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Metabolism of Selenium in Skeletal Muscle and Liver of Mice with Genetic Muscular Dystrophy[1, 3] (40406)

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Genetic muscular dystrophy is characterized by progressive muscular weakness and atrophy and necrosis of the skeletal muscle (1, 2). In several aspects, the disease in genetically dystrophic mice is thought to resemble that in human beings (3). For a variety of species, dietary selenium deficiency can result in a state of nutritional muscular dystrophy (4) which resembles the symptoms of inherited muscular dystrophy. However, nutritional muscular dystrophy has not been observed in animals given adequate vitamin E regardless of selenium status. Furthermore, it has been shown that genetic muscular dystrophy cannot be reversed by the addition of selenium to the diet (5).

Several investigators have shown that glutathione peroxidase (glutathione:hydrogen-peroxidase oxidoreductase, EC 1.11.1.9) activity is increased in mice and chickens with inherited muscular dystrophy (6, 7). It is well documented that this enzyme requires selenium for activity (8) and that its activity decreases markedly in response to dietary selenium deficiency (9). Thus, because the glutathione peroxidase activity is increased in dystrophic muscle, the metabolic basis for muscular dystrophy does not appear to be related to selenium deficiency.

This increased activity of glutathione peroxidase in dystrophic muscle suggests that both selenium and enzyme protein concentrations are increased. The elevated enzyme level in the dystrophic muscle may be explained by an alteration in the rate of selenium retention and absorption which permits selenium accumulation to abnormally high concentrations. The present studies were performed to determine if such an alteration in selenium metabolism occurs in muscle from genetically dystrophic mice.

Materials and methods. The animals chosen for this study were male dystrophic mice (129/ReJ-dy strain) and age-matched normal controls (129/ReJ strain). Mice were obtained from Jackson Laboratories (Bar Harbor, Maine). They were killed in pairs at 55 days of age, at which time the signs of muscular dystrophy were apparent in the hindlimbs. Liver and skeletal muscle (obtained from the hindlimbs and forelimbs) were removed and homogenized in a solution containing 0.01 M phosphate buffer (pH 7.0) with 0.135 *M* KCl added. These tissues were homogenized in a Virtis-45 homogenizer at 15,000–20,000 rpm for 3 min at 0°; further homogenized by four up-and-down strokes with a Dounce (A) homogenizer, and filtered through four layers of cheesecloth. The filtrate was centrifuged at 700*g* for 10 min and the pellet was discarded; the supernatant was centrifuged at 100,000*g* for 60 min and this pellet was also discarded. The supernatant from the 100,000*g* centrifugation was analyzed for glutathione peroxidase activity.

Glutathione peroxidase was determined by the method of Little *et al.* (10) using 1.5 m*M* cumene hydroperoxide or by the method of Paglia and Valentine (11) using 0.25 m*M* hydrogen peroxide. In addition to hydrogen peroxide or cumene hydroperoxide, the reaction mixture contained 50 m*M* potassium phosphate (pH 7.0), 1 m*M* EDTA, 1 m*M* NaN_3, 0.25 m*M* NADPH, 1 E.U/ml GSSG-reductase, and 1 m*M* GSH in a total volume of 1 ml. All reactants except the enzyme source and peroxides were combined at the beginning of each day. The enzyme source (0.1 ml) was added to 0.8 ml of the above

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mixture and allowed to incubate 5 min at room temperature before initiation of the reaction by the addition of 0.1 ml of peroxide solution. Absorbance at 340 nm was recorded for 5 min, and the activity was calculated from the slope of these lines as μmoles NADPH oxidized per min. Blank reactions with the enzyme source replaced by distilled water were subtracted from each assay. Protein was measured by the method of Lowry *et al.* (12). The specific activity of glutathione peroxidase was measured in liver and skeletal muscle (from the hindlimbs and forelimbs) of control and dystrophic mice following (a) sodium selenite injections (b) exposure to selenium-free diet, or (c) no treatment with selenium.

