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Interaction of vitamin E and exercise training on oxidative stress and antioxidant enzyme activities in rat skeletal muscles☆

Chen-Kang Chang[a], Hui-Yu Huang[b], Hung-Fu Tseng[c], Yan-Der Hsuuw[d], Tim K. Tso[b,*]

[a]Sport Science Research Center, National Taiwan College of Physical Education, Taichung 404, Taiwan
[b]Graduate Institute of Food Science, Nutrition and Nutraceutical Biotechnology, Shih-Chien University, Taipei 104, Taiwan
[c]Institute of Medical Research, Chang-Jung Christian University, Tainan 711, Taiwan
[d]Department of Animal Science, National Pingtung University of Science and Technology, Pingtung 912, Taiwan

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Abstract

It has been shown that free radicals are increased during intensive exercise. We hypothesized that vitamin E (vit E) deficiency, which will increase oxidative stress, would augment the training-induced adaptation of antioxidant enzymes. This study investigated the interaction effect of vit E and exercise training on oxidative stress markers and activities of antioxidant enzymes in red quadriceps and white gastrocnemius of rats in a 2×2 design. Thirty-two male rats were divided into trained vit E-adequate, trained vit E-deficient, untrained vit E-adequate, and untrained vit E-deficient groups. The two trained groups swam 6 h/day, 6 days/week for 8 weeks. The two vit E-deficient groups consumed vit E-free diet for 8 weeks. Vitamin E–training interaction effect was significant on thiobarbituric acid reactive substances (TBARSs), glutathione peroxidase (GPX), and superoxide dismutase (SOD) in both muscles. The trained vit E-deficient group showed the highest TBARS and GPX activity and the lowest SOD activity in both muscles. A significant vit E effect on glutathione reductase and catalase was present in both muscles. Glutathione reductase and catalase activities were significantly lower in the two vit E-adequate groups combined than in the two vit E-deficient groups combined in both muscles. This study shows that vit E status and exercise training have interactive effect on oxidative stress and GPX and SOD activities in rat skeletal muscles. Vitamin E deprivation augmented the exercise-induced elevation in GPX activity while inhibiting exercise-induced SOD activity, possibly through elevated oxidative stress.

Keywords: Glutathione peroxidase; Superoxide dismutase; Glutathione reductase; Catalase; Swimming

1. Introduction

It has been shown in vivo, with electron spin resonance [1] and dichlorofluorescin [2], that free radical production is elevated after strenuous physical activities in humans and animal models. In addition, markers of free radical damage, such as exhaled ethane and pentane [3,4], thiobarbituric acid reactive substances (TBARSs) [5,6], conjugated diene [7], LDL susceptibility to oxidation [8], autoantibody against oxidized LDL [9], protein carbonyl concentration [10], and urinary 8-hydroxy-deoxyguanine secretion [11,12], were all elevated after strenuous aerobic exercises or long-term training and competition. The major sources of exercise-induced free radicals are mitochondrial electron transport chain, xanthine oxidase, membrane-bound NAD(P)H oxidase, and inflammatory response [13,14].

Most studies suggest that endurance training could elevate antioxidant enzymes, such as glutathione peroxidase (GPX) and superoxide dismutase (SOD) in humans [15–17] and rats [18,19]. These enzymes scavenge free radicals and peroxides and protect cells from oxidative damage [20]. The adaptation appeared to vary in different muscles [21–23]. However, contradictory results of the exercise effect on antioxidant enzymes do exist [24,25].

Vitamin E (vit E) is a potent lipid-soluble antioxidant in biological system with the ability to directly quench free radicals and function as a membrane stabilizer. The protective effect of vit E supplementation against exercise-induced oxidative stress in humans [26] and rats [27,28] has been

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* Corresponding author. Tel.: +886 2 25381111x6222; fax: +886 2 25334789.
E-mail address: timtso@mail.usc.edu.tw (T.K. Tso).

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reported. In addition, vit E deficiency may augment free radical production after exercise [29].

