal factors and nutritional status.

In the present study, we designed a nutritious breakfast that included one egg to provide high-quality protein, and we conducted an intervention study to evaluate the effects of consuming this breakfast for 4 weeks on the subjects' nutrient intake and blood parameters (including antioxidant status) in women university students.

# METHODS

## Subjects

Subjects were recruited from the training course for registered dietitians at Ochanomizu University through a recruitment poster with the exclusion criterion of allergy to eggs. The study protocol was approved by the Ethics Committee of Ochanomizu University and conformed to the Declaration of Helsinki. This trial was registered with the UMIN-CTR (http://www.umin.ac.jp/ctr/index.htm) as UMIN000011932. Fourteen woman students (ages 18-22 years) were enrolled. Written informed consent was obtained from the subjects, and parental consent was required and obtained for minor subjects (<20 years). According to the results of a questionnaire distributed before the study, 10 of the 14 subjects were eating breakfast every day, and the others sometimes skipped breakfast. The mean age of the subjects was  $19.6\pm1.2$  years, and the BMI was  $21.1\pm2.2$  kg/m<sup>2</sup>. None of the subjects had any degree of smoking habit.

# Study design and diets

A single-arm, open-design intervention lasting 4 weeks was performed in October and November 2013. All subjects were provided a nutritious breakfast at 8:00–9:00 am every weekday at their university. A breakfast menu was prepared consisting of one boiled egg, one piece of white toast with margarine/jam, vegetable salad, low-sugar yogurt, fruit juice and black tea. Boiled eggs were obtained from the Kewpie Corp. (Tokyo). This breakfast had 551 kcal energy content with 20.3 g protein (14.8% of energy), 15.9 g fat (25.8% of energy), and 82.3 g carbohydrate (59.4% of energy) on average. The subjects were asked to complete two visual analog scales (VASs), one for sleepiness and one for appetite, before breakfast each weekday. These scales were 100-mm long with words anchored at each end expressing the most negative (score=0) and the most positive rating (score=100). On holidays the subjects were asked to consume breakfast containing a provided egg at home. The subjects were also asked to maintain their normal lifestyle throughout the 4-week study period.

# **Dietary** assessments

At the beginning and again after the study period, the subjects' dietary habits during the previous month were assessed using a food frequency questionnaire, i.e., a brief-type self-administered diet history questionnaire (BDHQ) which was validated by Kobayashi et al.<sup>21,22</sup> The subjects were also instructed to take pictures and record the contents of their daily meals, snacks, and beverages in a diet diary for 5 weeks (1 week before the intervention started and for the 4 weeks of intervention). The diet diarry was collected every weekday, and each subject's meal intake and daily intakes of energy, fat, protein, carbohydrate, and other nutrients were calculated from the diary record by an experienced dietitian on the basis of the 2010 Standard Tables of Food Composition in Japan.<sup>23</sup>

We also calculated a score for adherence to the foodbased Japanese dietary guidelines, using a procedure developed by Oba et al.<sup>24</sup> Servings of (1) grain dishes, (2) vegetable dishes, (3) fish and meat dishes, (4) milk, and (5) fruits, and (6) the energy intake from snacks and alcoholic beverages were calculated from the food frequency questionnaire, and the values were energy-adjusted by the density method to obtain the values per 1800 kcal of energy to enable a comparison with the recommended values.<sup>25</sup> The six scores (ranging from 0 to 10 each) were summed to provide the overall score on adherence to the food-based Japanese dietary guidelines (ranging from 0 to 60).

#### Blood sampling and measurements

Before and again at the end of the study period, each subject's body weight, abdominal circumference and blood pressure were measured, and blood samples were obtained between 7:00 am and 8:30 am after an overnight fast from 8:00 pm of the previous day. Total protein was measured by the Biuret method, and albumin was measured by the modified bromcresol purple (BCP) method. The blood glucose concentration was measured by the hexokinase UV method. The serum insulin and folic acid concentrations were measured by a chemiluminescent enzyme immunoassay (CLEIA). Total cholesterol, highdensity lipoprotein (HDL) cholesterol, triglyceride, nonesterified fatty acids (NEFAs) and magnesium were measured by enzymatic procedures.

