Scand J Rehab Med 21: 19–26, 1989

MUSCLE ADAPTIVE CHANGES IN POST-POLIO SUBJECTS

Gunnar Grimby, Gisli Einarsson, Marita Hedberg and Amelie Aniansson

From the Department of Rehabilitation Medicine, Gothenburg University, S-413 45 Gothenburg, Sweden

ABSTRACT. Nineteen post-polio subjects (9 men and 10 women) aged 41–65 years were studied by means of muscle strength measurements (Cybex) of knee extension and muscle biopsies of the vastus lateralis for morphometric, histopathological and enzymatic analyses. Data from a reference group of 10 male subjects, age 42–51 years, are also given. Fourteen of the post-polio subjects had experienced a post-polio syndrome-like drop in function. All had had polio at least 25 years earlier. In nine of the 19 subjects, type I fibers accounted for more than 70 % of the total. There was a significant negative correlation between muscle strength and the percentage of type I fibers in women. Large cross-section areas of muscle fibers were found, with an average mean fiber area of 8 $\mu m^2 \times 10^3$. It is assumed that the large muscle fiber areas are due to an extreme use of the remaining muscle fibers in post-polio subjects with low muscle strength. There were significant negative correlations between muscle strength values and mean fiber area in men. Most subjects had single atrophic fibers; groups of atrophic fibers were less common. Internal nuclei and splitting were seen in about half of the subjects. The activity of citrate synthase was low, but normal for glycolytic enzymes.

Key words: muscle strength, muscle morphology, muscle enzymes, muscle adaptation, poliomyelitis.

Polio-survivors may experience an onset of new muscular problems around 30 years after acute polio (8, 14). There are a number of hypotheses concerning the possible causes of the muscle changes. It has been suggested that many of the surviving motor neurons carry permanent scars from the polio virus infection and, with the greater load, are not able to keep pace with the metabolic demands of innervating all their muscle fibers (14, 24). Another cause of this late-onset weakness could be an effect of the natural loss of the neurons with aging. The loss of a few large motor units has a more significant effect on the muscle strength than in normals, as each one of these neurons innervates a much larger than normal proportion of the muscle fibers. However, in normal subjects there may not be any significant loss of motor neurons before the age of 60, as has been demonstrated in studies of the human lumbosacral cord (22). No study has yet been made whether there is an earlier onset of these normal aging processes in polio patients. Unstable neuromuscular connections, as suggested by electromyographic studies (24) and which are also evident from our own observations (Einarsson, Stålberg & Grimby, unpublished observations), could result in a thinning out of the number of muscle fibers in the large motor units. This is further supported by a recent report by Dalakas et al. (8) based on observations of jitter and blocking in EMG in combination with the finding of single atrophic fibers and lack of grouping of atrophic fibers. It is to note, however, that in a recent published study by Cashman et al. (6) electromyographic and muscle biopsy evidence of ongoing denervation did not distinguish between stable patients with prior polymyelitis and those with new weakness.

The present material could be utilized in a follow-up study to provide a better understanding of the reduction in muscle function in post-polio subjects. Furthermore, the present group gives the possibility to study adaptive changes in the muscle structure due to a long-term use of the reduced number of motor units in daily activities.

METHODS

Subjects

Nineteen subjects were included in the present study. They were selected from a larger group of post-polio persons (17 men, 24 women), which will be presented separately concerning clinical findings and function in various activities. Fourteen of the subjects had experienced a post-polio syndrome-like drop in function. The selection criteria for the whole group were that they had had their polio at least 25 years earlier and were 40 to 65 years of age. For the present study, the subjects had to have maintained muscle strength at least for a resisted full knee extension in the sitting position, as muscle strength measurements were performed on a Cybex dynamometer. Nine men (44 to 61 years, mean 52 years) and ten women (41 to 65 years, mean 56 years) volunteered for the muscle biopsy study. These 19 subjects had had acute polio 29 to 61 years earlier (mean 41 years). The age at onset of polio was 2–33 years (mean 13 years). All measurements were performed on the same day. Informed consent was given by all subjects and the procedure was approved by the Ethical Committee of the Faculty of Medicine, Gothenburg University, Sweden.

