Scand J Rehab Med 26: 121–130, 1994

CHANGES IN MUSCLE MORPHOLOGY, STRENGTH AND ENZYMES IN A 4–5-YEAR FOLLOW-UP OF SUBJECTS WITH POLIOMYELITIS SEQUELAE

Gunnar Grimby, Marita Hedberg and Gull-Britt Henning

From the Department of Rehabilitation Medicine, University of Göteborg, Sahlgrenska University Hospital, Göteborg, Sweden

ABSTRACT. Twenty subjects with polio sequelae were studied on two occasions 4–5 years apart by means of dynamometer measurements of knee-extension and flexion strength and muscle biopsy for histochemical and enzymatic analyses. The subjects were divided into those who reported (unstable, $n = 12$) and did not report (stable, $n = 8$) new or increased muscle weakness in the tested leg between the two examinations. Muscle strength decreased significantly in the unstable subjects, but only for knee-flexion in the stable subjects. However, the endurance test comprising 50 consecutive knee-extensions at 180°/sec showed increased fatigability at the second examination only in the stable subjects. Most subjects had markedly increased muscle fiber areas, which in some subjects increased further, but in those already with very extreme hypertrophy the fiber size decreased. Capillarization and activity of citrate-synthase were decreased at the initial examination, but no significant further reduction was observed at the second examination. The results demonstrate individual patterns in the compensatory process for the presumed loss of motor units.

Key words: post-polio syndrome, muscle strength, muscle morphology, capillarization, muscle enzymes, adaptive processes.

Subjects with poliomyelitis sequelae (late polio) are characterized by structural and functional muscular changes, which are main factors in the development of new symptoms such as joint pain, muscle weakness, and fatigue, defined as the post-polio syndrome (15).

In the literature, however, there are only few long-term data on the post-polio syndrome. Dalakas et al. (8) reported a follow-up in 27 patients after an average of 8.2 years, with biopsy data in 8 of the patients and without any systematic comparison of the repeated biopsies. They summarized their findings as presence of reinnervation of both type I and II fibers, new active denervation, indicated by isolated atrophic angular fibers, but no group atrophy. The rate of decline in muscle strength was 1% per year, but that estimate was based on "points" from manual muscle testing and not on a linear scale using quantitative measurements. Agre et al. (2) recently reported that symptomatic post-polio subjects do not lose neuromuscular function to any significant extent in one year. Munin and coworkers (21) found no change in strength in 7 patients with late polio followed for 3 years. Munsat et al. (22) reported that the muscle strength of 6 post-polio patients with complaints of progressive loss of neuromuscular function varied greatly as shown by dynamometer measurements during a period of 400–2,100 days, but without a systemic change with time.

We have previously reported functional compensation in patients with late polio showing a combination of very large motor units (10) and muscle fiber hypertrophy (12). In the present study, we were able to demonstrate different individual patterns in muscle strength and morphological changes over a 4–5-year period and further elucidate the extreme muscle fiber hypertrophy previously described. As preliminarily reported (13) there was also evidence of on-going denervation/reinnervation, resulting in still larger motor-units in most polio subjects.

MATERIAL AND METHODS

Study group

Of the original groups of 39 subjects with poliomyelitis sequelae studied previously (11,12), 20 subjects (10 men and 10 women) could be re-examined with muscle biopsies 4–5 years later. Of the remaining subjects, one had died, one had developed a non-polio-related serious disease, one had moved and 9 did not agree to a new biopsy. The biopsy did not provide adequate material for histochemical analysis in 7 of the subjects, especially in some subjects with severe atrophic muscles.

The subjects had had acute polio 29–56 years earlier and were now 43–70 (mean 54) years of age. At the previous examination, 12 of the subjects had already experienced new symptoms that fulfilled the criteria of the post-polio syndrome (PPS) (15). At the second examination, another 3 subjects fulfilled these criteria, leaving 5 subjects with stable polio and no new symptoms. In the present analysis, subjects were, however, divided into those with perceived new or increased muscle weakness in the tested leg between the examinations (unstable, 12 subjects) and those without such new symptoms (stable, 8 subjects). The occurrence of other new symptoms, as increased fatigue, muscle or joint pain and difficulties in ambulation was also more common in the unstable group (Table I). Body weight did not change in any of the two groups during the observation period. There was no significant difference in present age or age at onset between the two groups. Ten of the patients had participated in a resistance training study 4-4 1/2 years earlier (9), and data before training are used for comparison which minimize decrements in strength over the observation period. Six of the patients had participated in a group-training program with endurance as well as strength training components 4 years earlier (11), but with no change in thigh muscle strength with training. Also their data before training were used for comparison. The leisure time physical activity pattern was for most subjects in general unchanged during the observation period and could be characterized as rather light (Table I).

