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Influence of Exercise on Muscle Fibers in Rats with Steroid Myopathy

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The influence of mild exercise on skeletal muscle fibers was investigated histochemically to assess the effects of exercise on steroid myopathy and its efficacy for preventing this disease. Twenty male Wistar rats were divided into 4 groups of 5 each: group T, which received exercise alone; group S which received steroid alone; group ST which received both exercise and steroid; and group C, the control group. In groups S and ST, hydrocortisone was administered subcutaneously at a dose of 10 mg/kg/day for 4 weeks. In the exercise groups, the animals were made to run at a speed of 15 m/min for about 1 h/day for 5 days a week on a treadmill. After the completion of treadmill exercise and steroid administration for 4 weeks, the rats were anesthetized with Nembutal, the soleus muscle (SOL) and the extensor digitorum longus muscle (EDL) were removed and prepared for examinations. The area of type I fibers in the SOL was significantly larger in group ST than in group S. The area of type IIa fibers in the EDL was significantly larger in group ST than in group S. In group S, the proportion of type I fibers in the SOL was significantly lower than in the other three groups. There was little difference in fiber type distribution between groups ST and C. These results suggest that steroid myopathy can be prevented by even mild exercise.

Key words: exercise, muscle fibers, steroid myopathy

Steroids have been used in the treatment of autoimmune diseases, bronchial asthma and various other diseases since Hench *et al.* (1) reported their usefulness for treating rheumatoid arthritis in 1949. However, long term steroid therapy is usually required to treat these diseases and this often causes adverse reactions.

One of these adverse reactions is steroid myopathy. Steroid myopathy was first reported by Cushing (2) in 1932, who noted muscle weakness developing in patients with excessive endogenous steroid production. Perkoff *et al.* (3) later reported a similar muscle disturbance in patients receiving steroid therapy, and this condition is now known as steroid myopathy. Steroid myopathy has been reported in patients undergoing long term steroid therapy for various diseases, including rheumatoid arthritis, or when steroids are given as immunosuppressive therapy after transplantation (4). Although discontinuing steroid treatment may lead to a resolution of myopathic symptoms, it is sometimes difficult to discontinue steroid treatment in patients with these diseases. Thus, preventing the disease becomes crucial. Some reports (5-17) have been published on the efficacy of exercise in preventing steroid myopathy. However, there is insufficient data regarding the amount of exercise necessary to prevent steroid myopathy in patients.

In the present study, a steroid was administered to rats in order to induce experimental steroid myopathy. The dose used was not high enough to cause life-threatening side effects. The influence of moderate exercise on the skeletal muscle fibers was then investigated histochemically to assess the effects of exercise on steroid myopathy. In light of the data gathered, the efficacy of exercise for preventing steroid myopathy was discussed.

Materials and Methods

Twenty male Wistar rats (11 weeks old) were divided into 4 groups of 5 each: group T, which received exercise alone; group S which received steroid alone; group ST which received both exercise and steroid; and group C, the control group.

In groups S and ST, hydrocortisone was administered subcutaneously at a dose of 10 mg/kg/day for 4

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weeks. In the exercise groups, the animals were made to run at a speed of 15 m/min for about 1 h/day for 5 days a week on a treadmill for small animals (LE 8706, Letica S.A., Barcelona, Spain). During the experimental period, they were housed 2 to a cage and kept in a room maintained at a temperature of 25°C with a 12-h light/dark cycle (lights on from 6:00–18:00). Water and food were given ad libitum.

During the experimental period, the animals were weighed daily and body weight changes were assessed.

After the completion of treadmill exercise and steroid administration for 4 weeks, the rats were anesthetized with Nembutal, the soleus muscle (SOL) and the extensor digitorum longus muscle (EDL) were excised for examinations, immediately frozen in dry ice and acetone, and stored in a freezer at −80°C. Then, the muscle tissue blocks were sliced into serial sections of 10 μm on a cryostat (Tissue-Tek, Miles, Elkhart, IN, USA). Each section was stained with hematoxylin and eosin and subjected to ATPase staining (pH 4.2–4.5, pH 10.7 and pH 10.8) according to the method of Brooke *et al.* (18–19) and examined by microscopy.

More than 200 muscle fibers were examined and classified for each specimen. The cross-sectional area of the muscle fibers was measured and the fiber type distribution was investigated. Since the fiber type distribution varies between different parts of the EDL, measurements were obtained from an area which contains a mixture of fiber types.

