Cent. Eur. J. Med. • 8(4) • 2013 • 509-516
DOI: 10.2478/s11536-013-0173-6

VERSITA

Central European Journal of **Medicine**

Role of antioxidant vitamins administration on the oxidative stress

Research Article

Miki Tomoeda[1,2], Chiaki Kubo[2], Hidenori Yoshizawa[2], Michiko Yuki[2], Masanori Kitamura[2], Shigenori Nagata[2], Masahito Murakami[1], Yasuko Nishizawa[2,3], Yasuhiko Tomita[2]

1 Department of Rehabilitation, Kobe International University, 9-1-6, Koyocho-naka, Higashinada, Kobe 658-0032, Japan

2 Departments of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka 537-8511, Japan

3 Departments of Clinical laboratory, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka 537-8511, Japan

Received 22 September 2012; Accepted 19 March 2013

Abstract: The health-promoting effects of antioxidant vitamins C and E supplementation are unclear. This study investigated the effects of vitamins C and E on the activities of reactive oxygen species (ROS)-scavenging enzymes and protein and lipid peroxidation statuses under resting and exercise-induced conditions. Thirteen healthy, previously untrained males (age 20–21 years) participated in this study. Seven subjects performed physical exercise using a cycle ergometer, and six performed a 6-min walk test (6MWT) prior to vitamin administration and after 1-week oral administration of vitamin C (1000 mg/day) and vitamin E (300 IU/day). Venous blood samples were collected before and after exercise. Plasma vitamin C concentration, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, and protein carbonyl and thiobarbituric acid-reactive substance (TBARS) contents were measured. Antioxidant supplementation increased vitamin C concentration by 34% ($p<0.05$), decreased SOD activity by 17% ($p<0.05$), increased GPx activity by 13% ($p<0.05$), and increased the GPx/SOD activity ratio by 37% ($p<0.05$). Protein carbonyl and TBARS contents were unaffected. Antioxidant vitamins effectively increase the plasma GPx/SOD activity ratio, but fail to reduce protein carbonyl levels induced by exercise.

Keywords: *Reactive oxygen species • Antioxidant vitamin • Glutathione peroxidase (GPx)/superoxide dismutase (SOD) activity ratio • Protein carbonyl*

1. Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen in forms such as superoxide anions (O_2-), hydroxyl radicals, and hydrogen peroxide (H_2O_2), and are produced as a result of normal aerobic metabolism. Highly reactive radical species can oxidize lipids, proteins and DNA, potentially leading to various diseases including cancer, arteriosclerosis, and cardiovascular and inflammatory diseases [1].

Exhaustive exercise has been reported to increase ROS in skeletal muscles and other organs [2,3]. It has therefore been suggested that increasing the concentration of antioxidants in muscles could protect against ROS-induced damage [4]. Indeed, oral administration of vitamins C (L-ascorbic acid) and E attenuated exercise-induced oxygen stress [5].

Vitamins C and E are antioxidants that protect against oxidative stress [6]. Vitamin C is an aqueous antioxidant present in the cytosol and extracellular fluid, which interacts directly with free radicals. Vitamin E is

* *E-mail: yasuhiko-tomita@umin.ac.jp*

lipid-soluble and penetrates into cellular membranes to interrupt lipid peroxidation by transferring its own hydrogen. Vitamin E is thus converted to a vitamin E radical, which is reduced by vitamin C to regenerate vitamin E. This reaction in turn produces vitamin C radicals, which are regenerated by glutathione.

Various enzymes are involved in ROS-scavenging pathways, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [7]. SOD-deficient mice showed muscle atrophy [8,9].

However, recent studies have indicated a negative effect of vitamin C on training efficiency [10], and administration of vitamin C prevented the exercise-induced mRNA expression of cytochrome C, as well as SOD and GPx [10]. In addition, ROS have demonstrated a role as essential signaling molecules required for muscle adaptation [11]. Vitamins C and/or E, despite protection against oxidative stress interfere with the adaptive responses to endurance exercise training [10]. Most of the studies have reported that vitamins C and/or E supplementation does not affect redox status [12-14], less have reported attenuation of oxidant stress [10,15], and there are reports indicating a pro-oxidant effect [14]. These differences between responses and adaptation to exercise possible indicate the complexity of redox biochemistry [16].