Informed consent was given by all subjects. The procedure was approved by the Ethics Committee of the Faculty of Medicine at the University of Göteborg.

Table I. *Characteristics at the second examination of all polio subjects and divided into those acknowledging (unstable) and not acknowledging (stable) new or increased leg muscle weakness*

Mean values and standard deviation are given.

	All	Unstable	Stable
n	20	12	8
Age, years	54 (9)	57 (9)	51 (7)
Age at onset of polio, years	12 (10)	13 (11)	10 (8)
Body weight, kg	68 (11)	68 (14)	68 (6)
Number of subjects with			
increased fatigue	12	9	3
muscle or joint pain	12	8	4
increased walking difficulties	8	8	0
increased stair-climbing difficulties	7	7	0
Leisure time physical activity[1], number of subjects:			
grade 1	2	1	1
grade 2	5	4	1
grade 3	8	4	4
grade 4	5	3	2

[1]Grade 1 is hardly no activity, 2 is only very light activity, 3 is light activity 2–4 hours/week, 4 is moderate activity 1–2 hours/week or light activity > 4 hours/week (14).

Muscle strength

Knee-extensor and flexor strength for isometric concentraction at a knee-angle of 60° and for isokinetic concentric contractions at angular velocities of 60 and 180°/sec were measured using a Kin-Com dynamometer (Chattecx Co., Chattanooga, TN). Endurance was measured as the reduction in peak torque as well as the torque at 45° knee angle, between the first and the last three extensions in a series of 50 concentric maximal voluntary contractions with an angle of velocity of 180°/sec (23). Only measurements from the weaker side, provided that it was possible to perform a full knee-extension against gravity, are presented in the present paper. Warming-up submaximal exercise was performed on a bicycle ergometer for 6 minutes prior to the muscle tests. The knee-extensor torque values were recorded with a computerized system using compensation for the weight of the lower leg and the lever arm. Three curves were recorded, and the highest peak torque values are reported. At the first measurements in one of our subgroups (12) a modified Cybex II dynamometer with an identical type of gravity compensation had been used. The subjects were positioned in the same way and were attached to the lever arm in a similar manner. No significant difference in peak torque values was found between the two dynamometers in 23 healthy subjects aged 21-84 years in our laboratory.

There was no significant difference between duplicate sets of Kin-Com dynamometer measurements about 3 months apart in 30 post-polio ($r = 0.91$–0.94), the average difference being 3.9 (0.5–8.4)% (authors' unpublished observations). There was no systematic difference in duplicate determinations of the peak torque reduction at the fatigue test ($r = 0.69$) and an average different in torque reduction between the two test of 1.8%

Muscle biopsy

Muscle biopsies were taken with alligator forceps (16), under local anesthesia, from the same muscle as in the former examination (11,12) and close to the same site, i.e. from the middle portion of the vastus laterialis (half-way between the upper border of the patella and the anterior iliac spine). The same leg was used as for the strength measurements. 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 isopentane (−160°C) and used for histochemical and histopathological analysis. Both parts were stored at −80°C until analyzed.

Serial transverse sections (10 μm) for histochemical analysis were cut with a cryotom at −21°C. The myofibrillar adenosine-triphosphatase (ATPase) method was used for muscle fiber classification as described in the previous report (12). The reactions were carried out at pH 9.4 following alkaline preincubation (pH 10.3) to classify fibers into type I and type II. The type II fibers were subclassified into II A, II B and II C fibers using preincubation at pH 4.62 and 4.35. The average numbers of fibers counted in each subject was 415 ± 53 fibers.

The fiber areas were measured on photos of NADH tetrazolium reductase-stained transverse sections. For this purpose, a digitizer table connected to a microcomputer with a specially developed program was used. There was no significant difference at repeated measurements ($n = 22$) by two observers ($r = 0.94$) with an average difference of $3.4 \pm 0.9\%$. The results from the observer who made measurements in all specimens are presented. To allow

comparison, the fiber areas from the biopsies from the first examination were recalculated using the same equipment. Measurements from oblique sections were avoided and some biopsies were, thus, not used for measurements (see Study group). On average, fiber areas were measured for 463 ± 57 and 252 ± 46 fibers at the two examinations, respectively.