Data was analyzed with the analysis of variance (ANOVA) test, with $P < 0.05$ considered significant.

Results

Body weight. At the end of the experiment, the rats' body weight had increased to 120%, 114.9%, 110.7%, and 107% in groups C, T, S, and ST, respectively, compared with pre-experiment weights. The weight gain was significantly lower in groups ST and S than in group C. In group T, it was almost the same as in group S for 2 weeks, but thereafter it was greater. No significant difference was noted between groups ST and S (Fig. 1).

Muscle fiber area. The cross-sectional area of the muscle fibers was measured for each fiber type. The areas of type I and IIa fibers in the SOL were, respectively, 3474.7 and 3380.0 μm^2 in group C, and 3123.0 and 3657.8 μm^2 in group T. The area of type I fibers was

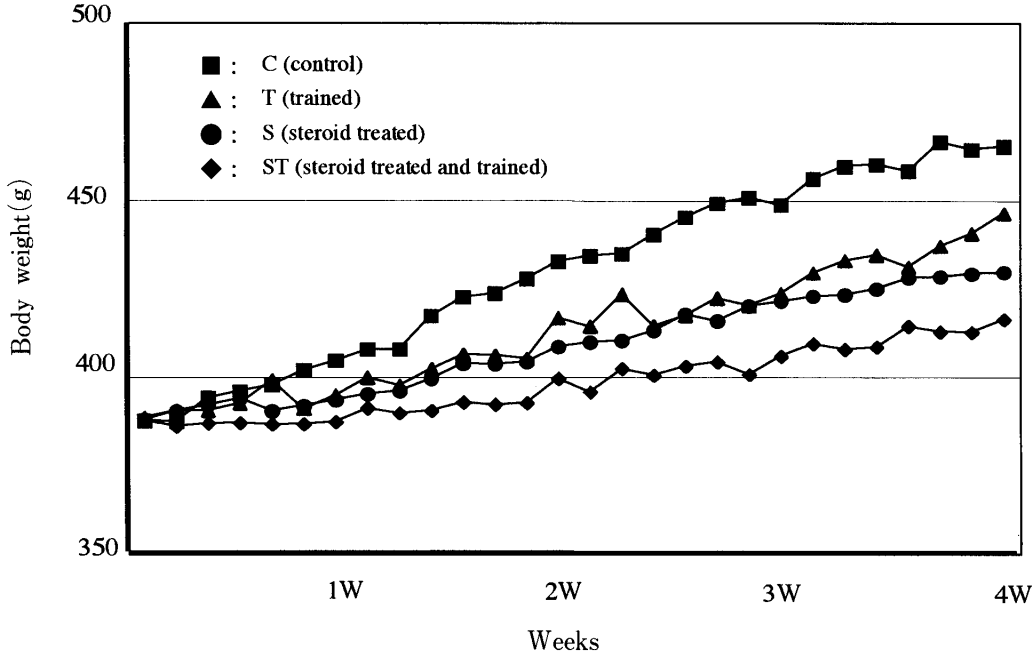

Fig. 1 Changes in body weight. The weight gain was significantly lower in groups ST and S than in group C. No significant difference was noted between groups ST and S.

significantly larger in group C than in group T. In the steroid groups, the areas of type I and IIa fibers were, respectively, 2516.5 and 2310.9 μm^2 in group S, and 2829.0 and 2354.9 μm^2 in group ST. The areas of type I and IIa fibers were, respectively, 72.4% and 68.4% of the control group's value in group S versus 81.1% and 72.9% in group ST. Both type I and IIa fibers showed significant atrophy in groups S and ST as compared with group C. The area of type I fibers was significantly larger in group ST than in group S (Figs. 2 and 3).

The areas of type I, IIa and IIb fibers in the EDL were, respectively, 1064.4, 3140.6 and 1377.4 μm^2 in group C and 1078.4, 3166.4 and 1438.0 μm^2 in group T. There were no clear differences in the area of each fiber type between groups C and T. In the steroid groups, the areas of type I, IIa, and IIb fibers were, respectively, 1035.2, 2513.4 and 1152.0 μm^2 in group S and 1058.7, 2962.4 and 1239.2 μm^2 in group ST. In group S, the areas of type IIa and IIb fibers were, respectively, 70.4% and 85.3% of the control group's values and significant atrophy was observed. In group ST, the area of type IIb fibers was 87.5% of the control group's value and significant atrophy was also noted. In contrast, type I fibers showed little decrease in area in groups S and ST and no significant differences were noted between groups C, T, S and ST. The area of type IIa fibers was significantly larger in group ST than in group S (Figs. 2 and 3).