Each subject's low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. The serum iron concentration was measured by the Nitroso-PSAP method, and the serum ferritin concentration was measured using a latex agglutination immunoassay. The serum calcium concentration was measured by the chlorophosphonazo-III method. The serum lutein+zeaxanthin and vitamin C concentrations were measured by highperformance liquid chromatography, and the vitamin E concentration was measured by the fluorometric method. The malondialdehyde modified LDL (MDA-LDL) concentration was measured by an enzyme-linked immunosorbent assay (ELISA). The serum iron, ferritin, calcium and magnesium levels were measured using an automated clinical analyzer (BIOLIS 24i, Tokyo Boeki Machinery, Tokyo). The serum lutein+zeaxanthin level was measured by Nikken Seil (Shizuoka, Japan), and the other basic biochemistry tests were performed by the laboratory SRL (Tokyo).

LDL was separated from plasma by single-spin density gradient ultracentrifugation at 100,000 rpm for 40 min at 4°C. The LDL oxidizability was determined by lag time assay as described.<sup>26</sup> The prepared LDL samples (final concentration of protein: 70 µg/mL) were oxidized by 200 2,2-azobis-4-methoxy-2,4-dimethylvaleronitrile μM (Wako Pure Chemicals, Osaka, Japan). The kinetics of LDL oxidation were determined by monitoring the absorbance of conjugated dienes at 234 nm using a spectrophotometer (DU800, Beckman Coulter, CA, USA) at 4min intervals at 37°C.

The total antioxidant capacity was determined by a 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay (Cayman Chemical,

Ann Arbor, MI) according to the manufacturer's instructions.

# Statistical analyses

All data are presented as mean±SD. Comparisons of the data obtained before (baseline) and after the 4-week intervention were performed using a two-tailed Wilcoxon signed rank test. Differences were considered significant when p < 0.05. The statistical analyses were performed using the GraphPad Prism 5 software package (GraphPad Software, La Jolla, CA).

# RESULTS

#### Nutritional intakes

All 14 subjects completed the intervention and did not miss the one egg per day requirement during the 4-week intervention. The subjects' nutritional intakes from their weekday breakfasts and their daily nutritional intakes calculated from the dietary records are shown in Table 1. All that the subjects ate or drank before 9:00 am was considered breakfast.

The results of our analyses revealed that the subjects' energy intake at breakfast during the intervention was not significantly different from that before the intervention, but that the intakes of protein  $(12.7\pm2.2\% \text{ to } 14.8\pm0.3\%)$ , p < 0.01) and some vitamins such as  $\alpha$ -tocopherol, folate and ascorbic acid, dietary fiber, and cholesterol were significantly higher during the intervention.

As for daily intakes, the subjects' energy intake during the intervention was not significantly different from that before the study. However, the energy percentage from