For the histopathological evaluation, hematoxyline-eosine and modified Gomori-trichrome stainings were also used, as well as periodic acid Schiff (PAS) reaction for glycogen. The criterion for atrophic fibers was a lesser diameter of $< 20\mu$m. Large grouping was defined as more than 16 fibers of the same type grouped together. The occurrence of internal nuclei in more than 10% of the fibers in the whole cross-section was reported. Amylase-periodic acid-Shiff (PAS) staining was used to visualize capillaries (1) as in the previous report (12), and the number of capillaries per fiber and the fiber area per capillary were calculated for the different fiber types.

Enzymatic analyses were made in 10 subjects of 3-hydroxy-CoA-dehydrogenase (HAD), citrate synthase (CS), triose-phosphate dehydrogenase (TPHD), lactate dehydrogenase (LDH) and myokinase (MK), as described earlier (12), by means of fluorimeter technique using a Farrand ratio-fluorimeter-2 (Farrand Optical Co., Valhalla, NY). The reactions catalyzed by the enzymes under investigation were coupled to NAD, NADP-linked reactions (20). The assays were performed at 24°C. The protein content was determined in order to express the activities per gram of protein. The methodological errors, as presented in the previous paper (12), for the determination of enzyme activities were TPDH 5.8%, LDH 5.2%, MK 3.8% and CS 3.8%.

Statistics

Wilcoxon's non-parametric test was used for paired observations. Mean and standard error of the mean were calculated using conventional formulas.

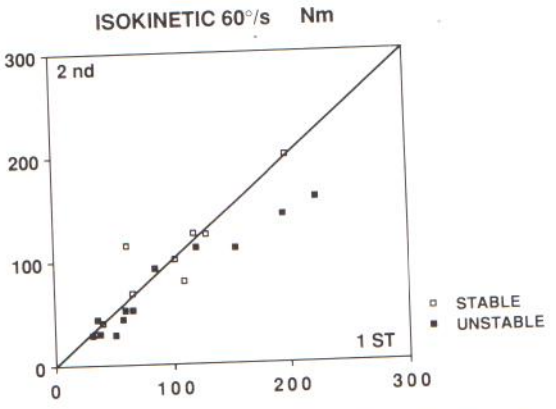

Fig. 1. Peak torque values at isokinetic concentric knee-extension with an angular velocity of 60°/sec at the 1st and 2nd examinations 4–5 years apart. Subject with new or increased muscle weakness (unstable) and those with no new weakness (stable) are shown separately.

RESULTS

As seen in Fig. 1, the individual variation in peak torque values was large. The values for knee-extension were below around half the expected values in this age range (6,12) in 11 of the subjects. The average values for isokinetic knee-extension at 60 and 180°/sec were 55 (SD 29) and 58 (SD 29)% of predicted normal values (12,19) at the first examination, and 49 (SD 27) and 53 (SD 27)% at the second examination. For isometric knee-extension at 60° knee-angle, the corresponding values were 54 (SD 28) and 47 (SD

Table II. *Muscle strength expressed as peak torque (Nm) in 20 polio subjects before (1st exam.) and after (2nd exam.) a 4–5 year follow-up period*

The subjects are divided in those with perceived new or increased weakness in the studied leg (unstable) and those without new symptoms (stable).

Mean values and standard error of the mean are given. Significance levels for differences for paired observations are presented.

	Unstable ($n = 12$)		Stable ($n = 8$)	
	1st exam.	2nd exam.	1st exam.	2nd exam.
Knee-extension				
Isometr. 60° knee angle	103 ± 18	83 ± 15^{xx}	127 ± 21	117 ± 22
Isokinetic conc.				
60°/s	94 ± 19	75 ± 13^{xx}	100 ± 19	97 ± 20
120°/s	75 ± 14	59 ± 12^{xx}	71 ± 15	68 ± 14
Knee-flexion				
Isometr. 60° knee angle	54 ± 7	35 ± 6^{xx}	52 ± 9	43 ± 8^{x}
Isokinetic conc.				
60°/s	55 ± 8	34 ± 6^{xx}	50 ± 10	42 ± 8
120°/s	42 ± 6	28 ± 4^{xx}	40 ± 8	31 ± 7^{x}

Significant differences between the two examinations are indicated. $^{x}p < 0.05$. $^{xx}p < 0.01$.

