Changes in Total Fiber Numbers of Disused Soleus Muscle on Rats

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In this study, by use of technique that was modified from Morey method, we discussed the histological influence on the soleus muscle of the rats caused by disuse. This study is characterized by the calculating of total numbers of muscle fibers. ST(slow-twitch) and FT(fast-twitch) fibers in total muscular cross-sectional area were classified by their difference in intensity of staining of actomyosin adenosinetriphosphatase (myosin ATPase).

During the experiment, average fiber diameter of ST and FT fibers declined when compared to control group($p < 0.01$). A 54% decrease in the total number of ST fibers was observed in the experimental group($p < 0.01$). Conversely, the total number of FT fibers increased to 362% of the control value($p < 0.01$). These results of the changes evoked in ST and FT fibers indicate 34% decrease in total muscular cross-sectional area, and showed that muscular function shifted toward a faster muscle in disused soleus muscle.

Key words: Disuse atrophy, Histochemistry, Fiber type, Fiber size

When we examine the influence exerted on skeletal muscle fiber by atrophy resulting from disuse of muscle, the methods used are immobilization (Booth and Kelso 1973), denervation (Goldberg 1969), tenotomy (Goldspink et al. 1983), and suspension (Morey 1979). With any of these methods, we are unable to reach a conclusion, because of the differences in the period of examination, the process of atrophy, type and age of animals and muscle. However, some reports indicate that there are characteristic changes of muscle atrophy in the soleus muscle. Namely, the function of the soleus muscle (maintaining of body posture) is lost, its composition shifting from that of slow muscle to faster muscle. Booth and Kelso (1973) reported a decrease in type I fibers in the rat soleus muscle. But Maier et al. (1976) contended that examination of the published data of Booth and Kelso (1973), instead of showing a selective decrease in type I fibers. They showed a 7% increase of type II fibers above the control, suggesting a conversion of type I to type II fibers.

However, the above mentioned histological results are not data calculated from the total number of muscle fibers. Templeton et al. (1988) reported for total fiber numbers in 2-4wk suspension of male Sprague-Dawley rats. Total fiber numbers observed to be unchanged by suspended rats. Desplanches et al. (1987) found biochemistry that hindlimb suspension reduced the amount of type I isoenzyme and increased the intermediate isoenzyme in rat soleus muscle after 5wk suspension.

This study is characterized by the calculating of total numbers of muscle fibers from a section of soleus muscle by using a myosin ATPase stain. i.e. for exact date, it is important to analyze the total numbers of muscle fibers. We examined the histological influence on the soleus muscle of Wistar strain rats by disuse.

MATERIALS AND METHODS

Male Wistar rats, weighing ~160g, were weight matched and then separated into 2 groups. These

were then designated as group 1-Control Group($n=5$), and group 2-Experimental Group($n=5$). All the animals were provided with food and water ad libitum and housed at room temperature ($23\pm1^{\circ}\text{C}$) with a 12-h light/dark cycle.

The rats in the experimental group were set up in a manner whereby they were unable to use their hindlimbs. The hindlimbs of the experimental rats were suspended using a similar method (Nakagawa et al. 1989) to that described by Morey (1979). The rats were suspended from a hook and swivel situated above the cage designed to allow the animals freedom to move rotationally through 360° by means of their front limbs. So that only the front limbs were able to make contact with the cage, the height of the hook was adjusted accordingly. In addition, they were suspended at an approximately 20° head down tilt angle in order to minimize lordosis. The rats of the experimental group were kept in this state for a period of 5 weeks.

At the end of 5 weeks, the rats were killed with an overdose of ethyl anaesthetic. The soleus muscles were removed immediately, trimmed of excess connective tissue and fat, blotted dry, and wet weighed. A 5–10 mm cross section was taken from the mid-belly of each muscle, mounted on cork, and quick-frozen with isopentane cooled to -160°C by liquid N_2 . Serial cross sections, $10\mu\text{m}$ thick, were cut from the sample in a cryostat maintained at -20°C . Stained for myofibrillar actomyosin ATPase with preincubations of acidic (pH4.3) and alkaline (pH10.5), ST (slow-twitch fiber) and FT (fast-twitch fiber) fibers permitted classification of the fibers in serial cross sections (Dubowitz and Brooke 1973). FT (alkali-stable) and ST (acid-stable) fibers were identified from the intensity of the myosin ATPase reactions (Gollnick et al. 1972).

Measurements of the ST and FT fiber diameters, fiber type composition, total fiber number, and total muscular cross-sectional area were counted from a photographic montage taken from a transverse section through comparable regions at the largest

girth (Gonyea et al. 1977). In this way, the total number of ST and FT fibers was established. The total muscular cross-sectional area and diameter of the muscle fibers were measured using a measuring device (planimeter). The mean average diameter of the muscle fibers was calculated from the shortest and longest diameter length.