Daily

Table 1. The subjects' nutritional intakes for breakfast and daily

	Baseline	Egg period	Baseline	Egg period
Energy (kcal)	$478 \pm 179$	$556 \pm 29$	$1950 \pm 388$	$1946 \pm 247$
Protein (g)	$15.7 \pm 7.5$	$20.5 \pm 1.1$ *	$66.3 \pm 13.6$	$70.3 \pm 9.1$
Protein (% energy)	$12.7 \pm 2.2$	$14.8 \pm 0.3$ **	$13.7 \pm 1.1$	$14.5 \pm 0.7$ *
Fat (g)	$15.2 \pm 7.7$	$16.0 \pm 1.3$	$64.9 \pm 17.8$	$68.3 \pm 11.1$
Fat (% energy)	$28.5 \pm 7.1$	$25.7 \pm 1.3$	$29.4 \pm 3.0$	$31.0 \pm 2.6$ #
Saturated fatty acids (g)	$5.3 \pm 4.1$	$4.0 \pm 0.6$	$20.5 \pm 6.8$	$21.1 \pm 4.5$
Monounsaturated fatty acids (g)	$4.9 \pm 2.8$	$6.0 \pm 0.4$	$23.5 \pm 7.6$	$25.8 \pm 4.3$
Polyunsaturated fatty acids (g)	$2.7 \pm 1.2$	$3.7 \pm 0.3$ *	$11.7 \pm 3.0$	$12.3 \pm 1.4$
Cholesterol (mg)	$91 \pm 77$	$216 \pm 3.0$ ***	$360 \pm 103$	495 ± 72 ***
Carbohydrate (g)	$68.8 \pm 24.6$	83.1±4.7 *	$266 \pm 42.9$	$256 \pm 32.4$
Carbohydrate (% energy)	$58.8 \pm 7.3$	$59.5 \pm 1.2$	$56.9 \pm 2.8$	54.5 ± 2.9 *
Soluble dietary fiber (g)	$0.8 \pm 0.3$	$1.0 \pm 0.1$ **	$3.5 \pm 0.5$	$3.4 \pm 0.6$
Insoluble dietary fiber (g)	$2.3 \pm 0.9$	$2.8 \pm 0.2$ *	$10.2 \pm 1.5$	$9.8 \pm 1.9$
Dietary fiber (g)	$3.2 \pm 1.3$	$3.8 \pm 0.3$ #	$14.3 \pm 2.3$	$13.6 \pm 2.0$
Calcium (mg)	$187 \pm 124$	$194 \pm 26$	$552 \pm 179$	$558 \pm 103$
Magnesium (mg)	$63 \pm 31$	$67 \pm 6$	$257 \pm 43$	$246 \pm 30$
Iron (mg)	$1.7 \pm 0.9$	$2.1 \pm 0.1$	$8.1 \pm 1.4$	$7.8\pm0.8$
Vitamin A (retinol equiv.) (µg)§	$123 \pm 71$	$155 \pm 16$	$560 \pm 117$	$569 \pm 111$
Vitamin D (µg)	$0.7 \pm 0.7$	$0.9 \pm 0.1$ *	$3.6 \pm 1.5$	$4.8 \pm 1.2$ **
α-Tocopherol (mg)	$1.9 \pm 0.8$	$2.6 \pm 0.2$ **	$8.6 \pm 2.3$	$8.7 \pm 1.2$
Vitamin B-12 (µg)	$0.8 \pm 0.7$	$0.7 \pm 0.1$	$3.7 \pm 1.4$	$4.7 \pm 0.8$ *
Folate (µg)	$68 \pm 30$	$103 \pm 6$ ***	$305\pm70$	$306 \pm 46$
Vitamin C (mg)	$19 \pm 11$	52±9 ***	$102 \pm 65$	$113 \pm 28$ *

Breakfast

Values are mean±SD (n=14). Comparisons of the values between baseline and during the intervention period were performed using a two-tailed Wilcoxon signed rank test. \*\*\*p < 0.001, \*\*p < 0.01, \*p < 0.05, \*\*p < 0.10.

<sup>†</sup>Nutritional intakes from the subjects' weekday breakfasts.

<sup>‡</sup>Their daily nutritional intakes.

<sup>§</sup>Sum of retinol,  $\beta$ -carotene/12,  $\alpha$ -carotene/24, and cryptoxanthin/24.

Table 2. Adherence to the	food-based Japan	nese dietary gui	idelines for a	healthy diet
		20		2

	Baseline	Egg period	
Servings			
Grain dishes (servings/1800 kcal)	$3.4 \pm 1.0$	$3.7 \pm 1.0$	
Vegetable dishes (servings/1800 kcal)	$5.7 \pm 1.9$	$7.2 \pm 1.8$	#
Fish and meat dishes (servings/1800 kcal)	$6.4 \pm 2.6$	$6.2 \pm 1.7$	
Milk (servings/1800 kcal)	$2.0 \pm 1.3$	$2.0 \pm 1.1$	
Fruits (servings/1800 kcal)	$0.6 \pm 0.7$	$1.6 \pm 0.7$	**
Snacks and alcoholic beverages (kcal/1800 kcal)	$226 \pm 63$	$129 \pm 51$	**
Scores			
Grain dishes	$8.5 \pm 2.3$	$8.7 \pm 1.2$	
Vegetable dishes	$8.3 \pm 1.7$	$7.2 \pm 1.7$	
Fish and meat dishes	$5.3 \pm 3.6$	$5.3 \pm 3.0$	
Milk	$5.1 \pm 3.2$	$6.2 \pm 3.4$	
Fruits	$3.6 \pm 3.2$	$7.5 \pm 2.5$	*
Snacks and alcoholic beverages	$7.9 \pm 2.0$	$9.9 \pm 0.3$	*
Total score	$38.9 \pm 7.9$	$44.9 \pm 5.5$	#

Values are mean±SD (n=14). Comparisons of the values between basal and during the investigation period were performed using a two-tailed, Wilcoxon signed rank test.  $p^* = 0.01$ ,  $p^* = 0.05$ ,  $p^* = 0.05$ .

