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EFFECT OF FORCED RUNNING ON RAT SKELETAL MUSCLE WITH ACRYLAMIDE NEUROPATHY

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ABSTRACT. This study was performed to evaluate the effect of prolonged forced running on rat lower limb muscles with acrylamide neuropathy. Twenty-four 4-week-old rats were divided into three groups of eight rats. Acrylamide was given to two groups of sixteen rats to induce mild paralysis. Eight rats with acrylamide injections were forced to run 3 200 m/day on a treadmill for five weeks. Running activities slowed the rate of body weight gain and aggravated paralysis. Although the wet weight of tibialis anterior (TA) and extensor digitorum longus (EDL) muscles was reduced by running, that of soleus (SOL) muscles was unchanged. The ratio of their weight to their body weight (BW) remained constant regardless of exercise. Protein content (PC) of muscles was not altered by exercise, either. We postulated that exercise-induced worsening of paralysis in acrylamide neuropathy rats was not caused by muscle pathology. Deterioration of neuropathic condition due to exercise was suggested.

Key words: acrylamides, exercise, muscle, rat.

There were several experimental reports about exercise effects on skeletal muscle of healthy rats (4, 6, 11, 12), but few were available about those effects on that of neuropathy rats. Hie (18) showed that weight and twitch tension increase in exercised rat skeletal muscle during the process of reinnervation, and suggested that dynamic exercise was effective in the treatment of patients recovering from peripheral nerve injuries. However, exercise sometimes cause muscle weakness in patients with lower motoneuron lesions (2, 3, 16, 23).

Reitsma (30) and Vihko (31) demonstrated histological evidence of muscle damage after running exercise of already weakened muscles. Herbison (19, 21) observed the same kind of adverse effect of exercise (swimming and weight-lifting) in his biochemical studies of nerve-injured rat skeletal muscles.

The purpose of this study was to evaluate the exercise effect of prolonged forced running on the degree of paralysis, muscle weight, and protein content in partially paralyzed rats and to hypothesize the mechanism of exercise-induced muscle weakness which is sometimes seen in some patients with lower motoneuron disease.

METHODS

Experimental paralysis was produced by acrylamide injections, a well studied neuropathy (8, 27, 29). Chronic mild intoxication (10, 15) was induced while maintaining the rats' running ability.

Twenty-four 4-week-old Wistar rats were equally divided into three groups: Group 1, sedentary without acrylamide; Group 2, sedentary with acrylamide; and Group 3, exercised with acrylamide. A dose of 50 mg/kg of a 2% acrylramide solution (Sigma Chemical Co.) in saline was administered intraperitoneally via a Tuberculin syringe three times a week (on Mon, Wed, and Fri) to Groups 2 and 3 for the first four weeks. This schedule was followed by a once weekly injection for the next six weeks. The same schedule of saline injections was applied to the controls in Group 1. Group 3 was exercised on a treadmill for the last five weeks before sacrifice. Standard Purina rodent diet and water were given ad libitum. Plastic animal housing was $10.5'' \times 19'' \times 8''$ deep. Animal wards were maintained at $72 \pm 2°F$. Lighting was controlled by automatic light timers set on a 12 hour on/off cycle.

An endless belt type treadmill was used for running activities on which rats were conditioned to run by an electrical shock of 120 V, 3–5 mA, 30–40 msec of duration. The interval of the electrical current was set at 3–5 sec. In order to avoid exhaustion and frequent electrical shocks, rats were removed immediately from the treadmill when they were shocked three times consecutively. Rats were planned to run 3 200 m/day, 5 times a week on this 5° inclined treadmill. Running speed was set at 10–15 m/min.

Body weight and paralysis of each rat were evaluated weekly. Paralysis was rated by the appearance of four signs of weakness. One of them was the splaying phenomenon (8, 10, 27) of hind limbs when rats were dropped from a few inches onto a flat surface. The other three signs, namely flip-over tendency of the hind feet (9), crossed or wide-based hind limbs, and waddling gait (9, 27), were observed on a 20° inclined treadmill. Each of these paralysis signs was rated as 1