It has been shown that chronic exposure to oxidative stress could induce the expression of antioxidant enzymes in tissue cultures [30,31]. Although there is growing evidence that free radicals, other than inducing oxidative modifications of biomolecules, may serve as an important signal for training-induced adaptations [32], few studies have examined the effect of antioxidant status on training-induced change in antioxidant enzymes. Therefore, the present study investigated the interaction effect of vit E and exercise training on oxidative stress and antioxidant enzyme activities in a 2×2 design. We hypothesized that vit E deficiency, which will increase oxidative stress, would augment the training-induced adaptation of antioxidant enzymes. Specifically, oxidative stress markers TBARS and protein carbonyl concentrations and activities of antioxidant enzymes, including GPX, SOD, glutathione reductase (GRd), and catalase, in red quadriceps and white gastrocnemius of rats were measured.

2. Materials and methods

2.1. Animal care

Thirty-two adult male Sprague–Dawley rats, 3–4 months old, were randomly assigned to the trained vit E-adequate (YTYE), trained vit E-deficient (YTNE), untrained vit E-adequate (NTYE), and untrained vit E-deficient (NTNE) groups with eight animals in each group. Rats were housed individually in a temperature-controlled (22 °C) room with a 12:12-h light–dark cycle. Animals were given free access to water and their respective diet. The animal protocol was approved by Laboratory Animal Care Committee, Fooyin University.

2.2. Experimental diets

The two vit E-adequate groups were given AIN-93G diet for rodents [33] (ICN Biomedicals, Costa Mesa, CA). The two vit E-deficient groups were given a diet with the same compositions as AIN-93G, but without vit E (ICN Biomedicals). All diets were put in small feed cups and changed daily to prevent lipid oxidation.

2.3. Animal training program

The daily training program was consisted of two 3-h swimming sessions in water tanks with 45 min of rest in between. Each tank contained only one rat. The two exercise groups were trained 6 days/week for 8 weeks. Both groups swam for the same amount of time in each session. The training was conducted in a temperature-controlled (22 °C) room.

2.4. Tissue preparation

The two trained groups were sacrificed 2 days after their last exercise session to prevent any acute exercise effect. Rats were anesthetized with pentobarbital sodium (6 mg per 100-g body mass) by intraperitoneal injection. Red quadriceps and white gastrocnemius were collected and frozen in liquid nitrogen as soon as possible.

Muscles were homogenized in 0.1 M potassium phosphate buffer (pH 7.4) containing 90 μM butylhydroxytoluene with a motor-driven homogenizer in an ice bucket. The supernatant was collected after centrifuging at 1000×*g* for 5 min at 4 °C and stored at −70 °C for further analyses. Total protein content of muscle samples was measured immediately prior to each TBARS and enzyme assays with biuret method using a commercial kit (total protein liquicolor, Stanbio Laboratory, Boerne, TX).

2.5. Determination of TBARS and protein carbonyl concentration

Thiobarbituric acid reactive substances were measured, according to Oteiza et al. [34], with slight modifications. A mixture of 150 μl of muscle homogenate, 0.5 ml of 30 g/L sodium dodecyl sulfate, 2 ml of 0.1 M HCl, 0.3 ml of 10 g/L phosphotungstic acid, and 1 ml of 7 g/L 2-thiobarbituric acid was incubated in boiling water for 30 min. After cooling, 5 ml of 1-butanol was added. The organic layer was collected after centrifuging at 1000×*g* for 10 min at 4 °C. The absorbance was measured at 532 nm and compared with a standard curve constructed with known concentrations of 1,1,3,3-tetramethoxypropane. The data were expressed as nanomoles MDA per milligram protein.

Protein carbonyl concentration was measured with ELISA using Zentech PC test kit (Zenith, Dunedin, New Zealand). The absorbance at 450 nm was read with a microplate reader (Dynex, Chantilly, VA).

2.6. Antioxidant enzyme activities

Glutathione peroxidase activity in muscle homogenate was assayed as NADPH decreased per minute by a coupled reaction with GRd [35]. The data were expressed as units per milligram protein (nanomole NADPH per minute per milligram protein).