One serving of grain dishes contained 40 g carbohydrate; one serving of vegetable dishes was 70 g; one serving of fish and meat dishes contained 6 g protein; one serving of milk contained 100 mg Ca; and one serving of fruits was 100 g.

Recommended values are as follows: grain dishes, 4–5 servings/1800 kcal; vegetable dishes, 5–6 servings/1800 kcal; fish and meat dishes, 3–4 servings/1800 kcal; milk, 2 servings/1800 kcal; fruits, 2 servings/1800 kcal; and snacks and alcoholic beverages,  $\leq$ 200 kcal/1800 kcal. Total score ranging from 0 to 60.

protein (13.7±1.1% to 14.5±0.7%, p<0.05), vitamin D (3.6±1.5 µg/day to 4.8±1.2 µg/day, p<0.01), vitamin B-12 (3.7±1.4 µg/day to 4.7±0.8 µg/day, p<0.05), vitamin C (102±65 mg/day to 113±28 mg/day, p<0.05) and cholesterol intake (360±103 mg/day to 495±72 mg/day, p<0.001) were significantly higher during the intervention compared to the baseline values.

# Food group intake

The food group intakes were estimated using the results of the food frequency questionnaire. The frequencies of fruit and egg consumption were significantly increased due to the consumption of the fruit juice and eggs provided at breakfast. In contrast, the subjects' consumption of confectionery (e.g., candy and sweets) during the intervention was significantly lower than that at the baseline  $(44\pm18 \text{ g/day to } 30\pm18 \text{ g/day}, p<0.05)$ .

# Healthy diet score

The total score of adherence to the food-based Japanese dietary guidelines for a healthy diet during the intervention tended to increase compared with that at baseline  $(38.9\pm7.9 \text{ to } 44.9\pm5.5, p<0.10)$  (Table 2). The number of servings of fruits was significantly increased  $(0.6\pm0.7 \text{ servings/1800 kcal to } 1.6\pm0.7 \text{ servings/1800 kcal}, p<0.01)$ , and the numbers of snacks and alcoholic beverages were significantly decreased during the intervention  $(226\pm63 \text{ kcal/1800 kcal to } 129\pm51 \text{ kcal/1800 kcal}, p<0.01)$ .

#### Feeling of sleepiness and appetite

The subjects' feeling of sleepiness and their appetite before breakfast were evaluated using the VAS scores. The sleepiness score remained unchanged, but the appetite score after the 4-week intervention period was slightly but not significantly higher than that at baseline, indicating that the subjects tended to feel more hungry in the morning (63±19 to 74±15 points, p<0.10).

#### **Biochemical analysis**

The subjects' baseline characteristics were all within the normal ranges. As anthropometric measurements, the abdominal circumference, systolic blood pressure and diastolic blood pressure values did not change with the intervention, whereas the subjects' body weight and BMI were slightly decreased at the end of the intervention. The results of most of the biochemical tests such as total protein and albumin levels showed no significant changes after 4 weeks compared to the baseline values (Table 3). In addition, the serum lipids such as total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were not altered by the 4-week intervention, despite the significant increase in the intake of dietary cholesterol ( $360\pm103$  mg/day to  $495\pm72$  mg/day).

#### Serum concentrations of vitamins and carotenoids

Although the subjects' serum vitamin C and vitamin E levels remained unchanged, the folic acid concentration was significantly increased by the dietary intervention (15.4  $\pm$ 5.9 nmol/L to 18.1 $\pm$ 4.8 nmol/L, *p*<0.05). The serum lutein+zeaxanthin level was slightly increased after the intervention, but the difference was not significant (0.98 $\pm$ 0.43 µmol/L to 1.14 $\pm$ 0.46 µmol/L) (Table 3).