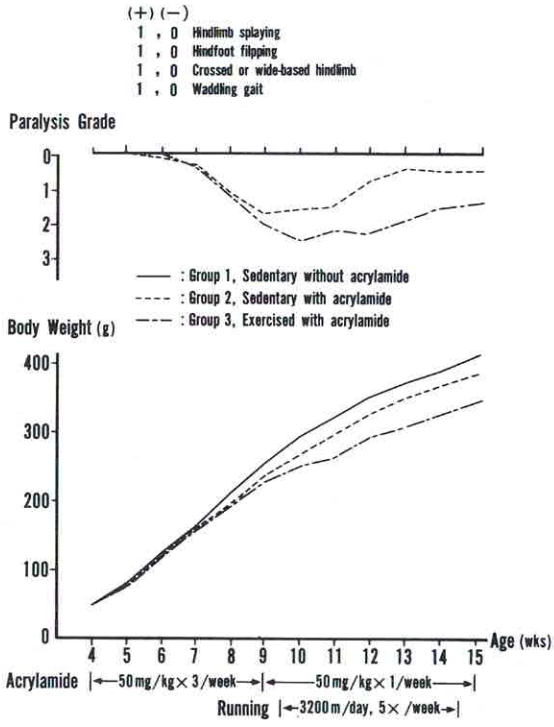

Fig. 1. Paralysis grade: Paralysis was graded by the appearance of hind limb splaying, hind foot flipping, waddling gait pattern, crossed or wide-based hind limbs. Paralysis was noted 2–3 weeks after the initiation of acrylamide injection. Paralysis was worsened by running activities. Body weight: Body weight increased continuously. Acrylamide and/or running activities slowed the rate of weight gain.

(positive) or 0 (negative). This scale ranged from 0 (normal) to 4 (severe paralysis) as a total.

Rats were sacrificed under CO_2 gas when they reached 15 weeks of age. Tibialis anterior (TA), soleus (SOL), and extensor digitorum longus (EDL) muscles were carefully removed from the left limbs and their wet weight of muscles (WWM) was measured. The soleus muscles were homogenized and centrifuged for Biuret's protein assay. Protein content (PC) was calculated from a bovine serum albumin standard curve. Data were analyzed with Student's *t*-test for statistic significance of difference among groups and with Mann-Whitney's U-test for paralysis grades.

RESULTS

Two rats in Group 3 did not run continuously without having frequent electrical shocks and were excluded from this experiment.

The body weight of all rats increased steadily as is depicted in Fig. 1. Acrylamide treatment and/or running activities slowed the rate of weight gain, but the acrylamide-treated Group 2 was not significantly lighter than Group 1. However, the rats exercised in Group 3 weighed significantly less ($p<0.05$) than Groups 1 and 2 (Table I).

Table I. *Muscle weight difference*

BW: body weight, TA: tibialis anterior, SOL: soleus, EDL: extensor digitorum longus, PC: protein content, WWM: wet weight of muscle

	Group		
	1	2	3
Number	8	8	6
BW (g)	411±41	381±44	341±14[b]
TA (mg)	825±41	672±53[b]	613±34[b, c]
SOL (mg)	227±22	201±22[b]	196±12[b]
EDL (mg)	177±14	171±13	157±11[b, c]
TA/BW (mg/g)[a]	2.03±0.10	1.76±0.11[b]	1.79±0.11[b]
SOL/BW (mg/g)[a]	0.55±0.11	0.52±0.11	0.57±0.11
EDL/BW (mg/g)[a]	0.43±0.11	0.45±0.11	0.46±0.11
PC/WWM (mg/g)	137±13	132±16	130±8

[a] Individual values converted to logarithms. Mean & S.D. reconverted by antilogs.
[b] Significantly different from group 1 ($p<0.05$).
[c] Significantly different from group 2 ($p<0.05$).

Paralysis was first observed 2–3 weeks after acrylamide administration was initiated. The acrylamide-treated groups showed their greatest paralysis at 9–10 weeks of age. The paralysis grade of the exercised rats in Group 3 was significantly worse ($p<0.05$ with Mann-Whitney's U-test) than the sedentary rats in Group 2 from the age of 12 to 14 weeks (Fig. 1).

The wet weight of TA, SOL, and EDL were lighter in Group 2 and lightest in Group 3 (Table I). Significant weight differences ($p<0.05$) were found in TA and SOL muscles between Groups 1 and 2 and in TA and EDL muscles between Groups 2 and 3. However, the ratio of the WWM to BW was constant except for TA/BW which was significantly less ($p<0.05$) in acrylamide-treated groups compared with controls. Protein content was the same for all three groups.