As a reference group for the male subjects, ten sedentary living men (age 42 to 51 years) without any clinical symptoms from the neuromuscular or locomotor systems were studied with the same methods. Their average body weight was 80±11 kg and their body height 178±7 cm. Measurements of enzymatic activities were not performed in the reference group.

Muscle strength measurements

Muscle strength was measured using a modified Cybex II dynamometer (Lumex, NY) with a specially designed computer program, including compensation for the torque due to the weight of the lower leg and the lever arm of the dynamometer (13). The subjects sat with a hip angle of 90° and the lower leg attached to the lever arm of the dynamometer. Isometric muscle strength was measured for knee extension at knee angles of 30° and 60°. Isokinetic strength during knee extension was measured with angular velocities of 30, 60, 180 and 300°/second. The range of angular movement of the knee joint was from 100° to 0° (i.e., full knee extension). Three curves were recorded, and the highest peak torque values are reported. In this presentation, only values from the weakest side, where the muscle biopsies were also performed, are given.

Muscle biopsy

Muscle biopsies were taken with an alligator forceps (15) from the middle portion of the vastus lateralis (halfway between the upper border of the patella and the anterior iliac spine) under local anesthesia. The muscle specimens were divided into two parts: one part was frozen immediately in liquid nitrogen and used for analysis of enzymatic activities, the other part was trimmed, mounted, and frozen in cooled isopentan (−160°C) and used for histochemical and histopathological analysis. Both parts were stored at −80°C until analyzed.

For histochemical analysis serial transverse sections (10 μm) were cut with a cryotome at −21°C. The myofibrillar adenosine-triphosphatase (ATPase) method was used for muscle fiber classification (5, 9). The reactions were carried out at pH 9.4 following alkaline preincubation (pH 10.3) to classify fibers into type I and type II (11). The type II fibers were subclassified into II A, II B and II C fibers using preincubation at pH 4.62 and 4.35 (5). The average number of fibers counted in each subject was 415±53 fibers.

Measurements of the fiber areas were made on photos of NADH tetrazolium reductase stained transverse sections. An optical illumination device ("particle size analyser", Carl Zeiss, Oberkochen, West Germany), projecting the muscle fibers as circles of varying size, was used and the total fiber area was approximated. This technique has shown good agreement with planimetric area measurement (3) with a standard error of the single determination expressed as a percentage of the mean being 1.8%. Fiber area was on an average measured in 175±20 fibers.

For the histopathologic evaluation, hematoxyline-eosine and modified Gomori-trichrome staining were also used, as well as periodic acid Schiff (PAS) reaction for glycogen (9). The criteria for atrophic fibres was a lesser diameter of <20 μm. Large grouping was defined as more than 16 fibers of the same type grouped together (16). The occurrence of internal nuclei in more than 3% or 10% (9) of the fibers in the whole cross-section was reported.

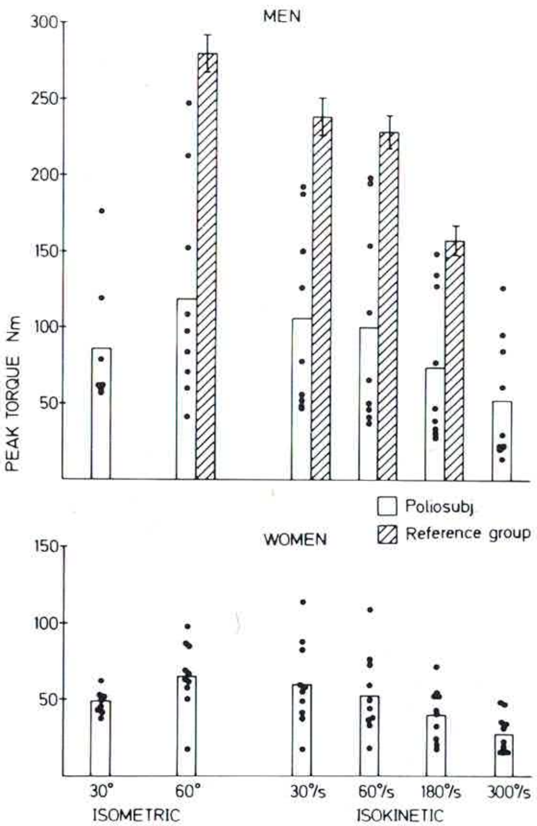

Fig. 1. Maximal torque during isometric knee extension with a knee angle of 30° and 60° and peak torques during isokinetic knee extension with angular velocities 30° to 300°/s in post-polio subjects and in a healthy male reference group. Mean values and standard error of the mean are given.