Selenium uptake studies. In selenium uptake studies, control and dystrophic mice (both groups at 55 days of age) received sc injections of sodium selenite at a concentration of 10 μg/100 g body wt on three consecutive days. On the fourth day, both groups of mice received sc injections of ^{75}Se-labeled sodium selenite (New England Nuclear, Boston, Mass.) at a concentration of 10 μCi/100 ng/100 g body wt. At 60 min after ^{75}Se injection, mice were killed; liver and skeletal muscle (from the hindlimbs and forelimbs) were removed, and radioactive selenium concentration was determined by means of a well-type gamma scintillation counter. The concentration of ^{75}Se in the tissue was calculated from the specific activity of the dose given. Tissues were then frozen and lyophilized to constant weight. Total selenium was measured on the lyophilized samples by neutron activation analysis as described by Lyon and Emory (13).

Selenium retention studies. In these studies control and dystrophic mice (both groups at 30 days of age) were fed a Torula yeast diet deficient in selenium (14) for 20 days. On day 21 both groups of mice were injected with ^{75}Se at a concentration of 10 μCi/100 g body wt. Mice in both groups were killed 60 min after injection, and radioactive and total selenium concentrations were measured as described in the section on selenium uptake studies.

In some studies control and dystrophic mice fed a normal diet were injected with ^{75}Se and radioactive and total selenium were measured as described in the section on selenium uptake studies.

For the experiments in which statistical analysis was done, results were analyzed by Student's *t* test. The expressions $P < 0.05$, $P < 0.01$, and $P < 0.001$ are used to indicate significance at the 5, 1, and 0.1% levels, respectively.

Results and discussion. The activity of glutathione peroxidase was significantly increased in muscle of forelimbs and hindlimbs from dystrophic mice (Table I), but its activity in the liver of dystrophic mice was not significantly different from that in controls. Bell and Draper (7) recently reported results similar to those shown in Table I. Since glutathione peroxidase requires selenium for activation, the selenium concentration was measured to determine if it increased along with the increase in enzyme activity in dystrophic muscle. As shown in Table I, selenium concentrations were significantly increased in the dystrophic (hindlimb) and nondystrophic (forelimb) muscles. When control and dystrophic mice were given a sc injection of ^{75}Se, both the hindlimb and forelimb muscles of dystrophic mice retained significantly more ^{75}Se than did the muscle from the paired control. This result suggests that skeletal muscle from the dystrophic mice absorbed and perhaps retained larger amounts of total selenium than the paired controls.

To investigate whether selenium uptake by skeletal muscle from dystrophic mice is significantly different from skeletal muscle of control mice, sodium selenite was injected on three consecutive days, followed on the fourth day by ^{75}Se injection. A marked increase was observed in total selenium concentration in skeletal muscle from the dystrophic mice (Table II); only a slight increase was noted for the control mice (Table I). In skeletal muscle from both groups the activity of glutathione peroxidase was increased following sodium selenite pretreatment, but only the increase in dystrophic mice was significant. It is interesting to note that the liver from the dystrophic mice showed a significant increase in both total selenium and glutathione peroxidase activity. The liver and skeletal muscle from the dystrophic mice also showed a significant increase in ^{75}Se absorption. This increased ^{75}Se absorption in tissues from dystrophic

TABLE I. GLUTATHIONE PEROXIDASE ACTIVITY AND CONCENTRATIONS OF RADIOACTIVE SELENIUM AND TOTAL SELENIUM IN LIVER AND SKELETAL MUSCLE FROM CONTROL (6) AND DYSTROPHIC MICE (8) FOLLOWING ^{75}Se INJECTION.[a]

	Tissue		
	Liver	Forelimb muscle	Hindlimb muscle
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; H_2O_2 as substrate)			
Control	360 ± 25[b]	17.8 ± 1.3	13.8 ± 0.9
Dystrophic	381 ± 31	34.3 ± 3.2***	36.7 ± 4.3***
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; cumene·OOH as substrate)			
Control	401 ± 43	24.2 ± 2.2	15.0 ± 1.1
Dystrophic	460 ± 30	36.6 ± 1.9*	24.0 ± 2.0*
Radioactive selenium content (pg ^{75}Se/g tissue wet wt)			
Control	108 ± 18	12.3 ± 0.4	8.6 ± 1.8
Dystrophic	122 ± 8	19.1 ± 1.6*	16.1 ± 0.7**
Total selenium content (μg selenium/g tissue dry wt)			
Control	1.44 ± 0.12	0.252 ± 0.012	0.207 ± 0.030
Dystrophic	1.49 ± 0.09	0.340 ± 0.011*	0.353 ± 0.021**

[a] ^{75}Se was injected sc (10 μCi/100 ng Se/100 g body wt) and animals were sacrificed 60 min post ^{75}Se injection and the above experiments performed.