DISCUSSION

Acrylamide causes dose-dependent muscle weakness secondary to peripheral neuropathy of the dying back type (10, 29). Under the circumstances of this study mild prolonged muscle weakness occurred without rat

weight loss. However, exercise aggravated the degree of paralysis and decreased the rate of BW gain (Fig. 1, Table I). We believe the running distance of 3200 m/day at 10–15 m/min was sufficient to show exercise effects on muscle when compared to other reports (22, 30). We thought deterioration of paralysis was caused by muscle atrophy. But the reduction of muscle weight was small as compared with the deterioration of paralysis.

Herbison (21) showed prolonged-exercise-induced weight reduction of synergistically tenotomized rat skeletal muscles although he found weight increased in less exercised muscles like other investigators (14, 26). Gordon (13) did not find weight increase in his exercised rat skeletal muscle. He thought that relative undernutrition from over-exercise was a causative factor to inhibit exercise hypertrophy of muscle. Carrow (4) found that the prolonged exercise of endurance type induced reduction in muscle fiber diameter and muscle volume. Hatano (17), Herbison (20), and Kernell (24) showed that electrical stimulation induced hypertrophy of rat skeletal muscles. However, Donselaar (7), Kernell (24) and Pette (28) demonstrated atrophy with more prolonged stimulation. They also showed that exercise-induced decrease of muscle fiber diameter was due to the fiber type conversion from Type 2 to Type 1 muscle fibers.

Our results did not show exercise-induced atrophy of muscles according to the values of WWM/BW (Table I), but the absolute WWM was significantly smaller in exercised neuropathy rats except for SOL muscles. It is well-known that SOL muscles are composed mostly of Type 1 fibers, while TA & EDL muscles contain Type 2 fibers. So the reduction of absolute weight in exercised TA & EDL muscles might be caused by fiber type conversion from Type 2 to Type 1 regardless of acrylamide neuropathy.

In muscle protein content, Crockett (5) and Helander (18) found that restricted activity resulted in a decrease of muscle PC. However, Herbison (19, 21) found lower muscle PC in intensely exercised rats with partial denervation. We did not detect such an exercise-induced PC change in exercised muscles. Our method of protein assay may be too gross to detect a minute change in muscle under these experimental conditions.

The mechanism of overwork weakness in peripheral nerve diseases still remains unsolved, but deterioration of nerve pathology (1) rather than muscle pathology (11, 13, 19, 22) due to heavy exercise might be a causative factor.

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REFERENCES

1. Anderson, Y. & Edström, J.: Motor hyperactivity resulting in diameter decrease of peripheral nerves. Acta Physiol Scand *39:* 553–558, 1975.
2. Bennet, R. L. & Knowlton, G C.: Overwork weakness in partially denervated skeletal muscle. Clin Orthop *12:* 22–29, 1958.
3. Bensman, A.: Strenuous exercise may impair muscle function in Guillain Barré patients. JAMA *214:* 468–469, 1970.
4. Carrow, R. E., Brown, R. E. & Van Haus V. D.: Fiber sizes and capillary to fiber ratios in skeletal muscle of exercised rats. Anat Rec *159:* 33–40, 1967.
5. Crockett, J. L. & Edgerton, V. R.: Exercise and restricted activity effect on reinnervated and cross-innervated skeletal muscles. J Neurol Sci *25:* 1–9, 1975.
6. Denny-Brown, D.: Experimental studies pertaining to hypertrophy, regeneration, and degeneration. Neuromusc Dis *38:* 147–196, 1961.
7. Donselaar, Y., Eerbeek, O., Kernell, D. & Verhey, B. A.: Fiber sizes and histochemical staining characteristics in normal and chronically stimulated fast muscle of cat. J Physiol (Lond) *382:* 237–254, 1987.
8. Edwards, P. M. & Parker, W. H.: A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol *40:* 589–591, 1977.
9. Fehling, C., Abdulla, M., Brun, A, Dictor, M., Schutz, A. & Skerfring, S.: Methylmercury poisoning in rat. Toxicol Appl Pharmacol *33:* 27–37, 1975.
10. Fullerton, P. M. & Barnes J. M.: Peripheral neuropathy in rats produced by acrylamide. Br J Industr Med *23:* 210–221, 1966.
11. Goldspink, G.: The combined effects of exercise and reduced food intake on skeletal muscle fibers. J Cell Comp Physiol *63:* 209–216, 1964.
12. Goldspink, G. & Howell, K. F.: Work induced hypertrophy in exercised normal muscles of different ages and the reversibility of hypertrophy after cessation of exercise. J Physiol (Lond) *239:* 179–193, 1974.
13. Gordon, E. E., Kowalski, K. & Fritz, M.: Protein changes in quadriceps muscle of rat with repetitive exercises. Arch Physical Med Rehab *48:* 269–303, 1967.
14. Gutmann, E., Schiaffino, S. & Hanzliokova, V.: Mechanism of compensatory hypertrophy in skeletal muscle of the rat. Experimental Neurol *31:* 451–464, 1971.
15. Hachisuka, K.: Overwork weakness induced by free-running in experimental acrylamide neuropathy. Rihabiliteishon Igaku (Japanese) *20:* 241–249, 1983.
16. Halsted, L. S. & Rossi, C. D.: New problems in old polio patients. Results of a survey of 539 polio survivors. Orthopedics *8:* 845–850, 1985.
17. Hatano, E., Juda, K. & Ikuta, Y.: Effect of electrical