For biochemical assays, the enzyme activity was determined by means of fluorimetric techniques using a Farrand ratio-fluorimeter-2 (Farrand Optical Co., Valhalla, NY). The reactions catalyzed by the enzymes under investigation were coupled to NAD–NADH-linked reactions and determined according to the principles given by Lowry & Passoneau (17).

Analyses were made of triosephosphate dehydrogenase (TPDH), lactate dehydrogenase (LDH), myokinase (MK), and citrate synthase (CS). The assays were performed at 37°C. The protein content was determined according to Lowry et al. (17) in order to express the activities per gram of protein. The methodological errors, as presented in a previous paper (4), for the determination of enzyme

Table I. *Correlation analysis of the relative occurrence of type I fibers and various strength measurements of knee extension*

Muscle strength measurement	Coefficient of correlation		Significance	
	Men	Women	Men	Women
Isometric 30° knee angle	−0.27	−0.59	NS	NS
Isometric 60° knee angle	−0.51	−0.75	NS	<0.05
Isokinetic 30°/s	−0.38	−0.82	NS	<0.01
Isokinetic 60°/s	−0.41	−0.81	NS	<0.01
Isokinetic 180°/s	−0.40	−0.71	NS	<0.05
Isokinetic 300°/s	−0.43	−0.63	NS	<0.05

NS means no significance.

activities were TPDH 5.8%, LDH 5.2%, MK 3.8% and CS 3.8%.

Amylase-periodic acid-Shiff (PAS) staining was used to visualize capillaries (1), and the number of capillaries per fiber and the fiber area per capillary were calculated for the different fiber types.

Statistics

For statistical analysis, Wilcoxon's nonparametric test was used for differences between groups; for analyses of correlation, Spearman's rank correlation test was used.

RESULTS

As can be seen in Fig. 1, the polio subjects had markedly reduced muscle strength compared with a reference group studied in our laboratory, the difference being significant ($p<0.05$). There was a large individual variation as seen from the presented individual values. All subjects showed a reduction in peak torque values with increasing angular velocity. The reduction between 30°/s and 300°/s did not differ between the male polio subjects and the reference group, nor was there any correlation between this reduction and the percentage type I fibers.

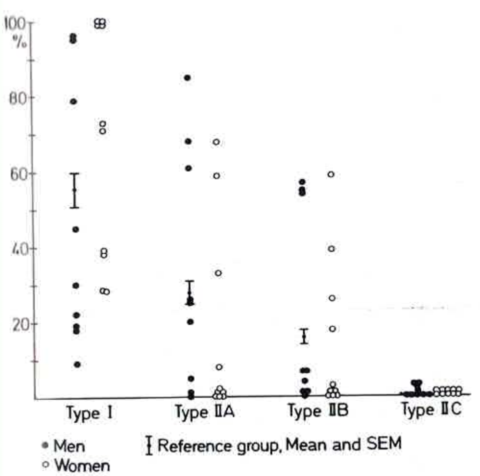

Fig. 2. Fiber frequencies in muscle biopsy from the vastus lateralis with individual values in male and female post-polio subjects. Mean values with standard errors of mean are given in the healthy male reference group.

In nine of the 19 subjects, more than 70% (range 70% to 100%) type I fibers were seen (Fig. 2). The rest of the subjects had less than 50% type I fibers (range 9% to 42%). Thus, the fiber frequency was separated into two groups. The subjects usually had somewhat more type II A than type II B fibers, but in some subjects, few or even no type II fibers were recorded. There were few type II C fibers in some of the subjects, but in none of them more than 3%. No sex difference was noted in fiber frequencies.

