Low-Volume Exercise Training and Vitamin E Supplementation Attenuates Oxidative Stress in Postmenopausal Women

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Summary The purpose of this study was to investigate the effects of low-volume exercise training (90 min/wk) and vitamin E supplementation on oxidative stress markers in postmenopausal women. The participants were non-randomly assigned the following four groups: control (C, n=8), vitamin E (S, n=8), exercise (Ex, n=6), or vitamin E and exercise (S+Ex, n=7). The S and S+Ex groups were instructed to take vitamin E (α-tocopherol, 300 mg/d) capsules for 12 wk. The exercise program of Ex and S+Ex groups consisted of walking for a 30–60 min/session 2 d per week for 12 wk. The serum derivatives of reactive oxygen metabolites concentrations were significantly decreased in the Ex. and S+Ex groups after 12 wk compared with the baseline values (three-factor ANOVA, an interaction between exercise and time, p<0.05). Conversely, serum biological antioxidant potential concentrations in the S and Ex groups were significantly higher at 12 wk than at the baseline, but not in the S+Ex group (three-factor ANOVA, an interaction between supplementation, exercise and time, p<0.05). Plasma thioredoxin concentrations in the S, Ex, and S+Ex groups were significantly higher at 12 wk than at the baseline values (three-factor ANOVA, interactions between exercise and time, and between supplementation, exercise and time, p<0.05). Our findings suggest that low-volume physical activity may improve resting oxidative stress status in postmenopausal women.

Key Words aging, physical activity, α-tocopherol, oxidative stress, atherosclerosis

Elevated oxidative stress leads to the development of cardiovascular diseases and atherosclerosis (1, 2). Oxidative stress in the vascular wall induces the oxidation of LDL and the expression of inflammatory mediators (3). These changes may be a fundamental causal pathway leading directly or indirectly to loss of elasticity in the arterial wall, and contribute to the initiation of atherosclerosis. Aging is also associated with elevated biomarkers of oxidative stress, and aggravates vascular function (4). These processes are probably related to a sedentary lifestyle and age-related decline in the production of endogenous antioxidants (3, 5). Postmenopausal women in particular do not experience the protective antioxidant benefits and anti-inflammatory effects of estrogen, and are therefore likely to show increased oxidative stress (6, 7). Therefore, it is important to ameliorate oxidative stress by increasing physical activity or taking antioxidants in postmenopausal women.

Cross-sectional studies have revealed an inverse association between physical activity and oxidative damage (8, 9). In addition, intervention studies have reported that endurance training attenuates the resting oxidative stress levels (10, 11). Most exercise intervention studies on oxidative stress markers and antioxidant capacity (12) have been conducted in accordance with physical activity guidelines for older adults (13–15) and World Health Organization’s 2010 Global Recommendations on Physical Activity for Health (≥150 min/wk) (World Health Organization, accessed, November 14, 2012). However, studies from many countries have reported that most older adults do not complete the required amount of physical activity to meet the guidelines set by expert panels (16). Therefore, determining the least amount of physical activity required to alter the levels of oxidative stress markers could have public health implications.

Various forms of vitamin E, especially α-tocopherol, exhibit antioxidant and anti-inflammatory effects and inhibit several diseases, including in atherosclerosis and cardiovascular disease (17, 18). Although findings from both animal and human studies support the hypotheses that both regular physical activity and vitamin E supplementation are useful for reducing oxidative stress, few studies have investigated the effects of combining exercise training with vitamin E supplementation for reducing oxidative stress levels in elderly persons (19). Most of these studies have only investigated the combined...
effects for relatively short periods of time (ranging from a few days to a few weeks) and with relatively high doses of antioxidants (20). Consequently, further research should be conducted to determine whether combining low-volume exercise training and the daily recommended doses of vitamin E supplementation would be effective for improving oxidative stress status in elderly individuals and whether the duration of effects is sustained for longer than a few weeks. Such research implications would be useful from the viewpoint of aging and prevention of diseases related to oxidative stress.

