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Oxidative stress during recovery from muscle atrophy

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Single ankle joints of male Wistar rats (15-week-old) were immobilized in the extended position for 7 days and remobilized for 5 days after the immobilization period. Atrophic and contralateral soleus, typical slow red muscles, were collected and their levels of thiobarbituric acid-reactive substance (TBARS) and glutathione were measured. Five-day remobilization did not increase muscle weight significantly. However, there were significant increases in TBARS and oxidized glutathione in the recovering muscle, which strongly suggested that enhanced oxidative stress occurred during the recovery from disuse muscle atrophy. Vitamin E injection accelerated the recovery from atrophy, thus showing that oxidative stress slowed it down.

Recovery; Muscle atrophy; Oxidative stress; Lipid peroxidation; Glutathione; Vitamin E

1. INTRODUCTION

Oxidative stress causes free radical reactions such as lipid peroxidation, which have a role in damaging biological structures and cellular functions. It has been shown for more than ten years that exhaustive exercise increases levels of lipid peroxidation in skeletal muscle, this being generally thought to be related to the pathogenesis of exercise myopathy [1]. Previously we found that muscle atrophy induced by immobilization is also accompanied by oxidative stress; thiobarbituric acid-reactive substance (TBARS) and oxidized glutathione (GSSG) were increased and total glutathione (GSH) was decreased in atrophied muscle [2]. Moreover, in the same report we proved that such oxidative stress accelerated muscle atrophy itself; vitamin E, an antioxidant, decreased the degree of atrophy.

It is generally accepted that some transition metals, such as iron and copper, are related to the production of free radicals [3]. We have found an increased iron level, especially in the microsomal fraction, in skeletal muscle atrophied by immobilization, and have suggested the possibility that increased iron may be responsible for the enhanced oxidative stress in atrophied muscle [4,5]. The role of iron has, since, been confirmed by the use of deferoxamine, an iron-chelating agent, which was shown to suppress the increased oxidative stress [6].

Oxygen consumption is expected to increase in the recovering muscle. Taking into account the catalytic action of the increased iron in atrophied muscle, we predicted that oxygen radicals might increase during the recovery from muscle atrophy [4]. In the present investigation, we measured some parameters of oxidative stress, such as TBARS and GSSG, and examined the effect of vitamin E on the recovery from atrophy.

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2. MATERIALS AND METHODS

2.1. *Animals*

Twenty-eight male Wistar rats (15-week-old) were used according to 'Guiding Principles in the Care and Use of Animals'; 12 rats for the assay of TBARS and glutathione, and 16 rats for the vitamin E injection experiment.

Under anaesthesia the ankle joint of one hind limb was immobilized in the fully extended position (i.e. with the soleus muscle in a shortened position), as described previously [2]. The procedure for limb-immobilization, as such, had no significant effects [4].

Some rats were exsanguinated after 7-day immobilization (Atrophy group). The ankle joints of the other rats were remobilized after 7-day immobilization and they were exsanguinated after 5-day remobilization (Recovery group). The soleus, typical slow red muscles from both hindlimbs (atrophic and contralateral) were collected. The water contents of both muscles were the same, and we measured wet tissue weight instead of dry weight.

2.2. *Assay of TBARS and glutathione*

The sample was assayed immediately after collection. Homogenization was done under argon gas flow to lessen the oxidative–reductive change.

TBARS was assayed by the fluorimetric method of Ohkawa et al. [7] with a slight modification, i.e. addition of 0.0125 vol. of ethanolic 2% butylated hydroxytoluene to the reaction solution to prevent further peroxidation of lipids during the assay.

Total GSH and GSSG were assayed by the method of Anderson [8].

2.3. *Vitamin E injection experiment*

Sixteen rats were divided into two groups of eight rats each and were injected intraperitoneally with either placebo or vitamin E one time daily during the remobilization period. Vitamin E was given in the form of *dl*-α-tocopherol solubilized in 10% polyoxyethylene hydrogenerated castor oil, 10% propylene glycerol buffered by sodium citrate (Eisai, Tokyo, Japan) at a dose of 30 mg/kg b.wt. Solubilization medium was given as placebo.

2.4. *Statistics*

The data were expressed as means ± S.E. A paired *t*-test was used for the comparison between atrophic and contralateral muscles, and a *t*-test for the other comparison.

3. RESULTS

The weight of soleus muscles varied from rat to rat, and so we calculated the degree of atrophy (%) as follows:

$$\frac{[\text{weight of contralateral}] - [\text{weight of atrophic}]}{[\text{weight of contralateral}]} \times 100$$

Fig. 1 shows the muscle weight and the degree of atrophy in the Atrophy and Recovery groups. The weight of atrophic muscles significantly decreased by ~ 45% in both groups and showed no significant difference between both groups. The degree of atrophy in the Recovery group was not significantly different from that in the Atrophy group.