Based on these findings, we examined the effect of vitamins C and E on ROS-scavenging enzyme activity under resting and occasional-exercise-induced conditions. Intensive exercise using a cycle ergometer and moderate exercise, with a 6-min walk test (6MWT), was employed. In parallel, plasma contents of protein carbonyl and thiobarbituric acid-reactive substance (TBARS) were measured as markers of protein and lipid peroxidations.

2. Materials and Methods

2.1. Subjects

Thirteen healthy, previously untrained males (age 20–21 years) participated in this study. Subjects were divided into two groups: one group exercised using a cycle ergometer (n=7), while the others performed a 6MWT (n=6). Height, weight and body mass index (kg/m^2) were measured before the experiment, and did not differ significantly between the two groups. All subjects were informed about the procedure and possible risks and the rights to terminate the experiment at any time, and all signed written consent forms. This study was approved by the ethical board of Kobe International University.

2.2. Experimental design

Subjects in both groups arrived at 9:30 a.m. and rested for more than 30 min. Venous blood samples were then collected under resting conditions, after which all subjects performed cycle ergometer or 6MWT exercise. Subjects rested for a further 30 min following exercise, after which venous blood samples were collected again. All subjects were then instructed to take vitamin C (1000 mg/day) and vitamin E (300 IU/day) orally for 1 week, and all the procedures (blood-sample collecting pre- and post-exercise) were repeated on the eighth day. Individuals performed the same types of exercise before and after vitamin administration. Participants were instructed to avoid alcohol and caffeine for 24 h preceding each exercise, and to eat no food for 3 h before each blood collection. All subjects were required to avoid strenuous exercise for 1 week preceding and during the course of the experiment.

2.3. Exercise conditions

2.3.1. Cycle ergometer exercise

This study used an electromagnetically controlled cycle ergometer (Konami Sports and Life Co. Ltd., Tokyo, Japan). A preselected workload (30 W) was imposed by a motor-driven flywheel and the subjects maintained pedaling at 90 rpm for 3 min. Thereafter, incremental loading of 10 W/30 sec was started until the subjects were exhausted.

2.3.2. 6MWT exercise

A straight walking course of 10 m with two cones set at both ends as turnaround markers was used. Before exercise, the participants sat in a chair for 3 min and their pulse was measured under resting conditions. During the exercise, all subjects were instructed to walk as fast as possible for 6 min, with no further encouragement during the exercise. Heart rate was measured after completion of the exercise.

2.4. Laboratory analysis

2.4.1. Plasma collection

Peripheral venous blood samples were collected from each subject and centrifuged in $_{K2EDTA}$ tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) at 1000 *g* for 10 min at 4°C. The supernatant plasma fraction was used in the following assays.

2.4.2. Vitamin C concentration

Five hundred-microliter aliquots of plasma were mixed with the same volume of 10% metaphosphoric acid (Wako Pure Chemical Industries Ltd., Osaka, Osaka, Japan) and centrifuged at 10,000 g for 15 min at 4°C. Vitamin C concentration was measured using a vitamin C assay kit (Shima Laboratories Co. Ltd., Itabashi, Tokyo, Japan) according to the manufacturer's protocol. Absorbance of each sample at 530 nm was measured by microplate reader, then plotted on a standard curve generated by serially diluted standard provided by the manufacturer to obtain vitamin C concentration (μg/ml).

2.4.3. GPx activity

GPx activity was measured colorimetrically using a glutathione peroxidase assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. This kit measures GPx activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of an organic hydroperoxide by GPx, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 355 nm. Thus the rate of decrease in absorbance at 355 nm measured by microplate reader is directly proportional to the GPx activity (nmol/min/ml) in the sample.