#### Serum antioxidant status

The parameters of the subjects' serum antioxidant status, MDA-LDL concentration, lag time, and antioxidant activity are shown in Table 3. Compared to the baseline values, a significant decrease in the MDA-LDL concentration, which is an index of LDL oxidation ( $124\pm30$  U/L to  $108\pm17$  U/L, p<0.05), and a significant prolongation of the lag time, which is an index of the resistance of free radical-induced LDL lipid peroxidation ( $33.7\pm3.5$  min to  $37.2\pm4.5$  min, p<0.01) were observed. The serum total antioxidant capacity (TAC) value after the intervention was slightly higher than that at baseline, but the difference was not significant ( $1.8\pm0.4$  mM Trolox equiv. to

**Table 3.** The blood biochemical parameters

	Before	After
Total protein (g/L)	$75 \pm 3$	$76\pm4$
Albumin (g/L)	$48\pm2$	$48\pm2$
Glucose (mmol/L)	$4.44 \pm 0.22$	$4.33 \pm 0.33$
Insulin (pmol/L)	$52.1 \pm 25.7$	$49.3 \pm 28.5$
Total cholesterol (mmol/L)	$4.74 \pm 0.67$	$4.87 \pm 0.57$
HDL-C (mmol/L)	$1.86 \pm 0.47$	$1.86 \pm 0.44$
LDL-C (mmol/L)	$2.54 \pm 0.57$	$2.69 \pm 0.41$
Triglyceride (mmol/L)	$0.73 \pm 0.08$	$0.65 \pm 0.05$
Phospholipids (mmol/L)	$2.67\!\pm\!0.32$	$2.62 \pm 0.21$
NEFA (µEq/L)	$414\pm148$	$416 \pm 179$
Iron (µmol/L)	$16.4 \pm 6.43$	$17.6 \pm 6.77$
Ferritin (pmol/L)	$48.1\!\pm\!38.0$	$51.2 \pm 34.8$
Calcium (mmol/L)	$2.42\!\pm\!0.05$	$2.38 \pm 0.05$ *
Magnesium (mmol/L)	$0.82 \pm 0.04$	$0.82 \pm 0.04$
Vitamin C (µmol/L)	$0.53 \pm 0.12$	$0.53 \pm 0.07$
Vitamin E (µmol/L)	$25.5 \pm 4.6$	$23.2 \pm 4.6$
Folic acid (nmol/L)	$15.4 \pm 5.9$	$18.1 \pm 4.8$ *
Lutein+zeaxanthin (µmol/L)	$0.98\!\pm\!0.43$	$1.14 \pm 0.46$
MDA-LDL (U/L)	$124 \pm 8$	$108 \pm 5$ *
Lag time (min)	$33.7 \pm 0.9$	37.2±1.2 **
TAC (mM Trolox equiv.)	$1.8 \pm 0.1$	$2.1 \pm 0.1$

NEFA: nonesterified fatty acids; MDA-LDL: malondialdehyde modified LDL; TAC: total antioxidant capacity.

<sup>\*</sup>Values are mean±SD (n=12–14). Comparisons of the values between before and after the investigation period were performed using a two-tailed, Wilcoxon signed rank test.  $*^{*}p<0.01$ ,  $*^{p}<0.05$ .

2.1±0.3 mM Trolox equiv.).

# DISCUSSION

The additional intake of an egg at breakfast successfully increased the healthy young women's dietary energy percentage from protein and some nutrients' intake, both for breakfast and daily. The 4-week consumption of such a nutritious breakfast led to (1) an increase in the score of adherence to the food-based Japanese dietary guidelines for a healthy diet, (2) a decrease in the consumption of confections, and (3) decreases in the serum MDA-LDL level and the oxidative susceptibility of LDL.

According to Japan's National Health and Nutrition Examination Survey in 2013, 25.4% of Japanese women in their twenties are skipping breakfast.<sup>27</sup> There were fewer breakfast skippers among the present study's subjects, as they were registered dietitians 'in the making' and might be more strongly aware of their meals than others of their generation. The provided breakfast had 556±29 kcal energy, which was enough for a breakfast for a young woman, but this kcal energy value was not significantly different from that of the breakfasts consumed by the subjects before the intervention. On the other hand, the dietary energy percentage from protein both at breakfast and daily was increased, and the daily intakes of some nutrients such as vitamins D, B-12 and C were significantly higher during the intervention.

During the intervention, a decrease of confectionery consumption was observed. Some studies suggested that breakfast consumption is important for the regulation of energy intake.<sup>7,28,29</sup> Tani et al showed that a higher proportion of total energy intake during the morning might reduce the consumption of confections as well as the absolute intake of energy within the day in Japanese women.<sup>7</sup> It is known that a high-protein breakfast that includes

an egg has a greater effect on the subjective feelings of appetite (increased hunger) compared to a normal-protein breakfast.<sup>30-33</sup> A nutritious breakfast with an egg might therefore increase satiety, which may have contributed to the present subjects' decrease in confectionery consumption.