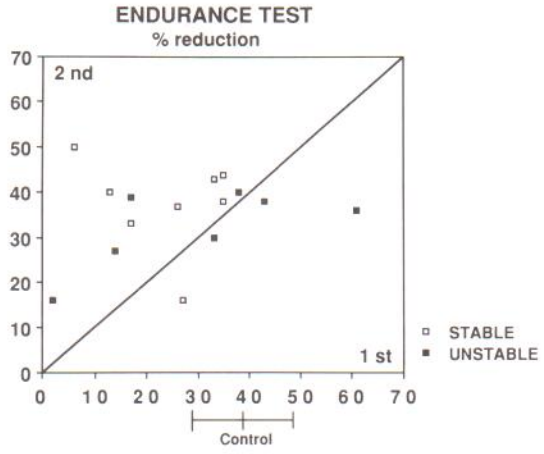

Fig. 2. Reduction in peak torque at 50 repeated maximal voluntary knee-extensions with an angle of velocity of 180°/sec. Stable and unstable subjects are shown separately, as in Fig. 1. Mean values ±SD in a female control group (40–48 years, $n = 13$), (19) are indicated.

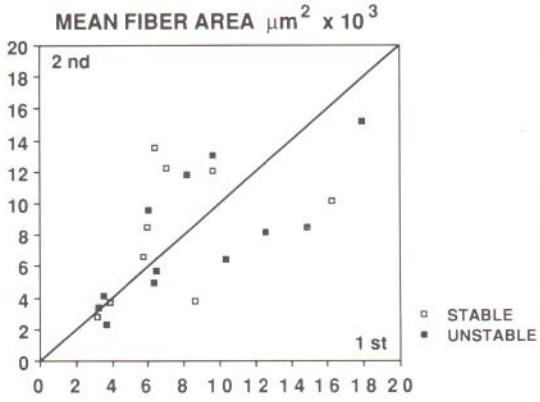

Fig. 3. Average mean fiber areas at the 1st and 2nd examination. Stable and unstable subjects are shown separately, as in Fig. 1.

27)%, respectively. Peak torque decreased significantly ($p < 0.001$) for isokinetic knee-extension and knee-flexion at 60 (11 and 29%) and 180°/sec (12 and 28%). Isometric knee-extension and knee-flexion at a knee angle of 60° showed a significant decrease (15%, $p < 0.01$ and 28%, $p < 0.001$, respectively).

When the subjects were separated into those with and without new or increased muscle weakness (unstable $n = 12$ and stable $n = 8$), significant reductions were only seen for knee-flexion in the stable, but for all measurements in the unstable subjects (Table II). There was no significant difference between the two groups for the initial values. Men and women are presented together, as only paired observations are included. The percentage reduction between the two measurements was also significantly larger for the unstable (22%) than for the stable (0%) subjects for isokinetic knee-extension at 180°/sec ($p < 0.05$) and for isokinetic knee-flexion at 60°/sec (38 and 17% respectively) ($p < 0.01$).

The reduction in peak torque as well as in torque at 45° knee angle (Fig. 2) in the endurance test was significantly ($p < 0.01$) larger at the second than at the first examination, indicating more fatigable muscles at the second examination. However, the reduction appeared only to be significant (12% larger reduction in torque at the second examination) in the stable subjects (with maintained strength) ($p < 0.05$) and not in the unstable subjects (4% larger reduction at the second examination).

As in the previous studies in the same subjects (11,12), the distribution of the different fiber types showed large individual variation but, still with the exception of a few subjects, rather similar occurrence of type I and II fibers at the two examinations, the average percentage of type I fibers being $40.9 \pm 7.3\%$ and $36.7 \pm 6.3\%$, respectively, on the two occasions. The distribution within type II fibers did not differ, and only a few intermediate (type IIC) fibers were recorded (around 1–2%). Most subjects showed large type grouping in one or both main fiber types, which naturally limits the possibility of obtaining a true estimate of the fiber distribution in a sample of the present size. It was also noted that there were sometimes difficulties in discrimination, especially between type IIA and IIB fibers.