2.4.4. SOD activity

SOD activity was measured colorimetrically using a superoxide dismutase assay kit (Cayman Chemical) according to the manufacturer's protocol. This assay used tetrazolium to detect superoxide radicals generated by hypoxanthine and xanthine oxidase. One unit of SOD is defined as the amount of enzyme needed to produce 50% dismutation of superoxide radicals. Absorbance of each sample at 440 nm was measured by microplate reader, then plotted on a standard curve generated by serially diluted standard provided by the manufacturer to obtain SOD activity (U/ml). The GPx/SOD activity ratio was calculated.

2.4.5. Protein carbonyl content

Protein carbonyl content was measured colorimetrically using a protein carbonyl assay kit (Cayman Chemical) according to the manufacturer's protocol. In this assay, 2, 4-dinitrophenylhydrazine reacted with protein carbonyls, forming a Schiff base to produce the corresponding hydrazone, which was then analyzed by colorimetrically. Concentration of protein carbonyl (nmol/ml) in each sample was measured by subtracted absorbance of non-reacted sample from reacted sample at 355 nm by microplate reader.

2.4.6. TBARS assay

TBARS were measured colorimetrically using a TBARS assay kit (Cayman Chemical) according to the manufacturer's protocol. Briefly, thiobarbituric acid was reacted with malondialdehyde (MDA) in the samples at 100°C, and the product, MDA-TBA2, was then measured colorimetrically. MDA is a naturally occurring product of lipid peroxidation derived from polyunsaturated fatty acids, and the results therefore reflected the lipid peroxidation in the samples. Absorbance of each sample at 530 nm was measured by microplate reader, and then plotted on a standard curve generated by serially diluted standard provided by the manufacturer to obtain the amount of TBARS (nmol/ml).

2.5. Statistical analysis

Group data are expressed as mean value ± S.E. Comparisons between values were made using paired T-test. Percent change compared to baseline values were analyzed using Wilcoxon signed-rank tests. The level of statistical significance was set at $p<0.05$. All statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Subject characteristics

There were no differences in age, height, weight or body mass index (BMI) between the groups.

3.2. Effects of vitamin administration

The effects of vitamin administration were examined by comparing pre- and post-administrationconcentrations of plasma vitamin C, activities of antioxidant enzymes, and indices of oxidative-stress-related parameters (Fig. 1). Compared to pre-administration conditions, post-administration vitamin C concentration was increased by 34% ($p<0.05$), SOD activity was decreased by 17% ($p<0.05$), and GPx activity was increased by 13% ($p<0.05$). The GPx/SOD activity ratio was increased by 37% ($p<0.05$; Fig. 2) post-administration.

The indices of protein carbonyl formation and TBARS contents showed slight decreases after the antioxidant administration, but the differences were not significant ($p=0.18$ and 0.38, respectively).

3.3. Effects of exercise

Heart rate was increased significantly after cycle ergometer exercise compared to 6MWT exercise ($p<0.05$; Fig. 3), indicating that the ergometer exercise was more strenuous than 6MWT exercise.

Figure 1. Plasma vitamin C concentration increased by 34% (a: $p<0.05$), superoxide dismutase (SOD) activity decreased by 17% (b: $p<0.05$), and glutathione peroxidase (GPx) activity increased by 13% (c: $p<0.05$) after vitamin administration. Protein carbonyl (d) and thiobarbituric acid-reactive substance (TBARS; e) contents were similar pre- and post-vitamin administration. Dotted lines indicate values of each subjects and solid line indicates means ± SE.

