Vitamin E Reduces Glucocorticoid-Induced Oxidative Stress in Rat Skeletal Muscle

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Summary The purpose of this study was to investigate the effect of vitamin E on oxidative stress in the skeletal muscle of glucocorticoid-treated rats. Male Sprague-Dawley rats (5 weeks of age) were fed a basal diet or a diet supplemented with vitamin E (5,000 mg DL-α-tocopherol acetate/kg diet) for 10 d. The rats of both diet groups received subcutaneous injections of corticosterone (CTC) (0, 25, and 100 mg/kg body weight/d) during the final 4 d. Weights of the extensor digitorum longus and gastrocnemius (GAST) muscles were dose-dependently reduced by CTC. However, the muscle weight losses in rats fed the vitamin E diet were smaller than those in rats fed the basal diet. Protein carbonyl content in the GAST muscle, which was determined as an index of oxidatively modified protein, was increased by 100 mg of CTC, and the increment was significantly (p<0.01) reduced by vitamin E supplement. Hyperglycemia was induced by 100 mg of CTC, but it was not affected by vitamin E. Lipid peroxide (TBARS) in plasma and in GAST muscle was elevated by 100 mg of CTC, and vitamin E significantly (p<0.001) suppressed the formation of TBARS in the muscle. The change in TBARS paralleled that in protein carbonyl. These results show that CTC leads to oxidative stress in rat skeletal muscles and that vitamin E has roles in reducing the oxidative stress which causes muscle atrophy.

Key Words vitamin E, corticosterone, oxidative stress, skeletal muscle

An administration of glucocorticoid to animals results in increased muscle proteolysis and growth inhibition (1,2). Our previous study has shown that corticosterone (CTC)-induced growth inhibition was accompanied by an increased lipid peroxidation in the liver, indicating that CTC develops oxidative stresses in tissues, which may accelerate muscle growth inhibition and atrophy. In contrast,

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vitamin E effectively reduces lipid peroxidation and growth inhibition (3). However, the effect of vitamin E on CTC-induced oxidative stress in muscle tissue has not been studied.

Lipid peroxidation is intimately involved in the pathogenesis of cellular injury by oxygen radicals (4), and evidence for an involvement of free radicals has been presented in the muscular dystrophy (5). Skeletal muscle proteins could be damaged (6) or modified (7) by oxidative stress. On the other hand, vitamin E is well known to have beneficial effects on skeletal muscle damage by protecting the cell membrane against oxidative stress (8, 9).

It has also been known that glucocorticoid induces hyperglycemia and that sustained excess glucose in blood can nonenzymatically react with amino groups of protein (10). Thus the muscle protein of glucocorticoid-treated rats may be susceptible to protein modification because of glycation. Furthermore, it is known that oxidatively damaged or modified protein can be degraded by proteinases, especially by the ubiquitin-proteasome system (11, 12), resulting in muscle atrophy.

Therefore in the present study, we examined the effects of vitamin E on muscle growth and protein carbonyl content and lipid peroxidation in plasma and in muscle of rats treated with CTC.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats at 3 weeks of age were obtained from SLC (Shizuoka, Japan) immediately after weaning and were individually housed in stainless-steel wire-mesh cages in a temperature-controlled room at 24°C with 55% relative humidity and a 12 h light-dark cycle. During a 14 d prefeeding period, the rats were fed a basal diet described below. They were allowed free access to the diet and water.

**Diets.** The experimental diet was prepared as previously reported (1, 3). The source of carbohydrate was cornstarch (Oriental Yeast, Tokyo, Japan). The source of protein was casein (Oriental Yeast). The source of fat was corn oil free of vitamin E (Nacalai Tesque, Kyoto, Japan). With the use of these nutrient sources, a basal diet was formulated (Table 1). The diet contained 6% mineral mixture and 2% vitamin mixture free of vitamin E (Oriental Yeast). DL-α-Tocopheryl acetate (Nacalai Tesque) was dissolved in the corn oil and mixed into the diet at a concentration of 60 mg/kg diet.

**Procedure.** After prefeeding of the basal diet, the rats were divided into two groups: a control group receiving the basal diet, and a vitamin E group receiving the same diet supplemented with a 5,000 mg DL-α-tocopheryl acetate/kg diet. After 6 d, rats of both diet groups were further divided into subgroups (n = 6) according to the dose levels of CTC treatment. CTC (Sigma Chemical, St. Louis, MO, USA) was dissolved in 0.2 mL of corn oil vehicle and administered to the rats by subcutaneous injection at 9:00–10:00 every day for 4 d. The dose levels were 0, 25, and 100 mg/kg body weight/d. At the end of the experimental period, all rats...
Table 1. Composition of basal diet.

| Ingredient                        | Amount (g/kg diet) |
|-----------------------------------|--------------------|
| Cornstarch                        | 480                |
| Casein                            | 250                |
| Corn oil                          | 60                 |
| Glucose                           | 50                 |
| Cellulose powder                  | 78                 |
| Mineral mixture<sup>1</sup>        | 60                 |
| Vitamin mixture<sup>2</sup>        | 20                 |
| Choline chloride                  | 2                  |
| DL-a-Tocopheryl acetate (mg/kg)   | 60                 |

<sup>1</sup>The mineral mixture was supplied as the following (mg/kg mineral mixture): CaHPO<sub>4</sub>·2H<sub>2</sub>O, 4.3; KH<sub>2</sub>PO<sub>4</sub>, 343; NaCl, 251; Fe-citrate, 6.23; MgSO<sub>4</sub>, 48.76; ZnCl<sub>2</sub>, 0.20; MnSO<sub>4</sub>·H<sub>2</sub>O, 1.21; CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.56; KI, 0.005; CaCO<sub>3</sub>, 293; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, 0.025.