We also assessed the score of adherence to the foodbased Japanese dietary guidelines for a healthy diet based on the Japanese Food Guide Spinning Top.<sup>34</sup> Our subjects' baseline scores were above the mean value of Nishimura et al's study,<sup>25</sup> and their scores during the intervention were slightly increased from baseline  $(38.9\pm7.9 \text{ to } 44.9\pm5.5 \text{ points})$ . The improvement of the score might be attributed to the decrease of confectionery consumption and the increase in the consumption of fruit.

There were no appreciable changes in our subjects' anthropometric measurements or basic biochemistry results during the investigation. Despite a significant increase in the dietary cholesterol ( $360\pm103$  mg/day to  $495\pm72$ mg/day, p<0.001), the subjects' blood cholesterol levels remained unchanged. The lack of the increase of serum cholesterol could be attributed to the relatively low amount of loaded cholesterol and to the subjects' characteristics as young and normolipidemic women. In addition to dietary cholesterol, the balance of saturated fatty acids and unsaturated fatty acids is well known to influence the serum cholesterol level.<sup>35-38</sup> In the present study, the ratio of polyunsaturated to saturated fatty acid (the P/S ratio) during the intervention period was not significantly different from that in the pre-intervention period.

The intake of antioxidants through the daily diet is known to be important in reducing oxidative stress and preventing many diseases. In our subjects, the serum vitamin C and vitamin E concentrations exhibited no significant changes after the dietary intervention, whereas the serum lutein+zeaxanthin level was slightly but not significantly increased ( $0.98\pm0.43 \mu mol/L$  to  $1.14\pm0.46 \mu mol/L$ ). It is known that vitamin E donates its phenolic hydrogen to scavenge free radicals,<sup>39</sup> and carotenoids neutralize singlet oxygen and free radicals and protect against oxidative damage.<sup>40</sup> Lutein and zeaxanthin are antioxidants that are commonly found in egg yolk, which may be important to decrease the susceptibility of the LDL particles to oxidation.<sup>41</sup>

The oxidation of LDL is well known as a risk factor for cardiovascular disease.<sup>42,43</sup> Several studies have shown that egg consumers had higher blood levels of lutein and zeaxanthin.<sup>19,44</sup> In the present study, one of the oxidative products of LDL, i.e., the MDA-LDL concentration, was decreased significantly, and the LDL oxidizability was significantly prolonged after the intervention. The slight increase in the subjects' serum TAC value might suggest improved antioxidant defense capacity. Wang et al. reported that the antioxidant capacity in the blood was associated with the dietary intake of antioxidants.<sup>45</sup>

In addition, the serum folic acid concentration of the subjects was increased after the intervention. Folate is one of the antioxidant vitamins, and its concentration is high in vegetables and fruits as well as eggs. An increased serum folic acid concentration could be beneficial; folate is an important nutrient especially for young women planning a pregnancy, because low folic acid status is known to be associated with neural tube defects,<sup>46</sup> and folate deficiency leads to increased plasma homocysteine concentrations, which are linked to the risks of cardiovascular disease<sup>47</sup> and osteoporosis.<sup>48</sup> The present study's breakfast including an egg provided the benefits of in-

creasing the intakes of protein and essential nutrients (e.g., vitamin D, vitamin B-12 and folate), and it is a good breakfast for all women of childbearing age.

Our study has several limitations. First, the open design and small sample size could be limiting factors for the interpretation of the results. There were both breakfast skippers and breakfast eaters among the subjects. In addition, our findings may not be limited to the effects of eggs because the provided breakfast contained other dietary factors such as vegetables and fruit juice. On the other hand, the study's strengths were that we investigated the dietary records in detail, and that we identified the association between the dietary intervention and blood antioxidant parameters.

In conclusion, eating one egg per day at breakfast could be good for supplying protein and maintaining one's nutritional balance, and we observed that consuming a nutritious breakfast with an egg for 4 weeks positively affected the dietary habits and two serum oxidative stress markers, i.e., the serum MDA-LDL level and the oxidative susceptibility of LDL, in healthy young women. Our findings indicate that eggs are a good source of protein and antioxidants that may be valuable in designing healthy and nutritious diets. Further studies with a larger number of subjects in a randomized crossover design are needed to precisely evaluate the effect of egg consumption on health in young women.

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# AUTHOR DISCLOSURES

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