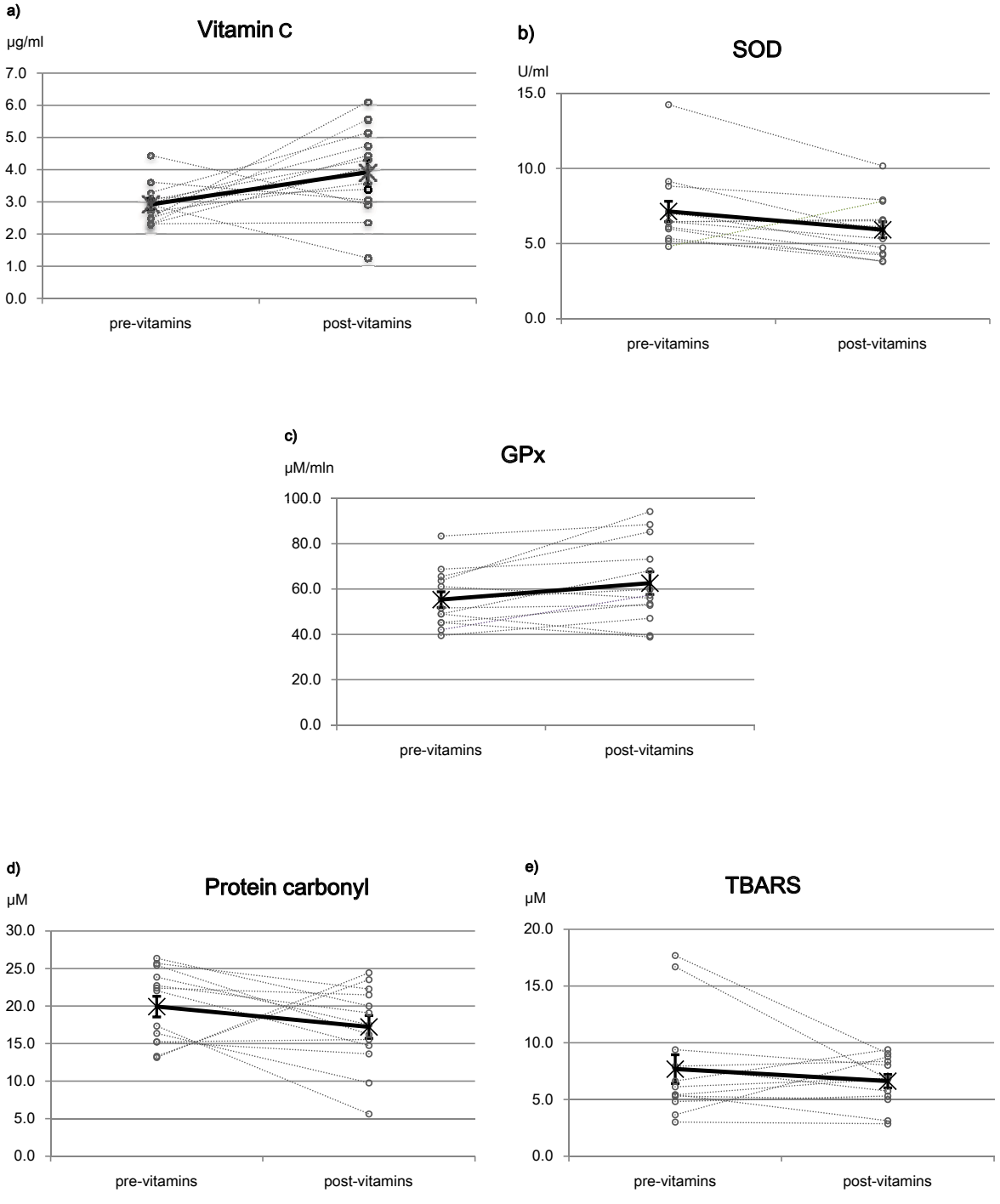
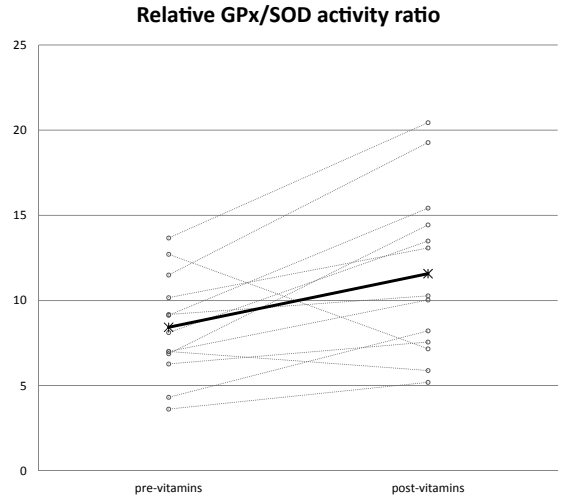


Figure 2. The relative activity ratio of plasma glutathione peroxidase (GPx) to superoxide dismutase (SOD) increased by 37% ($p<0.05$) after vitamin administration. Dotted lines indicate values of each subjects and solid line indicates means ± SE.