Fiber type distribution. In group S, the proportion of type I fibers in the SOL was 75.3%, which significantly lower than in the other three groups, while the proportion of type IIa fibers was increased (19-20). There was little difference in fiber type distribution between groups ST and C.

The proportion of type I fibers in the EDL was significantly lower in group S than in groups C and ST, while the distribution of type IIa or IIb fibers showed no obvious trends. There was little difference in the distribution of type I, IIa or IIb fibers between groups ST and C (Table 1).

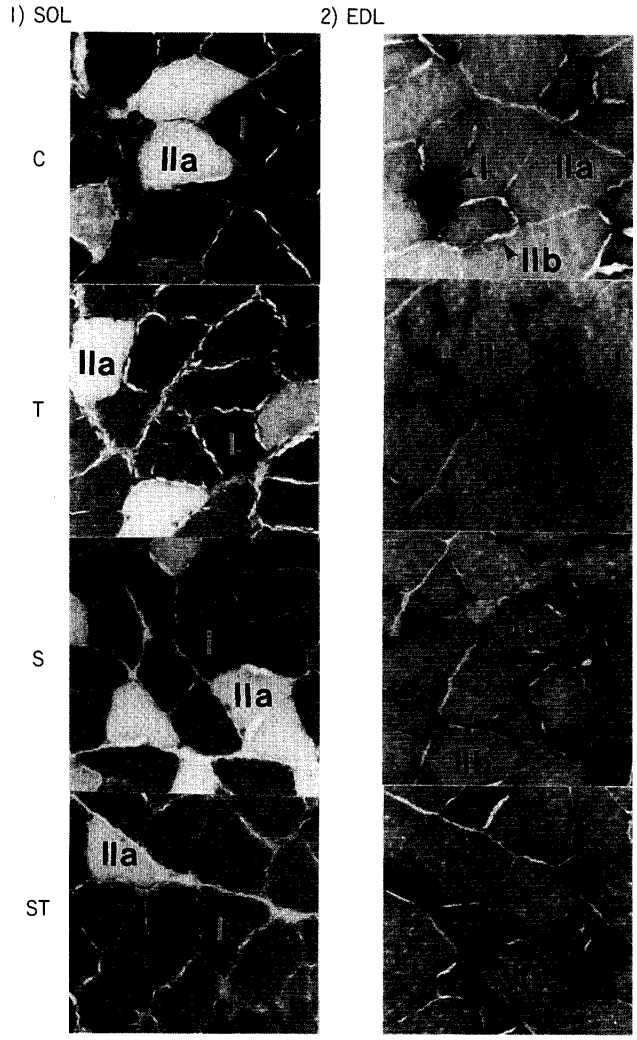

Fig. 2 ATPase staining of muscles 1) Cross sections of the soleus muscle (SOL) stained for ATPase after preincubation at pH 4.3. Magnification × 200. 2) Cross sections of the extensor digitorum longus muscle (EDL) stained for ATPase after preincubation at pH 4.5. Magnification × 200. I: type I fiber; IIa: type IIa fiber; IIb: type IIb fiber.

Discussion

Steroids cause muscle cells to produce glutamine synthetase. As a result, the synthesis of glutamine is enhanced and uptake of amino acids is inhibited, resulting in decreased muscle protein synthesis. This causes muscle atrophy and weakness, a condition known as steroid myopathy (21). In addition, a decrease in the activity of glycolytic enzymes and disturbance of mitochondrial oxidative phosphorylation have been reported (5, 13). Thus, it is thought that type II muscle fibers, which contain few mitochondria, are more readily influenced. Furthermore, diseases which are treated with steroid therapy may themselves be a cause of muscle weakness and disuse atrophy due to decreased exercise. In patients undergoing long-term steroid therapy, both types of muscle weakness may occur simultaneously. It has been