There were significant negative correlations between muscle strength and the percentage of type I fibers in women, on the basis of isometric as well as isokinetic strength values; the correlations not being significant in the men (Table I).

Large cross-sectional areas of the muscle fibers were found in a number of subjects, but with a large individual variation, as seen in Fig. 3. The variation coefficient was 41±8% for type I fibers (range 22–90), 53±10% for type II A (range 25–83), 64±15% for type II B (range 31–132%) in men. In women it was 35±4% for type I fibers (range 21–59), 38±5% for type II A (range 26–53) and 37±6% for type II B (range 22–53). As expected, the fiber areas in women were smaller than in men, especially for type II A fibers, and less so for type I fibers. On average, the fiber area values were high, with a mean fiber area in the order of 8 $\mu m^2 \cdot 10^3$, which can be compared with the men in the reference group, who had an average mean fiber area of

Table II. *Correlation analysis of mean fiber area and various strength measurements of knee extension*

Muscle strength measurement	Coefficient of correlation		Significance	
	Men	Women	Men	Women
Isometric 30°/s knee angle	−0.76	−0.08	<0.05	NS
Isometric 60°/s knee angle	−0.72	−0.14	<0.05	NS
Isokinetic 30°/s	−0.82	−0.32	<0.01	NS
Isokinetic 60°/s	−0.81	−0.36	<0.01	NS
Isokinetic 180°/s	−0.82	−0.43	<0.01	NS
Isokinetic 300°/s	−0.78	−0.36	<0.05	NS

NS means no significance.

$4.4 \pm 0.4\ \mu m^2 \cdot 10^3$. The variation coefficients for fiber areas were also significantly smaller in the reference group (25 ± 3, 24 ± 2 and 28 ± 4% for type I, II A and II B, respectively).

In women, there was a significant correlation between the mean area of type II A fibers and the relative number of these fibers ($r=0.87$, $p<0.05$); the corresponding correlation not being significant for type I fibers ($r=0.61$).

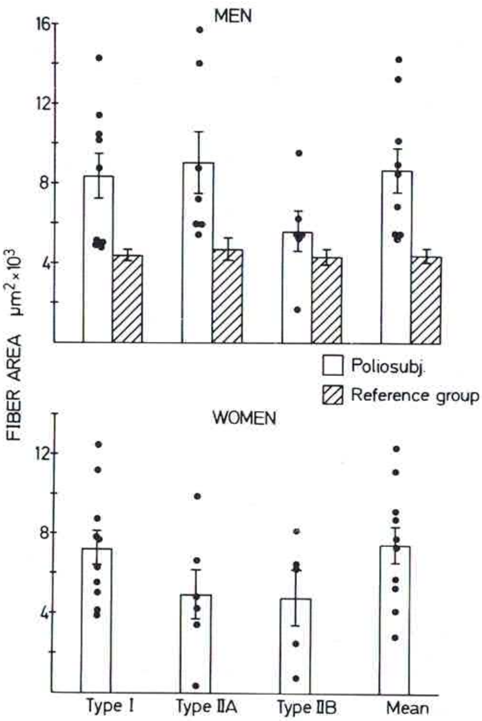

Fig. 3. Fiber area for various fiber types and mean fiber area in muscle biopsies from the vastus lateralis in male and female post-polio subjects. Individual values, mean values and standard error of the mean are given. In addition, mean values and standard errors of the mean are given from the healthy male reference group.

Correlation analyses between muscle strength values and mean fiber area (Table II) showed significant negative correlations in men, but not significant in women, and also significant correlations for type I fiber area in men ($r=0.75-0.71$).