Aim. The purpose of this study was to investigate the effects of a 12-wk low-volume walking program (>150 min/wk) below the current physical activity guideline and vitamin E supplementation on oxidative stress markers in postmenopausal women. We hypothesized that the combination of a 12-wk walking program with vitamin E supplementation would improve oxidative stress status in postmenopausal women to a greater extent than either exercise training or vitamin E alone.

METHODS AND PROCEDURES

Participants. Participants were recruited from the general populations of the local communities. For the baseline evaluation, we used a simple lifestyle-related questionnaire (physical activity, medication, sleep, alcohol intake, and smoking). Patients with a history of cardiovascular disease, physically active lifestyle, intake of lipid- and/or glucose-lowering medication in the previous 3 mo, smoking habit, or age below 60 were excluded from the study. A physically active lifestyle was defined as an exercise program, structured, and repetitive physical activity undertaken a minimum of 3 times per week, lasting at least 30 min per session, for the purpose of improving or maintaining one or more components of physical fitness described by Butcher et al. (21). In fact, none of the study participants were trained athletes competing in any sporting events but some participants were recreationally active. We recruited 32 postmenopausal women who initially met the criteria for the enrollment in the present study. Three participants could not complete the walking program of the present study because of injury or disease (not related to the present study). Consequently, 29 participants were included in the analysis. Twenty-nine postmenopausal women were non-randomly assigned the following four groups: control (C, n = 8), vitamin E (S, n = 8), exercise (Ex, n = 6), or vitamin E and exercise (S+Ex, n = 7). The physical characteristics of the participants are shown in Table 1. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the ethics committees of Waseda University. Informed consent was obtained from all participants after the experiment was described to them in detail.

Anthropometry. Anthropometric variables were measured at baseline and 12 wk. Body mass was measured to the nearest 0.1 kg using a digital balance (Inner Scan 50; Tanita Corporation, Japan). Height was measured to the nearest 0.1 cm at the level of the umbilicus using a wall-mounted stadiometer (YS-OA; As One Corporation, Japan). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured to the nearest 0.1 cm at the level of the umbilicus using a flexible plastic tape measure.

Arterial blood pressure. Arterial blood pressure was measured from the right arm with the subject in a seated position by a standard mercury sphygmomanometer (605P, Yagami Co. Ltd., Japan). Measurements were obtained at baseline and at week 12 in both groups. Participants were seated in a chair for 5 min before measurements. Two measurements were obtained at each time point, and the mean of these values was recorded.

Physical activity measurement. To assess the physical activity of participants, all participants were asked to wear a uniaxial accelerometer (Lifecode-EX; Suzuken Co. Ltd., Japan) for 12 consecutive weeks, i.e., for the entire study period. We collected accelerometer data from all participants simultaneously, indicating that seasonality was not an issue in the present study. The accelerometer measures the magnitude and frequency of accelerations and determines the intensity of activity (11 levels: 0, 0.5, and 1–9, where 0 is the lowest activity level and 9 is the highest) every 4 s. Data from participants who had worn the accelerometer for at least 10 h (total of 40 h) a day for at least 4 weekdays and 1 weekend day (total of 10 h) after calculation of wear time were considered valid (22, 23). The main physical activity variable used in this study was the time spent in moderate to vigorous physical activity (MVPA). MVPA was calculated on a daily basis, and a weighted average of daily weekday and weekend activity was used to calculate the weekly activity: weekly MVPA = (average daily weekday MVPA × 5) + (average daily weekend MVPA × 2). All the recorded minutes with a total of ≥4 activity levels were classified as MVPA. The level 4 activity threshold was derived from a calibration study and corresponded to approximately 3 metabolic equivalents (24).