3.3.1. 6MWT exercise

SOD activity was decreased by 17% after exercise in the absence of vitamin administration ($p<0.05$; Fig. 4), but the difference was not significant post-vitamin administration. Protein carbonyl content was significantly increased after exercise both pre- (30%; $p<0.05$) and post-vitamin (61%; $p<0.01$) administration. Other factors were unaffected by exercise load.

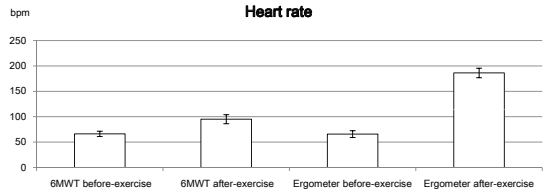
3.3.2. Cycle ergometer exercise

Protein carbonyl content was significantly increased after exercise both pre- (17%; $p<0.05$) and post-vitamin (41%; $p<0.01$) administration. Other factors were unaffected by exercise load.

4. Discussion

The excessive accumulation of ROS is biologically harmful. However, ROS also play important roles in various biological processes such as cell signaling and cellular defense pathways, including extracellular signal-regulated kinase 1/2 and nuclear factor-κB pathways [11,17]. ROS signaling thus contributes to muscle-fiber adaptation in response to both increased contraction activities and prolonged periods of muscle disuse [11]. ROS signals induced by muscle training increased antioxidant enzyme expression in healthy young adults, and the benefits of training were eliminated by antioxidant vitamin administration [10,15]. The benefits of antioxidant administration for reducing oxidative stress and DNA damage remain controversial [18].

Figure 3. Heart rate increased significantly after exercise in the cycle ergometer group compared to the 6-min walk test (6MWT) group ($p<0.05$). Values are means ± SE. bpm; beats per minutes.