stimulation on normal skeletal muscle. Rinsho Sports Igaku (Japanese) *3:* 1303–1308, 1986.
18. Helander, E. A. S.: Influence of exercise and restricted activity on the protein composition of skeletal muscle. Biochem J *78:* 478–482, 1961.
19. Herbison, G. J., Jaweed, M. M. & Ditunno, J. F.: Effect of swimming on reinnervation of rat skeletal muscle. J Neurol Neurosurg Psychiat *37:* 1247–1251, 1974.
20. Herbison, G. J., Jaweed, M. M. & Ditunno, J. F.: Electrical stimulation of sciatic nerve of rats after partial denervation of soleus muscle. Arch Phys Med Rehabil *67:* 79–83, 1986.
21. Herbison, G. J., Jaweed, M. M., Gordon, E. E. & Ditunno, J. F.: Overwork of denervated skeletal muscle. Effect on muscle proteins in rats. Arch Phys Med Rehabil *55:* 202–205, 1974.
22. Hie, B., van Nie, C. J. & der Zee, E. V.: Twitch tension, muscle weight, and fiber area of exercised reinnervating rat skeletal muscle. Arch Phys Med Rehabil *63:* 608–612, 1982.
23. Hortsmann, D. M.: Acute poliomyelitis: Relation of physical activity at the time of onset to the course of the disease. JAMA *142:* 236–241, 1950.
24. Kernell, D., Eerbeek, O., Verhey, B. A. & Donselaar, Y.: Effects of physiological amounts of high- and low-rate chronic stimulation on fast-twitch muscle of the cat hindlimb. J Neurophysiol *58*, 598–613, 1987.
25. Kinney, C. L., Jaweed, M. M., Herbison, G. J. & Ditunno, J. F.: Overwork effect on partially denervated rat soleus muscle. Arch Phys Med Rehabil *67:* 286–289, 1986.
26. Morgan-Hughes, J. A., Sinclair, S., Durston, J. H. J.: The pattern of peripheral nerve regeneration induced by crush in rats with severe acrylamide neuropathy. Brain *97:* 235–250, 1974.
27. Pette, D., Muller, W., Leisner, E. & Vrbova, G.: Time dependent effects on contractile properties, fiber population, myosin light chains and enzymes of energy metabolism in intermittently and continuously stimulated fast twitch muscle of the rabbit. Pfluegers Arch *364:* 103–112, 1976.
28. Prineas, J.: The pathogenesis of dying-back polyneuropathy. Part 2. J Neurol Neurosurg Psychiat *28:* 598–621, 1969.
29. Reitsma, W.: Skeletal muscle hypertrophy after heavy exercise in rats with surgically reduced muscle function. Am J Phys Med *48:* 237–258, 1948.
30. Vihko, V., Rantamaki, J. & Salminen, A.: Exhaustive physical exercise and acid hydrolase activity in mouse skeletal muscle. Histochemistry *57:* 237–249, 1978.

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