Statistical evaluations were made using the student-t test for comparison between the two sample means.

RESULTS

Table 1 shows the mean final body weight, the mean food intake per week and the mean soleus wet weight for each group. Compared with the controls, the body weight of the experimental group decreased by 28%. The experimental group revealed significantly lower values for the amount of food consumed and soleus wet weight.

Fibers were classified according to the terminologies of Dubowitz and Brooke (1973), and Gollnick et al. (1972) (ST fiber, FT fiber). Figure 1 and 2 shows the myofibrillar ATPase reaction of soleus muscle in the control and experimental groups. The mean fiber diameter of both the ST and FT fibers were significantly smaller in the experimental rats than in the control rats (Table 2). Also, Table 2 shows the ST fiber diameter to be less than the FT fiber diameter. These decreases in the ST and FT fibers result in a 66% decrease in the total muscular

Table 1 Body weight, food intake and soleus wet weight in control and experimental rats.

	Body weight(g)	Food intake /week(g)	Soleus weight(mg)
Control ($n=5$)	317.1 ± 14.8	184.0 ± 11.6	147.8 ± 11.6
Experimental group ($n=5$)	$228.7\pm28.9^*$	$161.1\pm5.7^*$	$118.3\pm9.8^*$

Significant difference from the control. Values are means $\pm$ S.D. * $P < 0.01$

Fig. 1 Total muscular cross-sectional area in the rat soleus muscle.
A : Control rat. B : Experimental rat.
Black stained : Fast-twitch fiber. White : Slow-twitch fiber.

Fig. 2 Muscle fibers in transverse serial sections in the rat soleus muscle.
A : Control rat. B : Experimental rat.
Black stained : Fast-twitch fiber. White : Slow-twitch fiber.

Table 2 Mean fiber diameter, total fiber number, and total muscular cross-sectional area in soleus muscles of rats.

	diameter of ST fiber(μ m)	diameter of FT fiber(μ m)	total number of ST fiber	total number of FT fiber	total number of fiber	total muscular cross-sectional area(mm ²)
Control (n=5)	60.1 ± 7.9	56.8 ± 2.7	2,341.0 ± 279.9	226.0 ± 91.3	2,567.0 ± 266.1	8.92 ± 2.15
Experimental group (n=5)	25.8* ± 1.4	30.7* ± 0.4	1,076.8* ± 118.9	818.4* ± 241.8	1,895.2* ± 230.2	3.03* ± 2.15

Values are means $\pm$ SD. * Significant difference between control and experimental group ($P < 0.01$). ST, slow-twitch; FT, fast-twitch

Fig. 3 The percentage of the number of slow-twitch fibers(ST) and fast-twitch fibers(FT) in the rat soleus muscles in control (upper) and experimental (lower) groups.

cross-sectional area (Table 2). In contrast, the total fiber number in the soleus muscles showed a 26% decrease in the experimental rats. The fiber type composition of the soleus muscles was altered as a result of suspension, i.e. a 54% decrease in the total number of ST fibers was observed in the experimental group when compared to the control group. Conversely, the total number of FT fibers increased to 362% of the control value the 5 weeks experimental period (Table 2). Also, in experimental group, the suspended rats showed a 56.8% ST fiber decrease compared with 91.2% in the control rats (Fig. 3).

DISCUSSION

The increase in the weight of the experimental rats was inhibited, which might be caused by the smaller amount of food consumed due to stress under experimental conditions.

The soleus is a postural muscle: the chronic disuse imposed upon the hindlimbs was expected to

have a greater effect on this muscle (Hargens et al. 1983). As previously reported, the wet weight of the soleus muscle showed a significant decrease. Several factors, such as growth failure or stress, may have been involved in the muscular atrophy observed after prolonged hypokinesia. In this report, the transformation of hormones in connection with stress has not been studied. Bikle et al. (1985) reported an increased corticosterone level after 1 day, with a return to the control levels on the 7th day of hypokinesia. Desplanches et al. (1987) explained that stress caused through using the suspension model was not a factor in muscle atrophy. Therefore, for the purpose of this experiment, changes in hormone level were not taken into account.

Atrophy of the soleus muscle was evident from the decrease in muscle wet weight and total muscular cross-sectional area. This seems to be caused by muscle fiber atrophy and the decrease in number of muscle fibers. Hauschka et al. (1987) reported that the average area of light ATPase fiber (ST) and dark ATPase fiber (FT) decreased in rats after 28 days of suspension. In this experiment, an average diameter of fiber, in both the ST and FT muscle fibers was found to be decreased. As to the change in the total number of muscle fibers, Templeton et al. (1988) reported a decrease in the number of type I fibers and an increase in type II fibers without change in the total number of muscle fibers as a result of 2-4wk rats suspension. This indicates that the composition of muscle fibers in type I and type