Exercise intervention. The exercise intervention occurred at the local community area between September 27 and December 16, 2011. All participants of the Ex and S+Ex groups were engaged in a walking program of a 30–60 min/session for 2 d every week for 12 wk. The exercise duration increased from a 30–40 min/session for the first 4 wk to a 60 min/session in the fifth week—a progressive approach is recommended by the physical activity guidelines for older adults (14). Exercise duration was defined as a 30–60 min/session described by the previous study (25). All the exercise programs were performed under trained supervision in the morning (9:00–10:00 AM). About halfway through the walking exercise during each session, the heart rate was measured using a heart rate monitor (Polar-RS400CX™, Polar Electro, Japan) and the perceived exertion (26) was assessed using Borg’s perceived exertion scale. To examine the effects of the walking exercise program and vitamin E supplementation on oxidative stress markers, participants were instructed not to change their lifestyle, including dietary habits, during the study period.
Participants of the control group were advised to maintain their normal lifestyle during the study.

**Vitamin E supplementation.** All S and S+Ex group participants were issued a package containing a 12-wk supply of capsules containing 100 mg vitamin E (α-tocopherol) and were instructed to take 1 capsule 3 times a day (total dose, 300 mg/d) with their morning, afternoon, and evening meals for the entire study period of 12 wk. A previous study has reported that this amount of α-tocopherol resulted in elevated serum α-tocopherol concentrations (27).

**Blood collection and laboratory assays.** After a 48-h period of physical activity avoidance, fasting (overnight for at least 10 h) venous blood samples were taken from the antecubital vein from all the participants at baseline and at 12 wk. In the Ex and S+Ex groups, post-intervention measurements were obtained at least 3 d after the subjects completed the exercise program. For measuring the serum blood markers, blood samples were allowed to clot for 30 min at room temperature and then centrifuged at 3,000 rpm for 10 min at 4°C. The obtained serum was dispensed into plain microtubes, and stored at −80°C until the assay. For measuring the plasma blood markers, venous blood samples were collected into sodium fluoride-ethylenediamine tetraacetic acid (EDTA)-containing tubes. Thereafter, samples were immediately centrifuged and treated as described above.

Serum concentrations of derivatives of reactive oxygen metabolites (d-ROMs) and the biological antioxidant potential (BAP) were measured using assay kits from Driacron (Milan, Italy). Serum concentrations of hexanoyl-lysine (HEL), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and plasma advanced oxidation protein product (AOPP) were measured using assay kits from Nikken Seil Co., Ltd. (Tokyo, Japan). Concentrations of plasma thioredoxin (TRX; Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan) were measured by enzyme-linked immunosorbent assay (ELISA). Plasma superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities were measured using assay kits from Cayman Chemicals (Ann Arbor, MI). The serum α-tocopherol levels were measured using high-performance liquid chromatography (HPLC).

**Dietary assessments.** To determine whether there were between-group differences in dietary α-tocopherol that might have affected levels of oxidative stress markers, the study participants weighed and recorded all the food and drink consumed starting 2 d before the initiation of the study and at week 10 during the study. Dietary records were analyzed using a computerized nutritional analysis system (Kenpakusha, Tokyo, Japan). All the dietary assessments were carried out by a registered dietitian.

**Statistical analyses.** Data were analyzed using Predictive Analytics Software (PASW) version 18.0 for Windows (IBM SPSS Japan Inc., Tokyo, Japan). One-factor analysis of variance (ANOVA) was used to examine the differences in the baseline physical and physiological variables, dietary and physical activity data, and blood markers among the 4 groups. Where significant effects were detected, post-hoc multiple comparisons were made using the Bonferroni method. Significant differences between variables were examined using three-factor ANOVA (supplementation, exercise, and time) with repeated measures. In addition, paired Student’s t tests were used to assess intra-group differences between pre- and post-intervention data. Statistical significance was accepted at the 5% level. The results are presented as means±standard error (SE) of mean.