Most polio subjects had large muscle fibers (Table III) with average values above those in a male (12) as well as female (40–48 years, $n = 13$, unpublished results) control group, in which the mean fiber areas were 4.4 ± 0.4 and $4.1 \pm 0.4\ \mu m^2 \times 10^3$, respectively. The average area both of type I and type II fibers decreased in most subjects, but the type I fiber area increased to very high values in 3 subjects, while the type II fiber area increased further in 4 subjects. The average values for the whole group, however, did not differ significantly between the two examinations, either for the different subgroups, or for the mean fiber areas (Table III, Fig. 3). The fiber area values or change in areas did not differ significantly between the unstable and the stable subjects. As in the previous studies (11,12), the variation coefficients for the fiber areas were large, but did not differ between the two examinations. The data are not included in this report, but histograms for the fiber areas are shown for 4

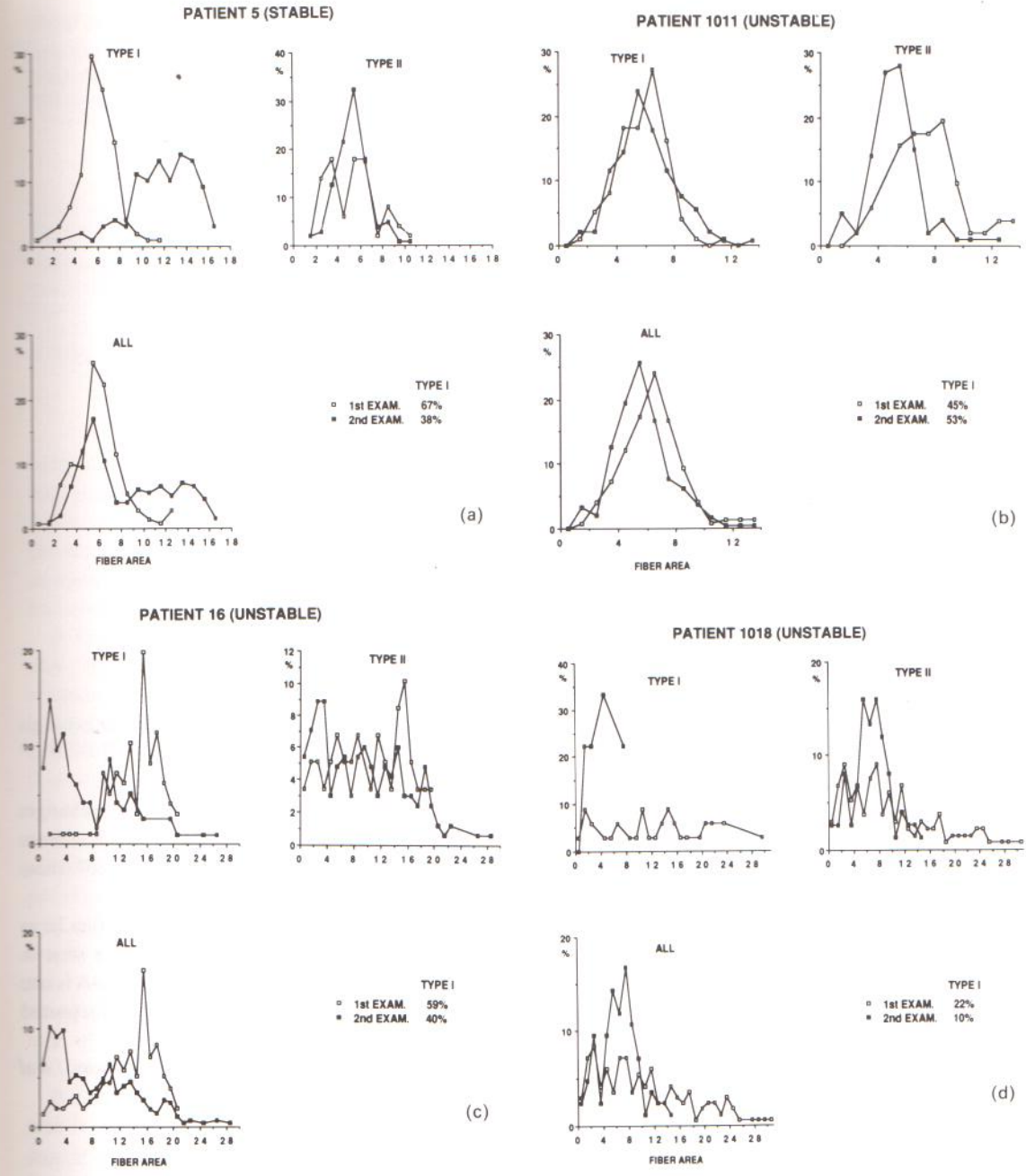

Fig. 4. Histograms of the distribution of areas ($\mu m^2 \times 10^3$) of individual muscle fibers at two examinations 4–5 years apart in 4 polio subjects, one stable (A, no. 5, median fiber area 6.0 and 7.1 $\mu m^2 \times 10^3$, respectively with 25–75 quartile ranges 4.7–6.8 and 5.1–12.0) and 3 unstable (B, no. 1011, C, 16 and D, 1018 with median fiber areas 6.5 and 5.4 (25–75% quartile ranges 5.1–7.8 and 4.4–6.7), 12.9 and 6.9 (ranges 8.9–16.2 and 2.9–12.6), 9.0 and 6.2 (ranges 4.3–14.9 and 4.2–8.4) $\mu m^2 \times 10^3$, respectively).