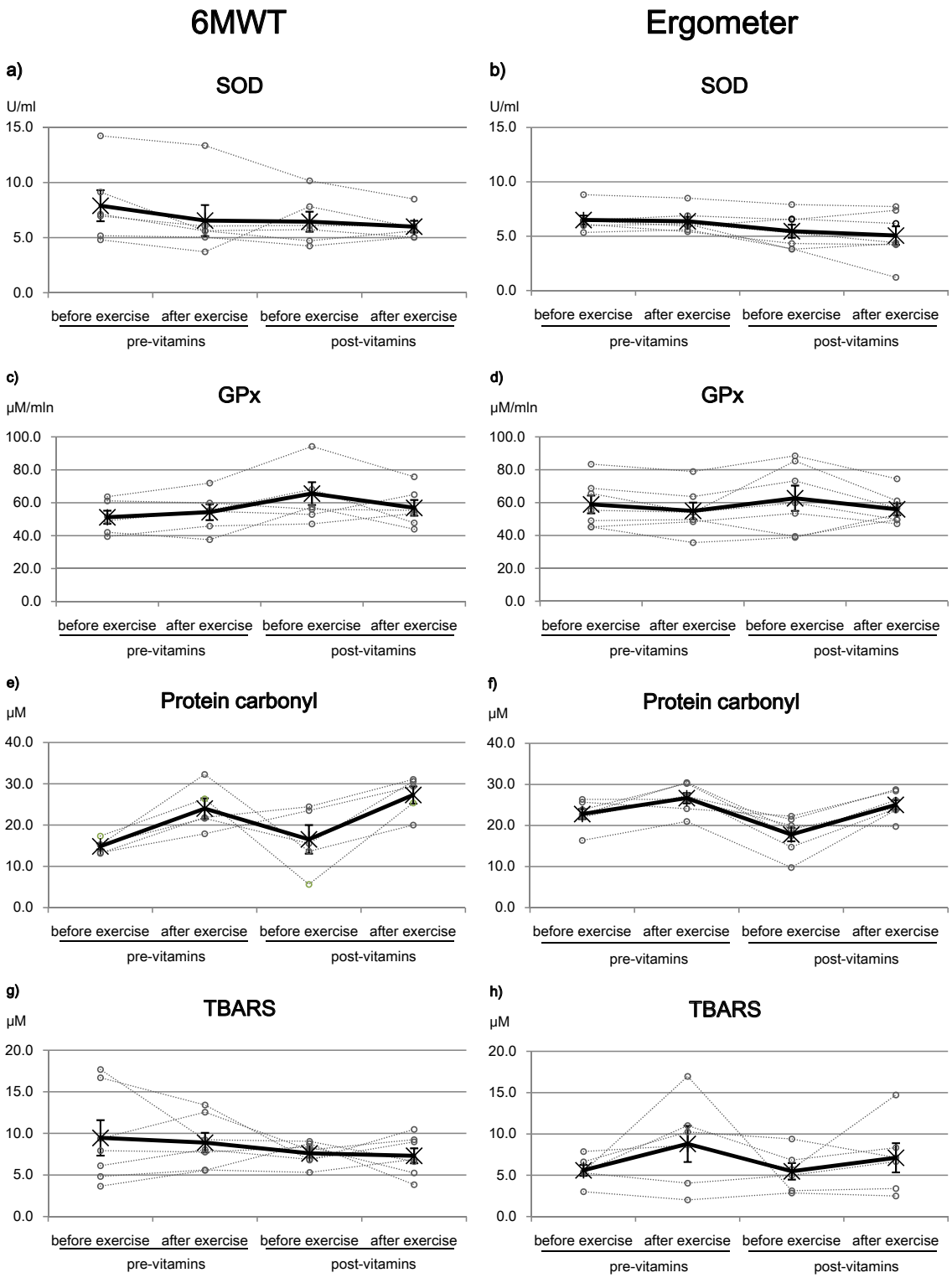
In contrast, antioxidant vitamins have also been reported to demonstrate health-promoting effects. Supplementation with antioxidant vitamins increased arterial elasticity and reduced blood pressure in patients with multiple cardiovascular risk factors [19], slowed down the atherosclerotic progression [20], and showed a beneficial effect on endothelial flow [21]. In a cohort analysis, antioxidant supplementation decreased total cancer incidence and total mortality in men [22]. However, the precise role of antioxidants in these health-promoting effects also remains unclear.

The present study was designed to elucidate the effects of antioxidant vitamins on ROS-scavenging enzyme activity and on protein and lipid peroxidation in healthy, untrained young adults. The duration of vitamins C and E administration was one week, shorter compared to previous reports with supplementation period with over three weeks [10,12-15,18], however, proved that one week antioxidant vitamin administration could increase the plasma concentration of vitamin C. Plasma SOD was significantly decreased while GPx activity increased, resulting in an increase in the GPx/SOD activity ratio. Although the indices of protein carbonyl and TBARS contents showed slight decreases, the differences were not significant.

A fine balance of antioxidant enzymes is necessary for reducing oxidative stresses. Over-scavenging of hydroxyl radicals by excess SOD reduces radical chain termination and results in increased lipid peroxidation [23], and enhanced SOD activity by mutated SOD causes familial amyotrophic lateral sclerosis [24]. Transfection of the Cu, Zn-SOD gene in cultured cells resulted in hypersensitivity to oxidants produced by xanthine/xanthine oxidase [25], while GPx gene transfection protected against hypersensitivity [26]. A low GPx/SOD ratio was associated with susceptibility to radiation pneumonitis in lung cancer patients [27].