[b] All values are given as much ± SE.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The numbers in parentheses indicate number of mice in each experimental group.

TABLE II. GLUTATHIONE PEROXIDASE ACTIVITY AND CONCENTRATIONS OF RADIOACTIVE SELENIUM AND TOTAL SELENIUM IN LIVER AND SKELETAL MUSCLE FROM CONTROL (9) AND DYSTROPHIC MICE (10) FOLLOWING SODIUM SELENITE[b] AND ^{75}Se INJECTIONS.[a]

	Tissue		
	Liver	Forelimb muscle	Hindlimb muscle
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; H_2O_2 as substrate)			
Control	448 ± 28[b]	23.3 ± 3.1	18.4 ± 1.9
Dystrophic	785 ± 52**	56.4 ± 2.2***	44.1 ± 3.0***
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; cumene·OOH as substrate)			
Control	487 ± 31	33.8 ± 5.6	20.1 ± 2.3
Dystrophic	728 ± 44*	59.0 ± 3.4**	40.6 ± 4.3**
Radioactive selenium content (pg ^{75}Se/g tissue wet wt)			
Control	159 ± 5	14.6 ± 1.7	11.2 ± 0.7
Dystrophic	303 ± 18**	35.5 ± 2.9***	29.4 ± 2.6***
Total selenium content (μg selenium/g tissue dry wt)			
Control	1.88 ± 0.21	0.281 ± 0.031	0.236 ± 0.038
Dystrophic	3.44 ± 0.39***	0.488 ± 0.044***	0.565 ± 0.091***

[a] Sodium selenite (10 μg/100 g body wt) was injected sc on 3 consecutive days followed on the fourth day by ^{75}Se sc injection (10 μCi/100 ng Se/100 g body wt). Animals were sacrificed 60 min after the ^{75}Se injection and the above experiments performed.

[b] All values are given as mean ± SE.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The numbers in parentheses indicate number of mice in each experimental group.

mice was greater than that observed for untreated dystrophic mice (Table I), which suggests that sodium selenite pretreatment may affect the threshold for selenium uptake into these tissues.

To measure the ability of skeletal muscle to retain selenium, dystrophic and control mice were placed on a selenium-deficient diet for 20 days. The concentration of selenium and the activity of glutathione peroxidase were decreased in all tissues from both groups, but the decrease was significantly greater for the control tissues (Table III). Thus it is suggested that tissues from the dystrophic mice retain more selenium when placed on selenium-deficient diet than do tissues from the control mice. These results demonstrate that liver and skeletal muscle from dystrophic mice, as compared with these same tissues in paired controls, show: (a) a significant increase in both glutathione peroxidase and total selenium, and (b) a significant increase in selenium absorption and retention.

The mechanism responsible for the change in selenium metabolism is not understood at present. Furthermore, it is not known if the changes in selenium metabolism represent changes within the skeletal muscle or other cellular elements. In the present study, an increase in macrophages was observed in dystrophic muscle. Macrophages contain considerable amounts of glutathione peroxidase (15). Furthermore, it has been shown that dietary selenium supplement stimulates a marked increase in the activity of this enzyme (15). Thus the changes reported above may simply represent an increase in macrophage activity in the dystrophic muscle. We have examined this possibility by autoradiographic methods. Following ^{75}Se injections, autoradiographs were made of sections of the skeletal muscle from the hindlimbs of both groups. ^{75}Se was observed in the muscle from both groups, although, qualitatively the muscle of the dystrophic mice showed increased ^{75}Se deposits. Both the macrophages and the dystrophic muscle contained ^{75}Se. Since the total muscle mass is larger than the mass of macrophage, the increase in ^{75}Se absorption