subjects (Fig. 4), where different patterns of changes are seen, as well as a large variation of fiber sizes. Even in subjects with a high number of very large fibers small atrophic fibers occurred. The stable subject (no. 5, Fig. 4A) showed a further increase in the size of type I fibers, but not in type II fibers. In the 3 unstable patients presented, no. 1011 (Fig. 4B) showed a decrease in fiber areas, especially for type II fibers,

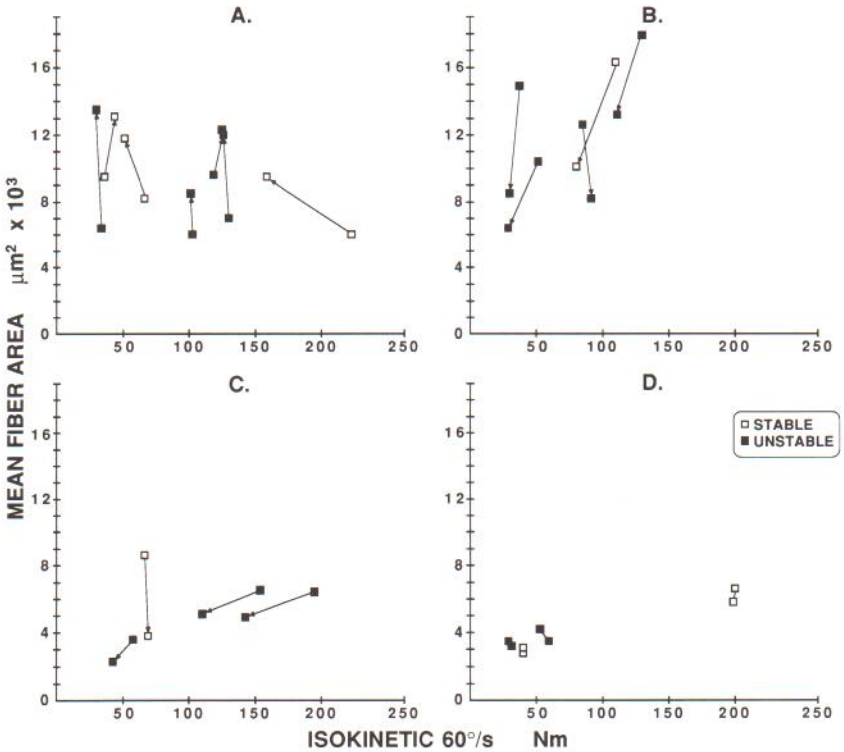

Fig. 5. Mean fiber area and isokinetic strength at an angular velocity of 60°/sec at the 1st and 2nd examinations. The changes are indicated by *arrows*. Stable and unstable subjects are shown separately. The subjects are divided into four panels as described in the text.

no. 16 (Fig. 4C) a reduction in areas for both fiber types, and no. 1018 (Fig. 4D) demonstrated a marked change in distribution in fiber areas between the first and the second examination, with loss, or reduction in size, of the previously very large type I and type II fibers.

Combining data from strength and fiber area measurements (Fig. 5) and dividing the subjects into four panels according to pattern of fiber area changes allows analysis on an individual basis, which is more appropriate than the statistical analysis of these changes in the group as a whole.