The TBARS levels in both groups are shown in Fig. 2. The TBARS level in atrophic muscle significantly increased in the Recovery group, but not in the Atrophy group. Its level in atrophic muscle in the Recovery group was significantly higher than that in the Atrophy group.

The level of total GSH and GSSG and the ratio of GSSG to total GSH in both groups are shown in Fig. 3. In both groups the level of total GSH in atrophic muscle decreased significantly. In the Recovery group the level of GSSG in atrophic muscle was significantly higher than that in contralateral muscle, but there was no significant difference in the Atrophy group. Its level

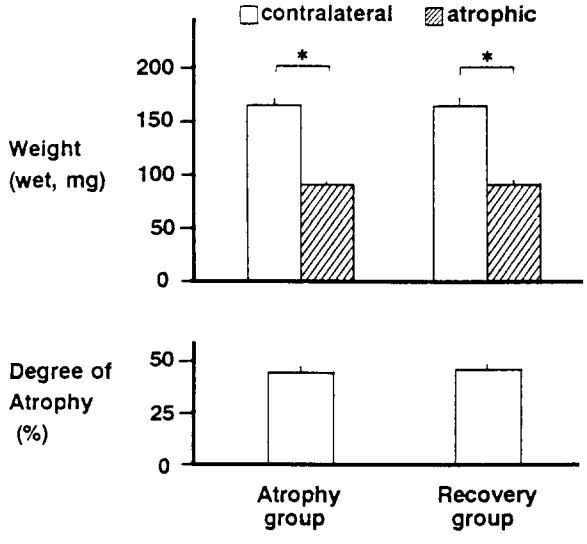

Fig. 1. Effect of 5-day remobilization on muscle weight and degree of atrophy. Atrophy group; single ankle joints were immobilized for 7 days. Recovery group; joints were remobilized for 5 days after 7-day immobilization. Data are mean ± S.E. (n = 6). *Significant difference at $P < 0.05$ between atrophic and contralateral muscles.

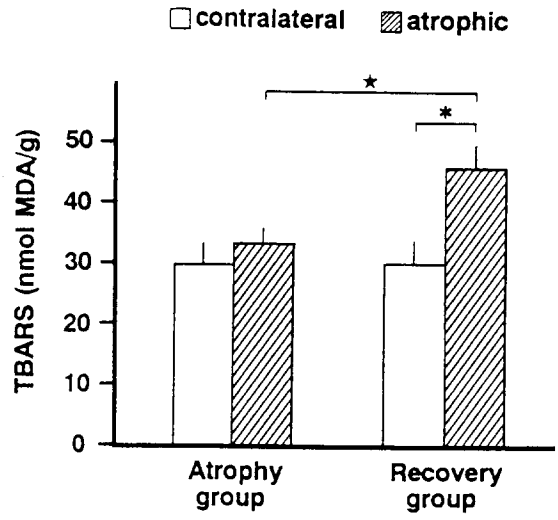

Fig. 2. Effect of 5-day remobilization on thiobarbituric acid-reactive substance (TBARS) level. Data are mean ± S.E. (n = 6). *Significant difference at $P < 0.05$ between Atrophy and Recovery groups. *Significant difference at $P < 0.05$ between atrophic and contralateral muscles.

in atrophic muscle in the Recovery group was significantly higher than that in the Atrophy group. Hence, the ratio of GSSG to total GSH in atrophic muscle increased significantly in both groups; the increase in the Recovery group was significantly larger than that in the Atrophy group.

Fig. 4 shows the effect of vitamin E on the recovery from muscle atrophy. The TBARS level of atrophic muscle in the vitamin E group was significantly lower than in the placebo group. In the vitamin E group, the muscle weight was significantly greater, and the degree of atrophy was significantly decreased by ~ 20% compared with the placebo group.

4. DISCUSSION

Five-day remobilization did not increase muscle weight and could not diminish the degree of atrophy (Fig. 1). This result agreed with the previous observation of Booth [9]; in his study there was no significant increase of muscle weight by 6-day remobilization after 10-day immobilization.

In the present investigation we found an increased level of TBARS and GSSG in atrophic muscles in the Recovery group (Figs. 2 and 3). The increase of TBARS level strongly suggests acceleration of lipid peroxidation. It is also generally accepted that, under conditions of increased oxidative stress to cells, the level of GSSG is increased [10]. Thus the increase of TBARS and GSSG prove the enhanced level of oxidative stress during the recovery from muscle atrophy. As far as we know, this is the first report on the oxidative stress in the recovering muscle. On the other hand, Gilbert [11] has reported the possibilities of enzyme regulation by

thiol-disulfide exchange and modulation of thiol/disulfide ratio in vivo to serve as a 'third messenger' in response to adenosine 3',5'-cyclic monophosphate levels. Our finding of an increased ratio of GSSG to total GSH during the recovery (Fig. 2) indicates a possible cause of metabolic changes in the recovering muscle [12].