The histopathologic findings in the 19 subjects studied are summarized in Table III, and cross-sections in two female subjects are shown in Fig. 4. Large grouping (>16 fibers) was seen in most of the subjects and for both fiber types in seven subjects who had 28–79% type I fibers. The five subjects with grouping of only type I fibers had 90–100% type I fibers. The five subjects with grouping of only type II fibers had 62–100% of that fiber type. Most subjects had single atrophic fibers, usually round, but groups consisting of more than three atrophic fibers were found in only four of the subjects, the lower part of Fig. 4 being from one of these subjects. In five subjects, more than ten atrophic fibers were noted in the whole cross-section. Four of these subjects had muscle strength values which were below average for the total group of post-polio subjects. Internal nuclei were

Table III. *Histopathologic findings (n=19)*

	No. of subjects
Small angular fibers	3
Small round fibers	18
Fiber atrophy (>10 fibers)	
Type I	1
Type II	4
Large grouping (>16 fibers)	
In type I and type II fibers	7
Only in type I	5
Only in type II	5
Internal nuclei (>10% of fibers)	7
Splitting	8
Fibrosis	2

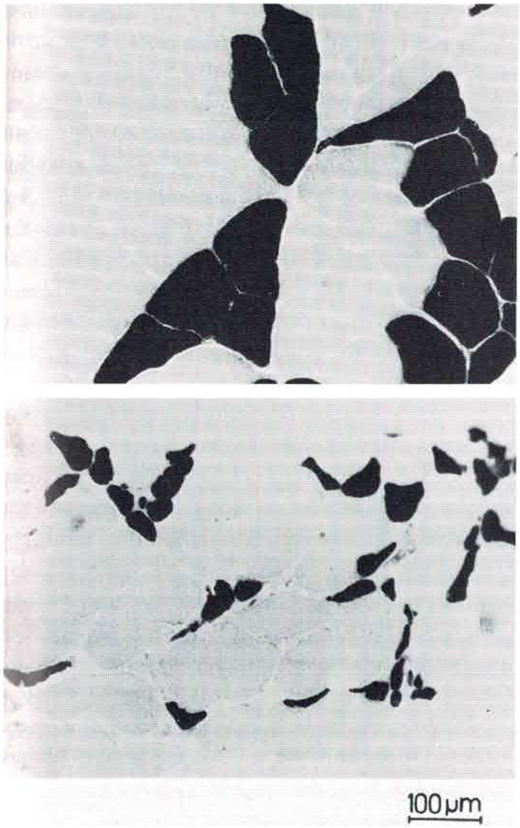

Fig. 4. Cross-section from the vastus lateralis muscle in two female post-polio subjects. (A) Upper part: age 42 years, polio 36 years ago; (B) lower part: age 61 years, polio 49 years ago. The sections are stained for myofibrillar ATPase using preincubation at pH 9.4. Type I fibers are stained lightly and type II fibers dark. There is a dominance of type I fibers with large type I as well as type II fibers (average area 5.06 and 5.85 $\mu m^2 \times 10^3$ respectively) in subject A. Small atrophic type II fibers are seen in subject B.

seen in more than 3% of the fibers in around half of the subjects, and in more than 10% of fibers in seven subjects. Splitting in a few fibers was seen in around half of the subjects. No other myopathic changes were noted. There were no differences in morphological findings between those who reported and those who did not report new muscular weakness.

The capillary density, expressed as capillaries per mm^2, was somewhat low in the post-polio subjects compared with the reference group, as seen in Table IV. The number of capillaries per fiber was, however, large in the post-polio subjects due to the large fiber areas. When the capillary density was expressed in relation to fiber area for the different types of muscle fibers, somewhat higher mean values, but not significantly different, were found in the male polio subjects compared to the reference group.

The activity of the different muscle enzymes studied showed large individual variations, as seen in Fig. 5. Enzymatic activities were not measured in the reference group presented in this paper. Low or very low values for citrate synthase were noted in the post-polio subjects in comparison with a group of clinically healthy men (n=22), 73 to 83 years of age (4), studied in our laboratory using an identical technique, where the average value was 26±2 mmol×g protein^{-1}×min^{-1}. The activity of the other enzymes (TPDH, LDH, MK) had average values of the same order as in that group of elderly men (average values 951±43, 609±41 and 724±35 mmol×g protein^{-1}×min^{-1}). The protein content in the polio subjects was 180±7 mg×g^{-1}. Correlation analyses were performed between the activity of

Table IV. *Capillary supply in post-polio subjects and in a male reference group*