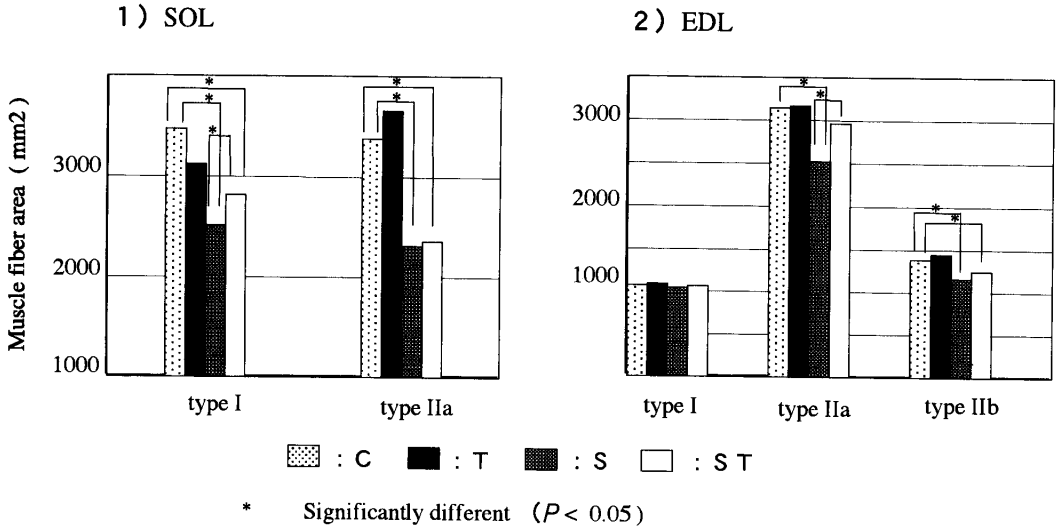

Fig. 3 Cross-sectional area of muscle fibers. 1) Both type I and IIa fibers showed significant atrophy in groups S and ST as compared with group C. The area of type I fibers was significantly larger in group ST than in group S. 2) Type I fibers showed little decrease of area in groups S and ST and no significant differences were noted between groups C, T, S and ST. The area of type IIa fibers was significantly larger in group ST than in group S. SOL: Soleus muscle; EDL: Extensor digitorum longus muscle.

Table I Fiber type distribution

1) SOL

	C	T	S	ST
Type I	87.9 ± 5.0	90.4 ± 4.0	75.3 ± 5.5*	84.6 ± 4.8**
Type IIa	12.1 ± 5.0	9.7 ± 4.0	24.6 ± 5.4*	15.4 ± 4.8

2) EDL

	C	T	S	ST
Type I	16.4 ± 0.9	10.0 ± 1.4*	7.4 ± 3.4*	16.5 ± 1.0**
Type IIa	46.3 ± 5.3	45.2 ± 2.8	45.8 ± 4.7	41.3 ± 1.1
Type IIb	37.3 ± 4.4	44.8 ± 1.4	46.3 ± 6.6	42.2 ± 2.0

Mean ± SD. (%)

* Significantly different from C ($P < 0.05$)

** Significantly different from S ($P < 0.05$)

1) In group S, the proportion of type I fibers in the soleus muscle (SOL) was 75.3%, which was significantly lower than in the other three groups, while the proportion of type IIa fibers was increased. There was little difference in fiber type distribution between groups ST and C. 2) The population of type I fibers in the extensor digitorum longus muscle (EDL) was significantly lower in group S than in groups C and ST.

reported that the number of steroid receptors was elevated and steroid sensitivity enhanced in muscles with disuse atrophy (22). These changes may contribute to steroid myopathy.

In the present study, examination of hematoxylin and eosin-stained sections showed no cellular infiltration or destruction of the muscle fibers in groups S and ST. In the steroid groups, weight gain was clearly slower than in the control group, and type II fibers of the EDL were clearly atrophic compared with the other types of fibers. These changes indicate that steroid myopathy developed in the steroid groups. In these groups, the areas of both type I and type IIa fibers of the SOL were decreased, as was the proportion of type I fibers, indicating disuse atrophy (23-24).

Some reports have been published on the efficacy of exercise therapy for preventing steroid myopathy. However, most studies assessed the influence of exercise on experimentally-induced steroid myopathy and only a few clinical studies have been carried out. Hober *et al.* (4) evaluated the effects of leg exercise using a cybex in patients undergoing long-term prednisolone therapy at a dose of about 12 mg/day after renal transplantation. They reported improvements in femoral muscular atrophy, increased knee-extensor power and decreased loss of the cross-sectional area of midthigh muscle. However, they did not examine the muscle fibers histochemically and did not confirm whether the muscular atrophy was ascribable to steroid myopathy or not. The effects of exercise

on experimentally-induced myopathy have also been assessed using weight lifting, running, treadmill exercise and other methods.