II fibers had become equal. But, in our data, total numbers of fibers decreased in the experimental group. It seems to us that the differences of the results may be due to the differences in age and type of animals when compared to the above reports. This decrease in the total number of muscle fibers is thought to have been caused by the loss of ST fibers, as an increase in number of FT fibers was found in the experimental group. It was not clear whether the increase in the number of FT fiber was due to the shift from ST to FT for contractile properties, or with hyperplasia of the FT fiber in growth. Since the FT fiber ratio in the soleus muscle of rats has a tendency to decrease with aging, the increase in FT fibers is thought to have resulted from properties shifting from ST fiber to FT fiber. Templeton et al. (1984) have reported the influence of suspension hypokinesia 2-4 weeks on contractile properties of the soleus. Soleus speeding after 2wk of suspension was evidenced by a shorter contraction time and a decreased rate of tension development (Templeton et al. 1984). This shifting of contractile properties can be explained as fiber type grouping (Dubowitz and Brooke 1973) in the FT fiber by re-control of the nerve as seen in Fig. 1,2. From the above, by the data calculated from the total numbers of muscle fibers, it is clear that the soleus muscle shifts muscular function from slow muscle to faster muscle through disuse as simulated in this experiment.

REFERENCES

- Bikle, D.D., B.P. Halloran, C.M. Cone, and E. Mory-Holton, 1985: Bone loss during simulated weightlessness: is it glucocorticoid mediated?. *Physiologist*, 28, Suppl., : S-123-S-124.
- Booth, F.W., 1977: Time course of muscular atrophy during immobilization of hindlimb of rats. *J. Appl. Physiol. Respirat. Environ. Exer. Physiol.*, 43 : 656-661.
- Booth, F.W., and J.P. Kelso, 1973: Effect of hind-limb immobilization on contractile and histochemical properties of skeletal muscle. *Pfluegers Arch.*, 342 : 231-238.
- Desplanches, D., M.H. Mayet, B. Sempore, and R. Flandrois, 1987: Structural and functional responses to prolonged hindlimb suspension in rat muscle., *J. Appl. Physiol.*, 63 : 558-563.
- Dubowitz, V., and M.H. Brooke, 1973: *Muscle Biopsy., A Modern Approach.*, Saunders, Philadelphia.
- Goldberg, A.L., 1969: Protein turnover in skeletal muscle. II. Effect of denervation and cortison on protein catabolism in skeletal muscle., *J. Biol. Chem.*, 244 : 3223-3229.
- Goldspink, A.L., P.J. Garlick, and M.A. McNurlan, 1983: Protein turnover measured in vivo and in vitro in muscles undergoing compensatory growth and subsequent denervation atrophy., *Biochem. J.*, 210 : 89-98.
- Gollnick, P.D., R.B. Armstrong, C.W. Saubert VI, K. Piel, and B. Saltin, 1972: Enzyme activity and fiber composition in skeletal muscle of untrained and trained men., *J. Appl. Physiol.*, 33 : 312-319.
- Gonyea, W., G.C. Ericson, and F. Bonde-Petersen, 1977: Skeletal muscle fiber splitting induced by weight-lifting exercise in cats., *Acta Physiol. Scand.*, 99 : 105-109, 1977.
- Hargens, A.R., C.M. Tipton, P.D. Gollnick, S.J. Mubarak, B.J. Tucker, and W.H. Akeson, 1983: Fluid shifts and muscle function in humans during acute simulated weightlessness., *J. Appl. Physiol.*, 54 : 1003-1009.
- Hauschka, E.D., R.R. Roy, and V.R. Edgerton, 1987: Size and metabolic properties of single muscle fibers in rat soleus after hindlimb suspension., *J. Appl. Physiol.* 62 : 2338-2347.
- Maier, A., J.L. Crockett, D.R. Simpson, C.W. Saubert IV, and V.R. Edgerton, 1976: Properties of immobilized guinea pig hindlimb muscle., *Am. J. Physiol.*, 231 : 1520-1526.
- Morey, E.R., 1979: Space flight and bone turnover: correlation with a new rat model of weightlessness., *Bioscience*, 29 : 168-172.
- Nakagawa, Y., M. Totuka, T. Sato, Y. Fukuda, and K. Hirota, 1989: Effect of disuse on ultrastructur-

- e of achilles tendon in rats., Eur. J. Appl. Physiol., 59 : 239-242.
- Templeton, G.H., H.L. Sweeney, B.F. Timson, M. Padalino, and G.A. Dudenhoefter, 1988 : Changes in fiber composition of soleus muscle during rat hindlimb suspension., J. Appl. Physiol., 65 : 1191-1195.
- Templeton, G.H., M. Padalino, J. Manton, M. Glasberg, C.J. Silver, P. Silver, D. DeMaTino, T. LeConey, G. Klug., H. Hagler, and J.L. Sutko, 1984 : The influence of rat suspension-hypokinesia on the gastrocnemius muscle., Anvi. Space Environ. Med., 55 : 381-386.

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