	Post-polio subjects		Male reference group
	Women n=10	Men n=9	n=10
No. of capillaries per mm^2	216±16	186±10	316±31
No. of capillaries per fiber	1.86±0.27	2.30±0.37	1.36±0.12
Fiber area in relation to cap. around each fiber ($\mu m^2 \cdot 10^3$)			
Type I	1.34±0.12	1.40±0.15	1.18±0.10
Type II	1.46±0.29	1.83±0.18	1.46±0.19
Type II A	1.81±0.11 n=4	1.77±0.22 n=5	1.46±0.20
Type II B	1.21±0.43 n=3	1.75±0.20 n=5	1.34±0.13

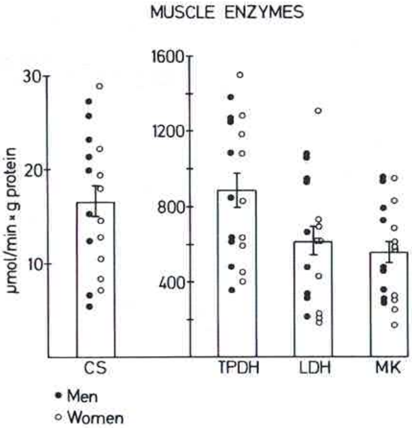

Fig. 5. Enzymatic activities (CS citrate synthase, TPDH triosephosphate dehydrogenase, LDH lactate dehydrogenase, MK myokinase) in biopsies from the vastus lateralis muscle in post-polio subjects. Individual values, as well as means and standard error of the mean, are given per gram protein.

the different enzymes and the relative occurrence of fiber types and the relative fiber areas, but no significant correlations could be demonstrated; neither could any significant correlations be demonstrated between the activities of the different enzymes and the capillarization or the muscle strength.

DISCUSSION

The reduced knee extensor muscle strength corresponds with the general reduction in strength seen in the post-polio condition and there is as expected large individual variation. The quadriceps muscle was chosen for this particular study, as muscle biopsies were included and could easily be taken in the vastus lateralis muscles and reference groups were available for comparison. It should be noted that strength values could only be measured with a Cybex dynamometer in those who could perform a resisted full knee extension, and biopsies were only taken in these subjects. The post-polio subjects demonstrated as in normal subjects a reduction in isokinetic peak torque values with increasing angular velocity, and the highest torque values were recorded with isometric measurements at a knee angle of 60°.

A striking finding in the morphological part of the present study is the presence of large muscle fiber areas (cf. ref. 2, 3, 12), but with large inter- and intraindividual variations, which are also expressed in the large variation coefficients of the fiber areas, calculated on individual measurements. The post-polio men had about twice as large fiber areas for both type I and type II A fibers compared with a reference group of middle-aged men, whereas the difference concerning type II B fibers was not as large. Also the female post-polio subjects had large type I fibers compared with healthy females of the same age (12). However, type II fibers were not as large in the women as in the men, and only moderately increased compared with the areas in healthy middle-aged women (12). As can be seen in Table II, a negative correlation could be found between muscle strength, and the mean fiber area in the male subjects. It is suggested that this could be explained by the excessive use of the remaining muscle fibers, leading to hypertrophy in the post-polio subjects with low muscle strength. The lack of significant correlations in female subjects could be due to sex-dependent hormonal factors or differences in the physical activity pattern, so that muscle fiber hypertrophy is a less consistent consequence. In healthy subjects such a large increase in fiber size with strength training can only be found in elite body builders and not in type I fibers (20). When a person rises from the sitting position, the loading moment for the knee joint at high knee angles can be around 50 Nm (21), which is near or below the maximal torque values for slow knee extension (60°/s) in four male and six female post-polio subjects (Fig. 1), illustrating the very high relative load for rising in a number of subjects.

The frequencies of the different fiber types showed large individual variation. One reason for this could be the uneven distribution of fiber types within the muscle due to the regeneration process with terminal sprouting. Even so, it was possible to demonstrate a significant negative correlation between the relative occurrence of type I fibers and the muscle strength in women. Thus, we would suggest that, at least in some of the subjects with a very high proportion of type I fibers, this is due to an adaptive process leading to transformation of type II to type I fibers in subjects with the most marked reduction in the number of muscle fibers. A similar finding has recently been demonstrated in anterior tibial muscle by Edström et al. (10). In their study, post-polio subjects with a marked loss of function in anterior tibial muscle compensated by increased recruitment and firing rates of the remaining motor units, and the biopsies from these subjects exhibited almost exclusively type I muscle fibers. On the other hand, post-polio subjects who were wheelchair-bound did not demonstrate any type I fiber dominance. It was therefore suggested, that the finding of type I fiber dominance in some of the subjects was due to excessive use of remaining muscle fibers causing a transition of type II to type I fibers.