<sup>2</sup>The vitamin mixture was supplied as the following (mg/kg vitamin mixture): retinyl acetate, 932; cholecalciferol, 5.83; menadione, 60; thiamine HCl, 590; riboflavine, 590; pyridoxine HCl, 290; cyanocobalamin, 2.0; ascorbic acid, 5,880; D-biotin, 650; folic acid, 520; D-calcium pantothenate, 2,350; niacin, 2,940; inositol, 11,760.

Chemical analysis. Plasma glucose concentration was determined by the method of Somogyi-Nelson. Lipid peroxidation in muscle and plasma was assessed as the concentration of thiobarbituric acid reactive substance (TBARS) by the modified method of Ohkawa et al. TBARS is a product of the oxidative degradation of polyunsaturated fatty acids, malondialdehyde (MDA) in particular. The protein carbonyl content of muscle homogenate was measured by using 2,4-dinitrophenylhydrazine. The carbonyl content was calculated as molar absorbance, 21,000 M<sup>-1</sup>·cm<sup>-1</sup>. These analyses, except protein carbonyl contents, were omitted in CTC 25 mg groups.

Statistical analysis. Data were analyzed by analysis of variance (ANOVA), using a General Linear Models procedure of the statistical analysis system with Duncan’s multiple range test. A p value < 0.05 was considered statistically significant.

RESULTS

Growth was markedly inhibited by CTC treatment in a dose-dependent manner in both diet groups. Feeding the vitamin E diet significantly (p < 0.05) reduced the CTC-induced growth retardation. Body weight gains for 4 d of the rats treated with
Table 2. Effect of feeding vitamin E-supplemented diet on oxidative stress in the muscle of rats treated with corticosterone (CTC).

| CTC (mg) | Basal diet | Vitamin E diet | ANOVA |
|----------|------------|----------------|--------|
|          | 0          | 25             | 100    |          | CTC  | VE   | CTC × VE |
| Muscle weights |          |                |        |        |
| EDL (mg)  | 93.5 ± 3.6a | 83.1 ± 7.5bc | 69.6 ± 5.6d | 93.6 ± 6.5a | 89.4 ± 5.1ab | 76.6 ± 7.2cd | <0.001 | 0.042 | NS |
| GAST (g)  | 1.17 ± 0.09a | 0.97 ± 0.13bc | 0.80 ± 0.04d | 1.18 ± 0.11a | 1.09 ± 0.08ab | 0.88 ± 0.09cd | <0.001 | NS   | NS |

Muscle (GAST)

| Protein carbonyl (µmol/g protein) | 1.47 ± 0.44b | 1.67 ± 0.36b | 2.70 ± 0.38a | 1.29 ± 0.30b | 1.55 ± 0.45b | 1.82 ± 0.36b | <0.001 | 0.008 | NS |
| TBARS (nmol MDA/g protein) | 183 ± 82b | — | 1,188 ± 501a | — | — | 260 ± 234b | <0.001 | <0.001 | — |

Plasma

| α-Tocopherol (µg/mL) | 14 ± 2b | — | 27 ± 5b | — | — | 136 ± 72a | NS | <0.001 | — |
| Glucose (µmol/mL) | 5.4 ± 0.9b | — | 15.4 ± 2.9a | — | — | 14.6 ± 4.4a | <0.001 | NS | — |
| TBARS (nmol MDA/mL) | 2.0 ± 0.3b | — | 4.8 ± 2.5a | — | — | 3.4 ± 0.8ab | 0.008 | NS | — |

Values are expressed as means ± SD (n = 4–6). Means in rows followed by different superscript letters are significantly different, p < 0.05.

1 EDL, extensor digitorum longus; GAST, gastrocnemius muscle.
2 Rats received a subcutaneous injection of 0, 25, and 100 mg CTC/kg body weight every day for 4 d.
3 Basal and vitamin E diets, containing 60 or 5,000 mg DL-α-tocopheryl acetate/kg diet, respectively.
4 Two-way analysis of variance. VE, vitamin E; NS, no significance.
0, 25, and 100 mg CTC were 30.6 ± 4.7 g, 9.1 ± 3.6 g, and −15.4 ± 3.7 g in the basal diet group, and 29.0 ± 3.1 g, 15.3 ± 6.0 g, and −10.1 ± 5.9 g (mean ± SD) in the vitamin E diet group, respectively.