| Group       | C (n=8) | S (n=8) | Ex (n=6) | S+Ex (n=7) | p-value (S×T, Ex×T, S×Ex×T) |
|-------------|---------|---------|----------|------------|-------------------------------|
| Age (y)     | Baseline: 68.4±1.4 | 66.5±2.0 | 72.0±2.1 | 65.9±2.0 | 0.899, 0.955, 0.915 |
|             | 12 wk: 1.51±0.02 | 1.53±0.01 | 1.53±0.02 | 1.57±0.02 | 0.495, 0.897, 0.551 |
| Height (m)  | Baseline: 1.51±0.02 | 1.53±0.01 | 1.53±0.02 | 1.57±0.02 | 0.495, 0.897, 0.551 |
| Body mass (kg) | Baseline: 51.5±2.1 | 50.2±1.7 | 51.0±1.5 | 58.5±3.3 | 0.899, 0.955, 0.915 |
|             | 12 wk: 51.9±2.0 | 50.6±1.8* | 51.8±1.6 | 58.3±3.4 | 0.886, 0.947, 0.907 |
| Body mass index (kg/m²) | Baseline: 22.3±0.5 | 21.3±0.7 | 21.7±0.4 | 24.4±1.5 | 0.886, 0.947, 0.907 |
|             | 12 wk: 22.5±0.4 | 21.2±0.7* | 22.0±0.4 | 24.3±1.6 | 0.455, 0.857, 0.551 |
| Waist circumference (cm) | Baseline: 78.6±2.0 | 80.9±2.8 | 79.1±2.6 | 84.4±2.8 | 0.564, 0.414, 0.937 |
|             | 12 wk: 78.8±2.5 | 80.5±2.3 | 82.7±2.5* | 82.2±3.8 | 0.374, 0.423, 0.717 |
| Systolic blood pressure (mmHg) | Baseline: 132±3 | 120±4 | 135±4 | 140±4 | 0.564, 0.414, 0.937 |
|             | 12 wk: 138±3 | 123±3 | 137±5 | 137±4 | 0.374, 0.423, 0.717 |
| Diastolic blood pressure (mmHg) | Baseline: 75±2 | 73±2 | 78±3 | 84±4 | 0.564, 0.414, 0.937 |
|             | 12 wk: 80±2 | 72±1 | 78±4 | 81±3 | 0.564, 0.414, 0.937 |

All data are presented as means±SE. C, control group; S, vitamin E group; Ex, exercise group; S+Ex, vitamin E and exercise group.

* Significantly different from the baseline value in the same group (Paired Student’s t-tests, p<0.05).
walking program: i.e. Tuesday and Friday), there was no difference on the days that the participants exercised (days of the groups (post-hoc tests; Ex vs. C and S groups; S Ex vs. C 16.4 min/wk) and S (139.2 1 min/wk) groups than in the C (228.2 22.8 min/wk) and significantly higher in the Ex (219.5 22.4 min/wk) groups.

that the MVP A during the study duration was significantly higher than the corresponding baseline values. These values corresponded to an exercise intensity of 49 6 min and 10.5 0.3 (light), respectively. These values corresponded to an exercise intensity of 49% heart rate reserve.

Exercise adherence and exercise amount

The duration of the walking program was 44.5±1.6 min/session. The heart rate and rate of perceived exertion during the walking program were 113±4 beats/min and 10.5±0.3 (light), respectively. These values corresponded to an exercise intensity of 49±5% heart rate reserve.

One-factor ANOVA revealed a significant difference in the MVPA among groups. Post-hoc tests showed that the MVPA during the study duration was significantly higher in the Ex (219.5±22.4 min/wk) and S+Ex (228.2±22.8 min/wk) groups than in the C (117.1±16.4 min/wk) and S (139.2±13.0 min/wk) groups (post-hoc tests; Ex vs. C and S groups; S+Ex vs. C and S groups). When we excluded the MVPA measured on the days that the participants exercised (days of the walking program: i.e. Tuesday and Friday), there was no difference in the MVPA among groups.