TABLE III. GLUTATHIONE PEROXIDASE ACTIVITY AND CONCENTRATIONS OF RADIOACTIVE SELENIUM AND TOTAL SELENIUM IN LIVER AND SKELETAL MUSCLE FROM CONTROL (7) AND DYSTROPHIC MICE (12) AFTER BOTH GROUPS WERE FED A SELENIUM-DEFICIENT DIET FOR 20 DAYS AND THEN INJECTED WITH ^{75}Se.[a]

	Tissue		
	Liver	Forelimb muscle	Hindlimb muscle
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; H_2O_2 as substrate)			
Control	98.6 ± 8.6[b]	9.4 ± 0.8	7.6 ± 1.0
Dystrophic	169.8 ± 13.6*	20.3 ± 1.9***	23.6 ± 0.9***
Glutathione peroxidase activity (μmoles NADPH oxidized/min/mg protein; cumene·OOH as substrate)			
Control	178.9 ± 11.2	13.3 ± 1.1	8.2 ± 1.6
Dystrophic	241.6 ± 18.4*	27.6 ± 2.3**	17.4 ± 1.3***
Radioactive selenium content (pg ^{75}Se/g tissue wet wt)			
Control	199 ± 13	17.6 ± 0.9	13.6 ± 0.9
Dystrophic	286 ± 20*	29.6 ± 2.6**	24.4 ± 2.4**
Total selenium content (μg selenium/g tissue dry wt)			
Control	0.48 ± 0.03	0.121 ± 0.017	0.100 ± 0.019
Dystrophic	1.08 ± 0.08***	0.237 ± 0.014***	0.248 ± 0.039***

[a] Mice were placed on selenium-deficient diet for 20 days and then injected sc with ^{75}Se (10 μCi/100 ng/100 g body wt). Animals were sacrificed 60 min after the ^{75}Se injection and the above experiments performed.

[b] All values are given as mean ± SE.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The numbers in the parentheses indicate number of mice in each experimental group.

shown in the tables is more representative of the changes in the dystrophic muscles than in the macrophagic cells. In nondystrophic muscles, in which macrophages were only occasionally observed, ^{75}Se deposits were increased for all treatment groups. The autoradiographic results support the suggestion that selenium metabolism is altered in the skeletal muscle of the forelimbs and hindlimbs from the dystrophic mice.

The results of this study support the observation of Taussky *et al.* (16) that selenium concentrations in the tissues of dystrophic mice are higher than in their normal littermates. These investigators (17) also observed a 10% increase in selenium in the eggs from dystrophic hens over that observed in eggs from normal hens. It has been shown that mammalian systems can reduce Na_2SeO_3 to H_2Se. This latter form of selenium is known to be extremely toxic (18). Since skeletal muscle in the dystrophic mice acts as a sink for selenium, it may be that this tissue is converting oxidized selenium into the highly toxic reduced form which may precipitate the necrotic changes observed in skeletal muscle from the dystrophic mice. An alternate interpretation of the higher levels of selenium in the dystrophic tissue may represent a compensatory change in the muscle due to an increase in metabolites such as H_2O_2 and lipid hydroperoxides. Further studies are in progress to determine if the amount of this reduced selenium is increased in the dystrophic muscle.

Summary. The specific activity of glutathione peroxidase and the concentration of selenium in muscles from mice with genetic muscular dystrophy were both significantly increased over those of control mice. Following pretreatment with sodium selenite (10 μg/100 g body wt) for 3 days, the specific activity of glutathione peroxidase and the concentration of selenium in muscle from the dystrophic mice were both significantly increased over values in muscle from control mice. When the dystrophic and control mice were fed a diet deficient in selenium, both the activity of glutathione peroxidase and the selenium concentration were significantly decreased in muscle from both groups. However, the magnitude of decrease was significantly greater in the muscle from the control mice than in the muscle from the dystrophic mice. These results suggest that muscle from dystrophic mice has a greater capacity for selenium absorption and retention than does muscle from control mice.

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