A. An increase in mean fiber area from rather large to very large values (10–13 $\mu m^2 \times 10^3$) was seen in seven subjects (Fig. 5A). Patient no. 5 in Fig. 4A is one of the stable subjects. Four of these subjects reported

Table III. *Areas ($\mu m^2 \times 10^3$) for type I and II fibers and mean fiber area in 20 polio subjects before (1st exam.) and after (2nd exam.) a 4–5 year period*
The subjects are divided into unstable and stable subjects (see Material and Table 1).
Mean values, ± standard error of the mean, median values and 25–75% quartile ranges are given.

	Unstable			Stable		
Area	*n*	1st exam.	2nd exam.	*n*	1st. exam.	2nd exam.
Type I	11	9.16 ± 1.52 8.04 (5.03–13.72)	7.25 ± 1.35 5.43 (3.28–11.95)	4	5.21 ± 0.66 5.73 (4.35–6.08)	6.38 ± 2.03 5.60 (3.40–9.37)
Type II A	9	9.28 ± 1.62 8.60 (5.93–12.02)	7.54 ± 1.88 5.13 (3.16–11.65)	4	7.32 ± 0.92 6.62 (6.12–8.51)	7.15 ± 2.19 6.02 (4.31–9.98)
Type II B	9	6.48 ± 1.97 5.02 (2.73–6.60)	7.50 ± 1.61 7.00 (4.34–9.68)	6	6.41 ± 0.69 6.85 (5.65–7.73)	7.84 ± 1.65 7.80 (4.08–10.53)
Mean	12	8.58 ± 1.35 7.33 (4.88–11.49)	7.73 ± 1.18 7.31 (4.56–10.69)	8	7.86 ± 1.39 6.71 (5.86–9.14)	8.71 ± 1.42 9.33 (5.19–12.16)

Table IV. *Capillary supply in 16 polio subjects (10 men, 6 women) before (1st exam.) and after (2nd exam.) a 4–5 year follow-up period*

Mean values and standard errors of the mean are given. Values from a male reference group (42–51 years, $n = 10$) (12) and from a female control group (40–48 years, $n = 10$, unpublished observations) are given for comparison.

	1st exam.	2nd exam.	Male reference group	Female reference group
No of capillaries per mm^2	233 ± 17	267 ± 26	316 ± 31	258 ± 15
No of capillaries per fiber	2.00 ± 0.25	1.93 ± 0.19	1.36 ± 0.12	1.13 ± 0.09
Fiber area in relation to cap. around each fiber ($\mu m^2 \times 10^3$)				
Type I	1.48 ± 0.16	1.20 ± 0.11	1.18 ± 0.10	1.36 ± 0.10
Type II	1.88 ± 0.18	1.58 ± 0.17	1.46 ± 0.19	1.41 ± 0.11
Average	1.73 ± 0.15	1.57 ± 0.14	1.31 ± 0.15	1.39 ± 0.10

increased muscle weakness, but muscle strength did not decrease or was only moderately reduced from the first to the second examination. Despite no perceived muscle weakness, one subject showed a reduction in strength but from values within the normal range, and had evidence of compensatory fiber hypertrophy.

B. Five subjects with the largest reduction in fiber area (type I and/or II) from the first to the second examination had very high values at the first examination, mean values being above $10\ \mu m^2 \times 10^3$ in all cases (Fig. 5B). Patients nos. 16 (Fig. 4C) and 1018 (Fig. 4D) are 2 of those subjects. Four of these 5 subjects also had lower strength values at the second examination, but one of them was characterized as stable, since no further muscle weakness was perceived.

C. Four subjects with moderate or no fiber hypertrophy showed a reduction in the mean fiber area; in the 3 unstable subjects this was accompanied by quite a marked reduction in muscle strength, indicating the lack of a compensatory process (Fig. 5C). Subject no. 1011 in Fig. 4B is one these subjects.

D. Unchanged muscle strength and fiber areas were seen in 4 subjects in Fig. 5D. Three of them had low muscle strength, indicating that unchanged muscular function can also be found in subjects with a rather marked loss of neuromuscular function. The subject with the highest torque values and fiber areas within the normal range (12) also showed stable values and was, thus, only slightly affected by polio.