RESULTS

Physical characteristics and dietary data

There were no differences in the physical and physiological characteristics among the groups at the baseline evaluation, except for the systolic blood pressure (SBP) (Table 1). This difference in the SBP among groups at baseline was found to be significant by one-factor ANOVA. The SBP was significantly lower in the S group than in the C and S+Ex groups (post-hoc tests; S vs. C, S vs. S+Ex). Intra-group analyses revealed that the body mass and BMI in the S group at 12 wk were significantly higher than the baseline values, and the waist circumference of the Ex group had significantly increased at 12 wk over that at baseline. Analysis of dietary data revealed that energy (energy, protein, fat, and carbohydrate) and antioxidant intake (vitamin C, vitamin E, and β-carotene) did not differ among groups or within the groups at baseline or during the study (Table 2).

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One-factor ANOVA revealed a significant difference in the MVPA among groups. Post-hoc tests showed that the MVPA during the study duration was significantly higher in the Ex (219.5±22.4 min/wk) and S+Ex (228.2±22.8 min/wk) groups than in the C (117.1±16.4 min/wk) and S (139.2±13.0 min/wk) groups (post-hoc tests; Ex vs. C and S groups; S+Ex vs. C and S groups). When we excluded the MVPA measured on the days that the participants exercised (days of the walking program: i.e. Tuesday and Friday), there was no difference in the MVPA among groups.

Serum α-tocopherol concentrations

At the baseline level, there was no difference in the serum α-tocopherol concentrations among groups (Fig. 1). Three-factor ANOVA with repeated measures showed a significant interaction between supplementation and time also existed. The serum α-tocopherol concentrations in the S and S+Ex groups after 12 wk were significantly higher than the corresponding baseline values.

Plasma and serum oxidative stress markers

There were no differences in the serum d-ROMs concentrations among the groups at the baseline level (Fig. 2a). Three-factor ANOVA with repeated measures showed a significant interaction between exercise and time for serum d-ROMs concentrations. Intra-group

Table 2. The energy and antioxidant intake at baseline and at week 10.

| Group     | Energy (MJ/d) | Protein (g/d) | Fat (g/d) | Carbohydrate (g/d) | Vitamin C (mg) | Vitamin E (mg) | α-Tocopherol (mg/mL) |
|-----------|---------------|---------------|-----------|---------------------|----------------|----------------|---------------------|
| Baseline  | 7.6±0.5       | 71.1±5.7      | 51.1±6.3  | 260.5±6.1           | 103.6±12.9     | 138.3±11.9     | 7.3±0.6             |
| week 10   | 7.4±0.3       | 74.2±5.0      | 56.4±4.4  | 248.6±9.1           | 138.5±14.4     | 146.3±20.3     | 4.0±0.6             |
| C (n=8)   | 228.2±22.8     | 228.2±22.8    | 228.2±22.8| 228.2±22.8          | 228.2±22.8     | 228.2±22.8     | 228.2±22.8         |
| S (n=8)   | 139.2±13.0     | 139.2±13.0    | 139.2±13.0| 139.2±13.0          | 139.2±13.0     | 139.2±13.0     | 139.2±13.0         |
| Ex (n=6)  | 219.5±22.4     | 219.5±22.4    | 219.5±22.4| 219.5±22.4          | 219.5±22.4     | 219.5±22.4     | 219.5±22.4         |
| S+Ex (n=7)| 286.3±19.7    | 286.3±19.7    | 286.3±19.7| 286.3±19.7          | 286.3±19.7     | 286.3±19.7     | 286.3±19.7         |

All data are presented as means±SE. C, control group; S, vitamin E group; Ex, exercise group; S+Ex, vitamin E and exercise group.
Table 4. Antioxidant capacity at baseline and after 12 wk.