Gardiner *et al.* (12) administered triamcinolone to rats at a dose of 1 mg/kg/day in order to induce steroid myopathy, and forced them to perform weight lifting with a load set at 80% of their body weight 4 times a week for 6 weeks. This training led to enlargement and increased strength of both fast- and slow-twitch fibers in the gastrocnemius. Takahashi *et al.* (17) administered triamcinolone (1 mg/kg/day) to rats and allowed them to perform free running over an 8-week period. As a result, atrophy of type IIa and IIc fibers of the SOL, and type I, IIa and IIb fibers of the EDL was prevented. The drug used in these experiments, triamcinolone, has 4 times the glucocorticoid effect of hydrocortisone and is well known to cause steroid myopathy. However, since it is not very soluble, it is often administered by local injection, such as intraarticular injection, and is rarely used systemically or by intramuscular injection. Further, it is difficult to quantify the amount of exercise performed with weight lifting and free running.

Hickson *et al.* (14) administered hydrocortisone at a dose of 100 mg/kg/day for the last 12 days of a 13–18-week treadmill training period during which rats were forced to run at a speed of 31 m/min. He found that this training prevented a decrease in the wet weights of the SOL, gastrocnemius, and plantaris. Faluduto *et al.* (8) administered hydrocortisone (100 mg/kg/day) to rats, forced the animals to run at a speed of 29 m/min for 90 min daily for 11 days, and compared the decrease in the cross-sectional area of muscle fibers between the steroid-only and steroid-plus-training groups. They found that training prevented atrophy in almost 100% of type IIa fibers and 67% of type IIb fibers in the deep layer of the plantaris where both types of fibers are mixed. In addition, they found that exercise prevented atrophy in 50% of type IIa fibers and 40% of type IIb fibers in the superficial layer which has more type II fibers.

However, the dosage of steroid administered by Faluduto *et al.* (8) to induce myopathy was 10 mg/kg/day. This is clearly higher than the actual clinical dosage range, meaning that the experimentally-induced steroid myopathy they studied was not clinically relevant. Further, in our own experiments, we found that the running speed they used (about 30 m/min) was very high. More than half of the rats used in the present study could not run at this speed. In group ST, some rats died during the training period. According to Shepherd & Gollnick (25), a running speed of 30 m/min corresponds to an exercise intensity of 84% $\dot{V}O_2$ max for rats. It would be very difficult for patients with diseases requiring steroid therapy to perform such intensive exercise.

In the present study, hydrocortisone was administered at a dose of 10 mg/kg/day and the level of exercise was set at 15 m/min for 60 min daily, which was lower than has been used previously. It was concluded from the results of our previous studies that a dose of 10 mg/kg of hydrocortisone would have little influence on survival, but would be high enough to cause steroid myopathy. The running speed of 15 m/min employed in the present study was about half of that used previously. At this speed, the rats were often walking rather than running, so all animals could perform the training easily and none of them died during the study.

We found no clear differences in fiber area or fiber type distribution between groups C and T. These results suggest that the exercise load tested was not sufficient to increase muscle bulk in normal rats and could easily be performed by patients with diseases requiring steroid therapy. Further, the type II fibers of the EDL showed significant atrophy in groups S and ST, which is evidence steroid myopathy. However, the area of type IIa fibers was significantly larger in group ST than in group S, so steroid myopathy was considered to have been prevented by training in the former group. Type I fibers of the SOL showed significant atrophy in group S, and the proportion of type I fibers was significantly decreased along with the presence of possible disuse atrophy. The atrophy of type I fibers was significantly prevented in group ST compared with group S, and changes in fiber distribution (decreased type I fibers and increased type IIa fibers) were prevented in group ST. These results suggest that the changes induced in the SOL by disuse atrophy were prevented by exercise.

When the decrease in the cross-sectional area of EDL fibers was compared between groups S and ST by the method of Faludute *et al.* (8), atrophy was found to have been prevented in 73.3% of type IIa fibers and 14.4% of type IIb fibers. These values were lower than those reported by Faludute *et al.* However, it is questionable whether the exercise load they used could be employed clinically. The fact that mild exercise can prevent muscle atrophy in steroid myopathy, as we demonstrated in this study, may be of more clinical significance since even patients receiving steroids can perform such exercise.

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