In the present study, several muscle fascicles could not be examined, which means that the histopathological findings cannot be accepted without some reservations. The occurrence of large grouping can for type I fibers not only be taken as an effect of a denervation/reinnervation process, but might also be caused by a transition from type II to type I fibers, as discussed above. Splitting and internal nuclei were seen, but cannot be considered as a dominant finding. All except one of the subjects with internal nuclei in more than 10% of the fibers also had a mean fiber area above the average, which might indicate a migration of nuclei, specially in very large fibers.

Among the subjects with atrophic fibers, some had also clusters of nuclei in pronounced atrophic fibers, usually indicating a previous neuropathy. In contrast to the findings by Dalakas and coworkers (7, 8), we noticed groups of atrophic fibers in a few subjects, but in accordance with these authors, only isolated atrophies in most subjects. However, electromyographic and muscle biopsy evidence of on-going denervation has been noted in stable patients with prior poliomyelitis as well as in those with new weakness (6). For a further understanding of the relationships between laboratory and clinical findings prospective studies are necessary. In our subjects no distinct difference in muscle morphology was noted between the small group of subjects, who did not report new drop in function, and the subjects with such symptoms.

The activity of the oxidative enzyme citrate synthase was low, or even very low, in comparison with values found in clinically healthy groups of subjects studied in our laboratory with the same technique. As aging does not markedly change the metabolic potential, comparison can be made with a group of healthy males 73 to 83 years of age (4). As seen in Fig. 5, only two of the post-polio subjects had values of citrate synthase above the average value 26 ± 2 mmol×g protein^{-1}×min^{-1} in the normal subjects. This is in accordance with a diluted mitochondrial volume in normal subjects after heavy resistance training (19). The other enzymes studied (TPDH, LDH, MK) had average values of the same order as in the study of clinically healthy elderly men (4), but for these enzymes, also with large individual variation. Thus, the functional demands on the muscles in the post-polio subjects might better maintain the activity of these enzymes than the activity of citrate synthase.

The number of capillaries per mm^2 was somewhat low in the post-polio patients in comparison with the reference group in the present paper and also with previous published data from our own laboratory (3, 4). An increased proportion of fibrous tissue might contribute to this finding. When the number of capillaries per fiber are calculated, high values will be noted in the post-polio subjects as an effect of the large fiber areas. Fiber area calculated in relation to the capillaries around each fiber gives a measure of the diffusion distance from each fiber and is somewhat increased, at least in the male post-polio subjects in comparison with the present reference group and also with the values from clinically healthy elderly men studied with the same technique in our laboratory (4).

Thus, measurement of the oxidative enzymatic activity, and to some extent, of the capillarization demonstrates the lack of adaptation—and reduced capacity—for endurance activity in the post-polio subjects. It is reasonable to assume that, due to the low muscle strength and also other functional consequences of their disease, their general pattern of physical activity is low, and thus the possibility for adaptation of the aerobic metabolism is limited. On the other hand, they demonstrated a good adaptative capacity concerning muscle fiber size as a consequence of the demand to develop high muscle tension in the remaining muscle fibers.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (project No. 03888), the King Gustav V and Queen Victoria's Foundation, the Greta and Einar Asker Foundation, and the Norrbacka-Eugenia Foundation. Thanks are due to Ms Gull-Britt Henning and Ms Elvy Lénberg for skilful technical assistance. We would also like to express our gratitude to Ass. Prof. Karl-Gösta Henriksson, M.D. for valuable advice considering the histopathological evaluation.

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Address for offprints:

Gunnar Grimby, M.D.
Department of Rehabilitation Medicine
Sahlgrenska Hospital
S-413 45 Göteborg, Sweden