| Group | C (n=8) | S (n=8) | Ex (n=6) | S+Ex (n=7) | p-value (S×T, Ex×T, S×Ex×T) |
|-------|---------|---------|----------|------------|-----------------------------|
| HEL (nmol/L) | Baseline | 1.2±0.1 | 1.0±0.2 | 1.1±0.2 | 1.2±0.2 | 0.678, 0.625, 0.774 |
| 12 wk | 1.0±0.1 | 0.7±0.1 | 0.9±0.2* | 0.8±0.1* |
| AOPP (μmol/mL) | Baseline | 40.6±3.4 | 32.2±1.4 | 42.2±8.6 | 51.4±9.5 | 0.231, 0.352, 0.270 |
| 12 wk | 34.3±1.2 | 48.3±6.6 | 37.6±5.6 | 47.7±7.6 |
| 8-OHdG (ng/mL) | Baseline | 1.9±0.2 | 1.1±0.1 | 1.2±0.1 | 1.0±0.2 | 0.947, 0.243, 0.902 |
| 12 wk | 1.7±0.1 | 0.9±0.1 | 1.3±0.1 | 1.1±0.2 |

All data are presented as means±SE. C, control group; S, vitamin E group; Ex, exercise group; S+Ex, vitamin E and exercise group.

HEL, hexanoyl lysine; AOPP, advanced oxidation protein products; 8-OHdG, 8-hydroxy-2‘-deoxyguanosine.

* Significantly different from the baseline value in the same group (Paired Student’s t-test, p<0.05).

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Table 3. Oxidative stress at baseline and after 12 wk.

| Group | C (n=8) | S (n=8) | Ex (n=6) | S+Ex (n=7) | p-value (S×T, Ex×T, S×Ex×T) |
|-------|---------|---------|----------|------------|-----------------------------|
| HEL (nmol/L) | Baseline | 1.2±0.1 | 1.0±0.2 | 1.1±0.2 | 1.2±0.2 | 0.678, 0.625, 0.774 |
| 12 wk | 1.0±0.1 | 0.7±0.1 | 0.9±0.2* | 0.8±0.1* |
| AOPP (μmol/mL) | Baseline | 40.6±3.4 | 32.2±1.4 | 42.2±8.6 | 51.4±9.5 | 0.231, 0.352, 0.270 |
| 12 wk | 34.3±1.2 | 48.3±6.6 | 37.6±5.6 | 47.7±7.6 |
| 8-OHdG (ng/mL) | Baseline | 1.9±0.2 | 1.1±0.1 | 1.2±0.1 | 1.0±0.2 | 0.947, 0.243, 0.902 |
| 12 wk | 1.7±0.1 | 0.9±0.1 | 1.3±0.1 | 1.1±0.2 |

All data are presented as means±SE. C, control group; S, vitamin E group; Ex, exercise group; S+Ex, vitamin E and exercise group.

HEL, hexanoyl lysine; AOPP, advanced oxidation protein products; 8-OHdG, 8-hydroxy-2‘-deoxyguanosine.

* Significantly different from the baseline value in the same group (Paired Student’s t-test, p<0.05).

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Fig. 2. Serum derivatives of reactive oxygen metabolite (d-ROMs) (a) and concentrations of biological antioxidant potential (BAP) (b) measured at baseline and 12 wk in the control (C), vitamin E (S), exercise (Ex), and vitamin E and exercise (S+Ex) groups. Data are presented as means±SE. Supplementation×time (p=0.470), exercise×time (p=0.026), Supplementation×Exercise×time interactions (p=0.136) for serum d-ROMs concentrations. Supplementation×time (p=0.426), exercise×time (p=0.611), Supplementation×Exercise×time interactions (p=0.031) for serum BAP concentrations. * Significantly different from the baseline value in the same group (paired Student’s t-test, p<0.05).
analysis revealed that serum d-ROMs concentrations in the Ex, and S+Ex groups after 12 wk were significantly lower than the baseline values. There were no differences in the serum HEL, 8-OHdG, or plasma AOPP concentrations among groups at the baseline evaluation (Table 3). Intra-group analysis showed that serum HEL concentrations were significantly decreased in the Ex and S+Ex groups after 12 wk as compared with the baseline values. Further, plasma AOPP and serum 8-OHdG concentrations did not differ among the interventions or over time.