The result of the experiment was summarized in Table 2. Weights of EDL and GAST muscle were determined as an index of muscle atrophy. The weight of EDL muscle was dose dependently reduced by CTC treatment in both diet groups. However, the weight loss of EDL muscle in the vitamin E diet group was smaller (−4% and −18% of control (0 mg) in 25 and 100 mg CTC, respectively) than in the basal diet group (−11% and −26% of control (0 mg) in 25 and 100 mg CTC, respectively), and no significant difference was found between 0 and 25 mg CTC in the vitamin E diet group. When the data were subjected to ANOVA, a significant effect of vitamin E (p < 0.05) was observed. The weight of GAST muscle was also reduced by CTC, and vitamin E repressed the muscle weight loss as it did in the EDL muscle.

The protein carbonyl content in the GAST muscle, which was determined as an index of oxidatively modified proteins, was elevated by CTC when the rats were fed the basal diet. The increment in carbonyl modification was reduced by vitamin E. A negative correlation ($r^2 = 0.332, p = 0.0132$) between the muscle weight and carbonyl content was observed when the rats were fed the basal diet. TBARS in the GAST muscle was drastically increased by CTC (5.5-fold of control), and vitamin E completely suppressed this increase, which was similar to that previously observed in the liver of CTC-treated rats (3). The change in TBARS paralleled that in protein carbonyl.

Plasma α-tocopherol concentration was markedly elevated in the CTC + vitamin E group. Hyperglycemia was induced by CTC treatment, and no effect of vitamin E was observed. As expected, plasma TBARS concentration was increased by CTC treatment, but the elevation in TBARS concentration of the CTC + vitamin E group was smaller than in the CTC group.

DISCUSSION

In the present study, we demonstrated that oxidative stress, i.e., lipid peroxidation and protein modification, was enhanced by CTC in the rat skeletal muscle, as was previously shown in the liver (3), and vitamin E minimized these effects. It is clear that CTC developed oxidative stress in the whole body as plasma TBARS concentration was increased. We have also observed an impairment of the activities of antioxidant enzymes, superoxide dismutase (SOD), and glutathione S-transferase (GST) in the livers of CTC-treated rats (3). The contribution of antioxidative enzymes in the liver toward eliminating free radicals in the whole body is important (16). Therefore, the reduced activities of SOD and GST in the liver may be responsible for the CTC-induced oxidative stress in muscle. The effect of CTC on the antioxidative enzymes in skeletal muscle tissue, however, should be clarified.
It has been known that glucocorticoid induces hyperglycemia and that sustained excess glucose in blood nonenzymatically reacts with amino groups of protein and produces glycated (modified) proteins (10). Protein glycation ultimately produces advanced glycation end products (AGE), and the oxygen radicals derived from the intermediate product of glycation, amadori rearrangement products, can accelerate oxidative stress (17, 18). In the present study, increased carbonyl contents in the skeletal muscle suggested that glycation occurred in the muscle protein when rats were treated with CTC. Therefore lipid peroxidation in the muscle might be markedly increased by CTC.

It has been reported that an excessive accumulation of glycation-related products is closely associated with the development of diabetes (19). The role of free radicals in diabetes mellitus has been documented largely through the effects of free radicals on lipids and proteins. The metabolic changes occurring in the glucocorticoid-treated rats are similar to those in the streptozotocin-induced diabetic rats. Glucocorticoid treatment develops insulin resistance, hyperglycemia, hypertriglyceridemia, muscle proteolysis, and growth retardation, which are commonly seen in streptozotocin-induced diabetes mellitus (20, 21). Hyperglycemia might be responsible for protein glycation in skeletal muscle. Oxidatively damaged or modified myofibrillar proteins have been demonstrated in the skeletal muscle of Fe-nitrilotriacetate-treated rats (7). Oxidatively modified protein is susceptible to be degraded by proteinases, especially by the ubiquitin-proteasome system (11, 12).

It was shown that protein carbonyl in the GAST muscle was dose-dependently increased by CTC and that it negatively correlates with the muscle weight, indicating a relationship between protein modification and muscle atrophy in CTC-treated rats. The muscle weight loss of CTC-treated rats was improved by vitamin E, though the extent was small, probably by decrease of the free radical-mediated acceleration of muscle proteolysis. Vitamin E has beneficial effects on muscle injury or dystrophy (22), which are mainly explained by the effects of membrane protection. Because it is known that membrane fluidity must be preserved to maintain insulin action (23), vitamin E may also modify insulin activity that is retarded by CTC. In the glucocorticoid-treated animals, insulin resistance is developed, and the coadministration of insulin is effective to improve the growth retardation by reducing the accelerated muscle proteolysis (1). In the present study, the lipid peroxidation in GAST muscle might reduce insulin activity, accounting for reduced synthesis and the increased breakdown of muscle protein. Preventing lipid peroxidation in the muscle cells may conserve insulin activity resulting in the reduction in proteolysis. Further investigation is needed to verify this possibility.

In conclusion, the present results demonstrated that oxidative stress was enhanced by CTC in rat skeletal muscle, and vitamin E was effective in reducing this stress.
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