Superoxide dismutase activity was measured according to Flohe and Otting [36]. An aliquot of 25 μl of muscle homogenate was incubated at 37 °C with 1.45 ml of PPB containing 50 μM xanthine, 20 μM cytochrome *c*, and 1 mM EDTA. Absorbance at 550 nm was measured for 3 min after adding 125 μl of 80 U/L xanthine oxidase. The change in absorbance per minute was compared to a standard curve constructed with known units of SOD, and the data were expressed as units per milligram protein.

Glutathione reductase activity in muscle homogenate was measured with a commercial kit according to manufacturer's procedures (Sigma, St. Louis, MO). A first-order reaction rate constant (k) was used as the indicator of catalase activity [37]. Briefly, 20 μl of muscle homogenate, 500 μl of 0.1 M PPB, and 480 μl of 37.5 mM H_2O_2 were incubated, and the absorbance at 240 nm was read for 15 s. The rate constant k was calculated as 0.143 ($\log A_1/A_2$), where A_1 is

A_{240} at $t=0$ and A_2 is A_{240} at $t=15$ s. The data were expressed as k per milligram protein.

2.7. Muscle α-tocopherol concentrations

Muscle α-tocopherol concentrations were measured, according to Bieri et al. [38], with modifications for tissue samples. Lipids were extracted from muscle homogenates with 2.5 ml of *n*-hexane containing 0.00125% butylhydroxytoluene. After centrifugation, the organic layer was collected and dried under nitrogen at 4 °C with an MGS-1000 pressured gas blowing concentrator (Eyela, Tokyo, Japan). The lipid was then redissolved in 250 μl of methanol and analyzed using a high-performance liquid chromatography (HPLC D-7000; Hitachi, Tokyo, Japan) equipped with a C_{18} column (Waters, Milford, MA). The mobile phase was methanol/water (98:2) at flow rate of 2.5 ml/min. The amount of α-tocopherol in the muscle was determined by comparing to the peak area of the internal standard and was expressed as micromoles per gram protein.

2.8. Statistical analysis

The data were expressed as mean values and their standard errors (S.E.). The variables were analyzed by two-way analysis of variance (ANOVA) with vit E status and training as factors. When a significant interaction effect was found, Scheffe post hoc tests were performed. When a significant vit E or training effect was found and the interaction effect was insignificant, one-way ANOVA was used to compare the means of the two groups combined. All analyses were performed using SPSS 11.0 for Windows (SPSS, Chicago, IL). The statistical significance level was set at $P<.05$.

3. Results

Table 1 shows α-tocopherol concentrations in red quadriceps and white gastrocnemius in the four groups. Vitamin E deprivation resulted in significant decreases in muscle α-tocopherol concentrations in both muscles.

Table 1
α-Tocopherol concentrations in red quadriceps and white gastrocnemius in different groups[a]

Group	Red quadriceps	White gastrocnemius
	α-Tocopherol (μmol/g protein)	α-Tocopherol (μmol/g protein)
YTYE	0.393±0.022	0.340±0.034
NTYE	0.393±0.050	0.297±0.096
YTNE	0.059±0.010	0.129±0.013
NTNE	0.034±0.070	0.118±0.015
Main effect (P value)[b]		
Vit E	0.001*	0.001*
Training	0.563	0.583
Interaction	0.727	0.569

[a] All values are means±S.E.
[b] Determined by two-way ANOVA.
* $P<.01$.