**Plasma and serum antioxidant capacity responses**

At baseline, there was no difference in the serum BAP concentrations among groups (Fig. 2b). Three-factor ANOVA with repeated measures showed a significant interaction between supplementation, exercise and time for serum BAP concentrations. Intra-group analysis showed that serum BAP concentrations were significantly increased in the S and Ex groups after 12 wk as compared with the baseline values. At the baseline evaluation, there was a significant difference in the plasma TRX concentration among groups (Table 4). Plasma TRX concentrations were significantly higher in the C group than in the other groups. Three-factor ANOVA with repeated measures showed a significant interaction between exercise and time for plasma TRX concentrations. In addition, three-factor ANOVA with repeated measures revealed a significant interaction between supplementation, exercise and time for plasma TRX concentrations. Intra-group analysis showed that plasma TRX concentrations were significantly increased in the S, Ex, and S+Ex groups after 12 wk as compared with the corresponding baseline values. At baseline, there was no difference in the plasma SOD, CAT or GPX activity among groups (Table 4). Plasma SOD activities did not differ among the interventions or over time (Table 4). Intra-group analysis showed that the plasma CAT activity was significantly increased in the Ex group after 12 wk as compared with the baseline values. Plasma GPX activities did not differ among the interventions or over time (Table 4).

**DISCUSSION**

The main finding of the present study is that a 12-wk walking program, albeit below the current physical activity guideline for the elderly, can decrease serum d-ROMs concentrations and increase plasma TRX concentrations. In addition, a 12-wk regimen of vitamin E supplementation increases serum α-tocopherol concentrations. However, there were no additive effects of combining the exercise regimen and vitamin E supplementation on the improvement of oxidative stress status in postmenopausal women.

Many studies have shown that resting oxidative stress markers decrease after endurance exercise (10, 11). In the present study, exercise training decreased the levels of the oxidative stress marker d-ROMs. The usefulness of d-ROMs as an oxidative stress marker has been demonstrated in several studies (28, 29), suggesting their association with several diseases (2, 30, 31). In addition, although there was no significant interaction of interventions and time, serum HEL concentrations were significantly decreased in the Ex and S+Ex groups after 12 wk as compared with the baseline values. HEL has been identified as a lipid hydroperoxide-modified lysine residue, which is considered to be a useful marker for lipid peroxidation-derived protein modification in the early stage (32). Some studies have reported that the concentration of circulating HEL is a very sensitive biomarker of oxidative stress (33, 34). To our knowledge, ours is the first study to examine the effects of exercise training on the serum HEL concentrations. Dietary antioxidant intake may influence the level of oxidative stress markers (35, 36). In the present study, antioxidant intake (i.e., vitamin C, vitamin E, and β-carotene) did not differ among groups or within the groups at baseline or during the study. These findings suggest that we could minimize the effects of dietary antioxidant intake on blood oxidative stress markers.

The exercise duration (average of 44.5 ± 1.6 min/session 2 d a week) in this study was below the current physical activity guideline for older adults (>150 min/wk) (13, 14). In addition, the exercise intensity (48.2% of HR reserve) we set may be applicable for many older adults who would find it easier (due to low physical fitness levels) to perform low- to moderate-intensity exercise rather than high-intensity exercise (14). Furthermore, Wen et al. demonstrated that approximately 90 min/wk (15 min/d) of physical activity is sufficient to reduce mortality rates and extend the life expectancy among Taiwanese men and women (37). A number of studies have suggested that oxidative stress is widely regarded as being an important component in developing cardiovascular disease and atherosclerosis (1, 38, 39). Thus, the findings in this study extend those of the previous study (37), which suggests that physical activity below the current physical activity guideline can effectively reduce the resting oxidative stress markers and risks of several diseases in postmenopausal women. Determining the minimum threshold of physical activity and exercise intensity required to improve the oxidative stress status could aid in encouraging exercise adherence in the general public.