Increased SOD activity together with low GPx activity might lead to increased levels of H_2O_2 and H_2O_2-derived reactive species such as hydroxyl radicals. In

Figure 4. Plasma superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, protein carbonyl and thiobarbituric acid-reactive substance (TBARS) in the 6-min walk test (6MWT) and cycle ergometer groups. In the 6MWT group, SOD decreased significantly after exercise pre-vitamin administration (a: $p<0.05$). Protein carbonyl content increased significantly after exercise both pre- and post-vitamin administration in both the 6MWT (e: 30%; $p<0.05$ and 61%; $p<0.01$, respectively) and ergometer groups (f: 17%; $p<0.05$ and 41%; $p<0.01$, respectively). Dotted lines indicate values of each subjects and solid line indicates means ± SE.



the present study, oral administration of vitamins C and E increased the plasma GPx/SOD activity ratio, which might help to protect against oxidative stress. However, further studies are needed to clarify the correlation between GPx/SOD activity ratio and disease occurrence, and the preventive value of increasing the GPx/SOD activity ratio.

Protein carbonyl content increased significantly after exercise, but there were no differences between pre- and post-vitamin administration in either exercise group. There are other trials indicating the protective role of other antioxidants. Methylsulfonylmethane supplementation has been reported to decrease protein carbonyl induced by physical exercise [28], but the results of the present analysis indicated no such effect of antioxidant vitamin supplementation.

Similar studies have provided conflicting data on the effect of antioxidant vitamin supplementation in relation to physical training, probably reflecting methodological differences in assessing oxidative stress or training adaptations. Factors that could also explain such diversities include nutrition, subject characteristics (e.g. gender, age, etc.), the type (aerobic or anaerobic) or the duration of exercise, possible methodological problems or experimental errors, arising from the complexity of the techniques, or finally the biological variability of redox biochemistry itself.

In conclusion, antioxidant vitamin administration effectively increased the plasma GPx/SOD activity ratio, but had no effect on the increase in protein carbonyl content induced by physical exercise.

5. Acknowledgements

The authors are thankful to Daisuke Ishitani, Kenta Iwai, Ken-ichi Umeda, Takashi Saeki, Atsushi Shimomura, Takenori Tani, Nao Nakamichi, Nagisa Honjyo, Saori Miyake, and Tatsuya Yasukawa (Kobe International University) for technical assistance. This work was supported by grants from the Japan Society for the Promotion of Science (24590459), the Osaka Foundation for Prevention of Cancer and Cardiovascular Diseases, and Kobe International University.

References

[1] Halliwell B., Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans, Free Radic. Res. 1996, 25, 57-74

[2] Davies K.J., Quintanilha A.T., Brooks G.A., Packer L., Free radicals and tissue damage produced by exercise, Biochem. Biophys. Res. Commun. 1982, 107, 1198-1205

[3] Commoner B., Townsend J., Pake G.E., Free radicals in biological materials, Nature 1954, 174, 689-691

[4] Sastre J., Asensi M., Gasco E., Pallardo F.V., Ferrero J.A., Furukawa T., et al., Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration, Am. J. Physiol. 1992, 263, R992-995

[5] Goldfarb A.H., McKenzie M.J., Bloomer R.J., Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation, Appl. Physiol. Nutr. Metab. 2007, 32, 1124-1131

[6] Ryan M.J., Dudash H.J., Docherty M., Geronilla K.B., Baker B.A., Haff G.G., et al., Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats, Exp. Gerontol. 2010, 45, 882-895

[7] Xu X.M., Moller S.G., ROS removal by DJ-1: Arabidopsis as a new model to understand Parkinson's Disease, Plant. Signal. Behav. 2010, 5, 1034-1036

[8] Elchuri S., Oberley T.D., Qi W., Eisenstein R.S., Jackson Roberts L., Van Remmen H., et al., CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life, Oncogene 2005, 24, 367-380

[9] Muller F.L., Song W., Liu Y., Chaudhuri A., Pieke-Dahl S., Strong R., et al., Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy, Free Radic. Biol. Med. 2006, 40, 1993-2004

[10] Gomez-Cabrera M.C., Domenech E., Romagnoli M., Arduini A., Borras C., Pallardo F.V., et al., Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance, Am. J. Clin. Nutr. 2008, 87, 142-149

[11] Powers S.K., Duarte J., Kavazis A.N., Talbert E.E., Reactive oxygen species are signalling molecules for skeletal muscle adaptation, Exp. Physiol. 2010, 95, 1-9