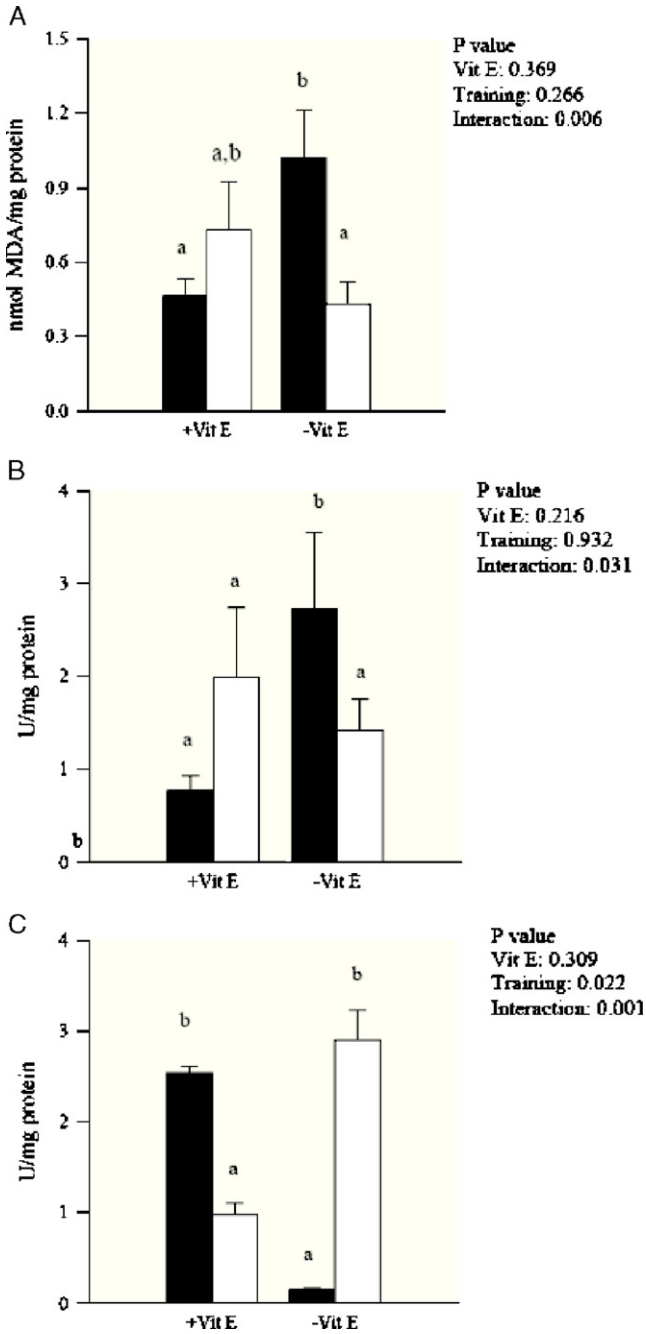

Fig. 1. Thiobarbituric acid reactive substance levels and GPX and SOD activities in red quadriceps in different groups: (A) TBARS, (B) GPX, (C) SOD. Closed bar, trained; open bar, untrained. Data were means±S.E. The P values were determined by two-way ANOVA. Bars with uncommon letters in the same figure were significantly different.

Fig. 1 shows the TBARS levels and GPX and SOD activities in red quadriceps in the four groups. Vitamin E–training interaction effect was significant on TBARS ($P=.0006$, Fig. 1A) and GPX ($P=.031$, Fig. 1B). The YTNE group showed higher TBARS levels than the YTYE and NTNE group. The YTNE group also had higher GPX activities than the other three groups. Training and vit E–training interaction effects were significant on SOD