One possible mechanism to explain this reduction in the resting oxidative stress markers after exercise training may be the increased activity of antioxidant enzyme and non-enzyme antioxidants in tissue. The circulating concentrations of TRX were significantly increased in the Ex group after 12 wk as compared with the baseline values. In addition, although there was no significant interaction of interventions and time, serum BAP concentrations, and CAT activity were significantly increased in the Ex groups after 12 wk as compared with the baseline values. BAP is measured as the reducative ability of antioxidants such as vitamin C, vitamin E, uric acid, and reduced glutathione in the serum (40). In addition, TRX plays an essential role in cellular function and protection by limiting oxidative stress directly via its antioxidant effects; TRX expression is enhanced by acute exercise (41). A previous study reported that
exercise training increased TRX levels in the rat brain (42). However, no information is available regarding the effect of regular exercise training on TRX in humans. Thus, it is noteworthy that increased physical activity may result in elevated plasma TRX concentrations in postmenopausal women.

Vitamin E supplementation resulted in an elevated level of serum α-tocopherol in the S and S+Ex groups. The recommended daily allowance for vitamin E is 15 mg/d and is required to maintain normal serum α-tocopherol concentration (Institute of Medicine, 2000). In Japan, vitamin E intake (i.e. from dietary foods) of 7.0 and 6.5 mg/d is considered adequate for men and women respectively, to maintain the normal serum α-tocopherol concentration of 12 μmol/L (Dietary Reference Intakes for Japanese, 2010). The participants maintained their diet during the intervention period with additional supplementation (i.e. 300 mg/d which is below the upper limit of 800 mg/d), and the serum α-tocopherol concentration increased in the S and S+Ex groups. Although few studies have investigated the effects of vitamin E supplementation alone (most studies have used the combination of antioxidant supplements) on oxidative stress status for older adults, one study has reported that 800 mg/d of vitamin E supplementation was effective for improving the resting oxidative stress status in older adults (19). In addition, a previous study has reported that the estimated relative contribution to the ferric reducing ability (i.e. BAP concentrations) of the blood is 5% for α-tocopherol (43). Consequently, intra-group analysis showed that serum BAP concentrations rose after 12 wk of vitamin E supplementation. These findings indicate that 300 mg/d of vitamin E supplementation alone could elevate the concentrations of serum α-tocopherol and improve antioxidant capacity status in postmenopausal women.

There is little consistency in the literature regarding the effects of the combination of exercise training and vitamin E supplementation on oxidative stress markers. ROS not only cause oxidative damage but they also play a role in cell signalling (20). ROS also activate the antioxidant response element, which controls both resting and exercise-induced expression of antioxidant enzymes (44). Antioxidant supplementation inhibits ROS production, and recent studies have also shown potentially negative effects of high doses of antioxidants on the adaptive responses of antioxidant enzymes (44, 45). In fact, we found no additive effects of combining walking with vitamin E supplementation on the improvement of oxidative stress status, or serum BAP or TRX concentrations in the S+Ex groups. Therefore, our findings indicate that the combination of exercise training and vitamin E supplementation could potentially attenuate the up-regulation of endogenous antioxidant capacity in postmenopausal women.

The present study had some limitations. First, although we applied some exclusion criteria, we could not strictly match the baseline value among groups. Consequently, there were significant differences in SBP. Thus, our results should be interpreted with caution because oxidative stress is related to the pathology of hypertension and arterial stiffness (1, 4). In addition, intra-group analyses revealed that body mass in the S and Ex groups and BMI in the S group significantly increased after the intervention. Although a direct relationship between oxidative stress, body mass and BMI has not been established, these changes may influence the oxidative stress status. Second, we evaluated the baseline physical activity levels of participants by using a simple questionnaire and found that some participants were recreationally active. It is not clear whether the findings of the present study were influenced by the baseline physical activity levels of participants. Thus, additional research will be required to replicate this work in sedentary or inactive older adults.

In conclusion, a 12-wk supervised walking program that was below the current physical activity guideline for older adults (<150 min/wk) improved oxidative stress status in postmenopausal women. However, combining the exercise program and vitamin E supplementation showed no additive effects on the improvement of oxidative stress status in postmenopausal women.

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Conflict of interest

All authors declare no conflicts of interest.

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