In the vitamin E group the TBARS level in atrophic muscle decreased significantly compared with that in the placebo group (Fig. 4), which shows that vitamin E injected intraperitoneally served effectively as an antioxidant to lessen the oxidative stress. The decrease of the degree of atrophy in the vitamin E group suggests that recovery proceeded rapidly with lower oxidative stress. In other words, this indicates that oxidative stress slowed down t6he recoveyr from atrophy.

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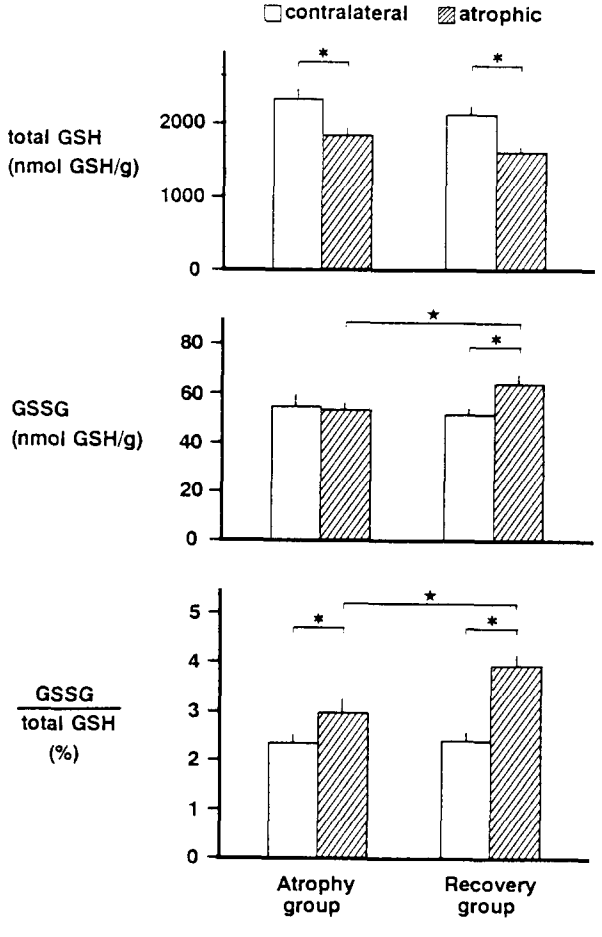

Fig. 3. Effect of 5-day remobilization on levels of total glutathione (GSH), oxidized GSH (GSSG), and ratio of GSSG to total GSH. Data are mean ± S.E. ($n = 6$). *Significant difference at $P < 0.05$ between Atrophy and Recovery groups. *Significant difference at $P < 0.05$ between atrophic and contralateral muscles.

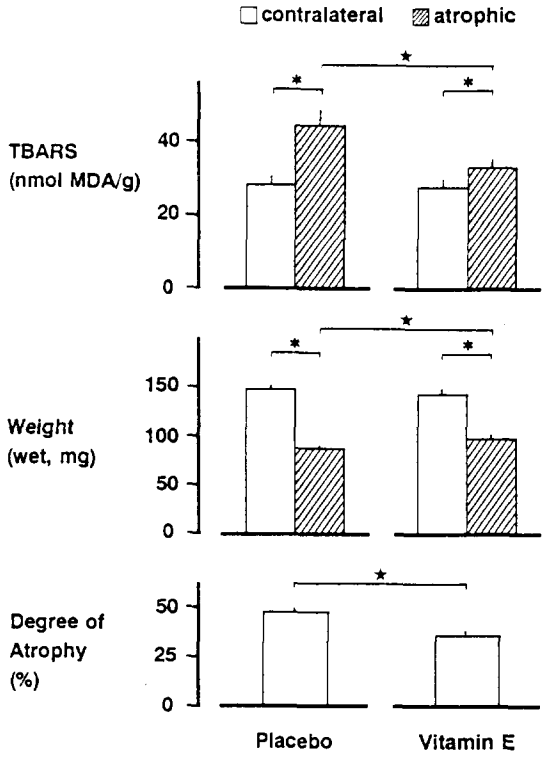

Fig. 4. Effect of vitamin E on recovery from muscle atrophy. In vitamin E and placebo groups, TBARS levels, muscle weights, and degree of atrophy in atrophic and contralateral muscles are shown. Data are mean ± S.E. ($n = 8$). *Significant difference at $P < 0.05$ between vitamin E and placebo groups. *Significant difference at $P < 0.05$ between atrophic and contralateral muscles.

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