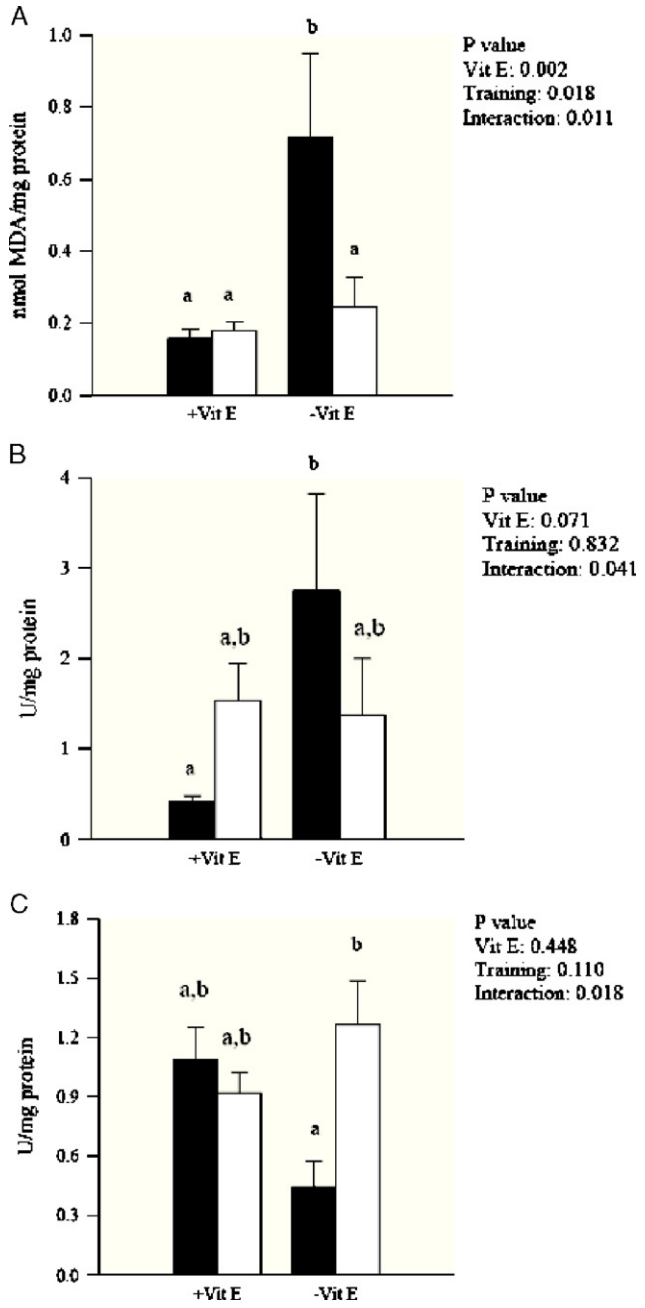

Fig. 2. Thiobarbituric acid reactive substance levels and GPX and SOD activities in white gastrocnemius in different groups: (A) TBARS, (B) GPX, (C) SOD. Closed bar, trained; open bar, untrained. Data were means±S.E. The *P* values were determined by two-way ANOVA. Bars with uncommon letters in the same figure were significantly different.

activities (*P*=.022 and .001, respectively, Fig. 1C). While SOD activity in the presence of vit E was significantly greater for the trained group, the reverse situation was found in the absence of vit E.

Thiobarbituric acid reactive substance levels and GPX and SOD activities in white gastrocnemius are presented in Fig. 2. Vitamin E, training, and vit E–training interaction effects were significant on TBARS (*P*=.002, .018, and .011, respectively, Fig. 2A). Similar to red quadriceps, the YTNE group showed higher TBARS levels than the other three groups. Vitamin E–training interaction effect was significant on GPX (*P*=.041, Fig. 2B) and SOD activities (*P*=.018, Fig. 2C). The YTNE group showed significantly higher GPX activity than the YTYE group. The YTNE group also had lower SOD activity than the NTNE group.

Table 2 shows protein carbonyl concentrations in red quadriceps and white gastrocnemius in the four groups. A significant vit E effect (*P*=.020) was found in red quadriceps. Since the interaction effect was absent, the individual effect of vit E can be analyzed. Protein carbonyl level was significantly higher in the two vit E-deficient groups combined than the two vit E-adequate groups combined (*P*=.013). No significant main effect was found in protein carbonyl levels in white gastrocnemius.

Glutathione reductase and catalase activities in red quadriceps and white gastrocnemius in the four groups are shown in Table 3. A significant vit E effect on GRd and catalase activities was present in both muscles. Training effect was significant on catalase activity in gastrocnemius. Vitamin E–training interaction effect was insignificant in both muscles. Therefore, individual vit E or training effect was analyzed. Glutathione reductase and catalase activities were significantly lower in the two vit E-adequate groups combined than in the two vit E-deficient groups combined in both muscles (red quadriceps: *P*=.001 and .003, respectively; white gastrocnemius: *P*=.045 and .041, respectively). In white gastrocnemius, the two trained groups combined had significantly higher catalase activity (*P*=.036) than the two untrained groups combined.