[12] Higashida K., Kim S.H., Higuchi M., Holloszy J.O., Han D.H., Normal adaptations to exercise despite protection against oxidative stress, Am. J. Physiol. Endocrinol. Metab. 2011, 301, E779-784
[13] Theodorou A.A., Nikolaidis M.G., Paschalis V., Koutsias S., Panayiotou G., Fatouros I.G., et al., No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training, Am. J. Clin. Nutr. 2011, 93, 1373-1383
[14] Yfanti C., Akerstrom T., Nielsen S., Nielsen A.R., Mounier R., Mortensen O.H., et al., Antioxidant supplementation does not alter endurance training adaptation, Med. Sci. Sports. Exerc. 2010, 42, 1388-1395
[15] Ristow M., Zarse K., Oberbach A., Kloting N., Birringer M., Kiehntopf M., et al., Antioxidants prevent health-promoting effects of physical exercise in humans, Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 8665-8670
[16] Nikolaidis M.G., Kerksick C.M., Lamprecht M., McAnulty S.R., Does vitamin C and E supplementation impair the favorable adaptations of regular exercise?, Oxid. Med. Cell. Longev. 2012, 2012, 707941
[17] Halliwell B., Biochemistry of oxidative stress, Biochem. Soc. Trans. 2007, 35, 1147-1150
[18] Retana-Ugalde R., Casanueva E., Altamirano-Lozano M., Gonzalez-Torres C., Mendoza-Nunez V.M., High dosage of ascorbic acid and alpha-tocopherol is not useful for diminishing oxidative stress and DNA damage in healthy elderly adults, Ann. Nutr. Metab. 2008, 52, 167-173
[19] Shargorodsky M., Debby O., Matas Z., Zimlichman R., Effect of long-term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q10 and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risk factors, Nutr. Metab. (Lond) 2010, 7, 55
[20] Salonen J.T., Nyyssonen K., Salonen R., Lakka H.M., Kaikkonen J., Porkkala-Sarataho E., et al., Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis, J. Intern. Med. 2000, 248, 377-386
[21] Lapointe A., Couillard C., Lemieux S., Effects of dietary factors on oxidation of low-density lipoprotein particles, J. Nutr. Biochem. 2006, 17, 645-658
[22] Hercberg S., Kesse-Guyot E., Druesne-Pecollo N., Touvier M., Favier A., Latino-Martel P., et al., Incidence of cancers, ischemic cardiovascular diseases and mortality during 5-year follow-up after stopping antioxidant vitamins and minerals supplements: a postintervention follow-up in the SU.VI. MAX Study, Int. J. Cancer 2010, 127, 1875-1881
[23] Omar B.A., McCord J.M., The cardioprotective effect of Mn-superoxide dismutase is lost at high doses in the postischemic isolated rabbit heart, Free Radic. Biol. Med. 1990, 9, 473-478
[24] Rosen D.R., Siddique T., Patterson D., Figlewicz D.A., Sapp P., Hentati A., et al., Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature 1993, 362, 59-62
[25] Amstad P., Peskin A., Shah G., Mirault M.E., Moret R., Zbinden I., et al., The balance between Cu,Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress, Biochemistry 1991, 30, 9305-9313
[26] Amstad P., Moret R., Cerutti P., Glutathione peroxidase compensates for the hypersensitivity of Cu,Zn-superoxide dismutase overproducers to oxidant stress, J. Biol. Chem. 1994, 269, 1606-1609
[27] Park E.M., Ramnath N., Yang G.Y., Ahn J.Y., Park Y., Lee T.Y., et al., High superoxide dismutase and low glutathione peroxidase activities in red blood cells predict susceptibility of lung cancer patients to radiation pneumonitis, Free Radic. Biol. Med. 2007, 42, 280-287
[28] Nakhostin-Roohi B., Barmaki S., Khoshkhahesh F., Bohlooli S., Effect of chronic supplementation with methylsulfonylmethane on oxidative stress following acute exercise in untrained healthy men, J. Pharm. Pharmacol. 2011, 63, 1290-1294