4. Discussion

Our results showed a significant vit E–training interaction effect on TBARS and GPX and SOD activities in red quadriceps and white gastrocnemius of rats. To our knowledge, this is the first study that suggested an interactive effect of vit E and exercise training on oxidative stress and antioxidant enzyme activities in rat skeletal muscle. This study

Table 2
Protein carbonyl concentrations in red quadriceps and white gastrocnemius in different groups[a]

Group	Red quadriceps	White gastrocnemius
	Protein carbonyl (nmol/mg protein)	Protein carbonyl (nmol/mg protein)
YTYE	0.039±0.007	0.031±0.009
NTYE	0.045±0.009	0.040±0.004
YTNE	0.061±0.007	0.043±0.009
NTNE	0.060±0.006	0.034±0.003
Main effect (*P* value)[b]		
Vit E	0.020*	0.162
Training	0.751	0.234
Interaction	0.675	0.500

[a] All values are means±S.E.
[b] Determined by two-way ANOVA.
* *P*<.05.

Table 3
Glutathione reductase and catalase activities in quadriceps and gastrocnemius in different groups[a]

Group	Red quadriceps		White gastrocnemius	
	GRd (U/g protein)	Catalase (k/mg protein)	GRd (U/g protein)	Catalase (k/mg protein)
YTYE	12.89±1.86	0.36±0.02	13.03±1.62	0.52±0.14
NTYE	16.74±2.71	0.26±0.02	11.33±1.62	0.19±0.04
YTNE	23.02±3.30	1.01±0.38	15.26±1.12	1.36±0.76
NTNE	30.99±4.26	0.79±0.21	21.19±4.18	0.65±0.12
Main effect (P value)[b]				
Vit E	0.001**	0.003**	0.043*	0.010*
Training	0.087	0.385	0.190	0.034*
Interaction	0.539	0.740	0.248	0.428

[a] All values are means±S.E.
[b] Determined by two-way ANOVA.
* $P<.05$.
** $P<.01$.

indicated that vit E status may influence the training-induced adaptation of GPX and SOD activities in rat skeletal muscle.

Thiobarbituric acid reactive substance level was the highest in the YTNE group in both red quadriceps and white gastrocnemius, which resulted from the combination of antioxidant deficiency and intensive exercise. A significant vit E–training effect on TBARS suggested that vit E deprivation would augment the oxidative stress induced by exercise training, while regular exercise training could protect muscles from oxidative stress with adequate vit E.

Most studies reported an increase in GPX activity after regular exercise training, and the adaptation seemed to be muscle specific in rats [39]. A 10-week running program on treadmill resulted in higher GPX activity in soleus and red gastrocnemius in rats [21]. Criswell et al. [40] showed an increased GPX activity in soleus, but not in rectus femoris and gastrocnemius after 12 weeks of interval or continuous training. On the contrary, Powers et al. [22] reported increased GPX activity in red gastrocnemius, but not in soleus after training. Leeuwenburgh et al. [23] also discovered an elevated GPX activity in deep vastus lateralis while no change in soleus after 10 weeks of aerobic training on a treadmill. Our results did not show significant training effect on GPX activity in either muscle group investigated. However, vit E–training interaction effect was significant in both muscles. The pattern of GPX activities in the four groups was parallel to that of TBARS. The YTNE group, which had the highest oxidative stress as indicated by the highest TBARS level, also showed the highest GPX activities among all groups in both muscles. It indicated that exercise-induced GPX adaptation may be augmented by vit E deficiency. The increase in GPX activity in response to oxidative stress may happen at transcriptional level as its mRNA expression was increased under elevated oxidative stress in rat diaphragm [41].

Activities of both cytosolic and mitochondrial isoforms of SOD (Cu-Zn-SOD and Mn-SOD, respectively) were increased in rat muscles in response to endurance training in most studies [22,42]. The adaptation of SOD also appeared to be muscle specific with inconsistent results. An increased SOD activity has been reported in soleus [22,40], but not in rectus femoris and gastrocnemius after training [40]. On the other hand, an elevated SOD activity was reported in deep vastus lateralis, but not in soleus after 10 weeks of aerobic training [23]. Some other studies reported no change in SOD activity after regular training [24,25]. The inconsistent results from previous studies may at least partially result from different antioxidant status of the experimental animals, as the current study showed a significant vit E–training interaction effect on SOD activity in both muscles investigated. In our study, SOD activities in the four groups showed an inverse pattern of TBARS in both muscles. The YTNE group, which had the highest oxidative stress as indicated by the highest TBARS level, showed the lowest SOD activity among all groups in both muscles. On the other hand, the NTNE group with low TBARS level showed high SOD activity in red quadriceps and white gastrocnemius. It has been suggested that Cu-Zn-SOD may be inactivated by reactive oxygen species, at least in vitro [43]. Itoh et al. [41] also reported that regular exercise resulted in lower Mn-SOD and Cu-Zn-SOD activities in diaphragm compared to the sedentary group in calcium-restricted rats, a condition with elevated oxidative damage. However, Mn-SOD mRNA was higher in exercised rats than that in the sedentary, suggesting that Mn-SOD may be inactivated by free radicals at posttranscriptional level [41].

There is little agreement in training effect on GRd and catalase activities in rat skeletal muscles. Glutathione reductase activity was reported to be increased [44], decreased [45,46], or unchanged [47] in rat muscles after exercise. Stimulatory [18,48], inhibitory [24,45], or no effect [22] has been reported on catalase activity after training or acute bout of exercise. Our results showed that the two vit E-deficient groups had significantly higher GRd and catalase activities than the two vit E-adequate groups in both muscles. These results indicated that vit E deficiency alone could up-regulate GRd and catalase activities. Glutathione reductase and catalase may be more

sensitive to the cellular antioxidant status rather than the free radical level. Catalase may also be stimulated by exercise training in white gastrocnemius, but not in red quadriceps.

The difference in adaptation of various antioxidant enzyme activities to vit E status and exercise training may result from their dissimilarity in cellular regulation pathways. Free radicals have been hypothesized to serve as a signal for adaptation to training, including increased antioxidative enzyme activities [32,49]. Free radicals could affect the expression of variety of kinases involved in several signal transduction pathways [50]. Furthermore, certain transcription factors such as nuclear factor-κB and activator protein-1 may be regulated by cellular redox states [13]. However, the detailed mechanism of regulation of the genes of these antioxidative enzymes and the role of free radicals on the adaptation response still remain to be elucidated.

Our results showed that consuming a vit E-deficient diet for 8 weeks resulted in approximately an 88% reduction in α-tocopherol concentration in red quadriceps and a 61% reduction in white gastrocnemius. This was similar to previous results that revealed a 35.8% decrease of vit E content in skeletal muscles after 5 weeks and a 61.2% decrease after 12 weeks in rats maintained on a vit E-deficient diet [51].

Protein carbonyl concentration, a marker of oxidative protein, has been shown to increase [10] or remain unchanged [2] after endurance exercise in rat skeletal muscles. This study showed that protein carbonyl concentration was elevated in the two vit E-deficient groups in red quadriceps, but remained unchanged in white gastrocnemius. The change in protein carbonyl concentration was not correlated with TBARS, a nonspecific marker of lipid peroxidation. The peroxidation of protein and lipid may involve different mechanisms [52].

The intensity of swimming exercise used in this study was similar to other studies that showed adaptation in antioxidant system [53] and energy metabolism [54,55]. Quadriceps and gastrocnemius were recruited during swimming exercise as suggested by the increase in citrate synthase activity and depletion of muscle glycogen in both muscles after such exercise [56,57].

In summary, this study shows that vit E status and exercise training have interactive effect on oxidative stress and GPX and SOD activities in rat skeletal muscles. Vitamin E deprivation augmented the exercise-induced elevation in GPX activity while inhibited the exercise-induced SOD activity, possibly through elevated oxidative stress. Further research is required to determine the role of free radical in exercise-induced adaptation in certain antioxidant enzymes.

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