The capillary density (Table IV), the number of capillaries per fiber and capillarization expressed in relation to fiber areas did not change significantly between the first and second examination. There was no difference in change between the stable and unstable subjects. At the first examination the number of capillaries per fiber was larger in the polio subjects than in controls, in accordance with larger fiber areas, but not proportionately to the fiber area. Thus, compared with the control groups (men ref. [12], women unpublished results, see Table IV) the average fiber area in relation to the number of capillaries around the fibers was significantly ($p < 0.05$) larger in the polio subjects at the first, but not shown at the second examination, indicating less favorable diffusion distances at the first examination.

The histopathological evaluation (Table V) showed essentially the same findings at the second as at the first examination. Large grouping (> 16 fibers) was

Table V. *Histopathological findings in 18 polio subjects before (1st exam.) and after (2nd exam.) a 4–5-year follow-up period*

	Number of subjects	
	1st exam.	2nd exam.
Small round fibers	18	15
Small angular fibers	3	2
Fiber atrophy (> 10 fibers)		
Type I	11	9
Type II	16	15
Large grouping (> 16 fibers)		
in both type I and II fibers	7	8
only in type I	4	2
only in type II	4	7
Internal nuclei (≥ 10% of the fibers)	6	7
Splitting	7	8

Table VI. *Muscle enzymatic activity in 10 polio subjects (4 men, 6 female) before (1st exam.) and after (2nd exam.) a 4–5 year follow-up period*
For abbreviations see Methods.
Values are expressed in μmol/min × g protein. Mean values and standard errors of the mean are given. Reference value from a female control group (age 25–48 years, $n = 9$, unpublished observations) are given for comparison.

	1st exam.	2nd exam.	Female reference group
HAD	26.5 ± 3.3	28.9 ± 1.5	33.5 ± 1.2
CS	23.1 ± 3.2	21.8 ± 1.9	35.7 ± 2.6
TPDH	1301 ± 182	1236 ± 109	1111 ± 47
LDH	857 ± 152	834 ± 111	761 ± 67
MK	865 ± 132	878 ± 74	890 ± 53
Protein	178 ± 5.5	169 ± 4.7	182 ± 3.1

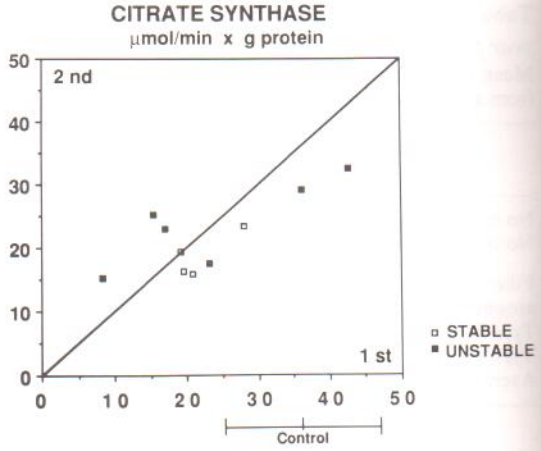

Fig. 6. Citrate synthase activity at the first and second examination. Stable and unstable subjects are shown separately. Mean values ±1 SD in a female control group from our laboratory (40–48 years, $n = 10$, unpublished observations) are indicated.

seen in all except 3 subjects at both examinations, and in 7 and 8 subjects, respectively, for both type I and II fibers. All subjects at the first examination and all but 3 at the second examination had single atrophic fibers, usually round, and in groups of more than three fibers in 9 subjects on both occasions. Internal nuclei were seen in more than 10% of the fibers in 6 subjects at the first examination and in the same plus an additional subject at the second examination. Splitting was also seen in about half of the subjects , and usually only in a few fibers in each biopsy.

There was no significant change in the enzymatic activity (Table VI) between the first and the second examination. As the total number of analyzed subjects was only 10, it was not statistically meaningful to analyze stable and unstable subjects separately. In relation to a female control group (unpublished results, see Table VI) the post-polio subjects had, on average, significantly lower values for citrate synthase (CS). The 4 polio subjects with the highest values, 3 of them unstable, reduced their values from the first to the second examination, as seen in Fig. 6. For the other enzymes studied, no similar tendency toward a fall was seen, and the polio subjects had enzymatic activities in the region of the control values.

DISCUSSION

The present results combined with previously published reports (10,12) demonstrate individually different patterns in the development of functional compensation and deterioration in the post-polio state. The average values for muscle strength decreased more than expected from the age-related changes. The expected decline in isokinetic muscle strength over this period can from available Swedish cross-sectional data (6,18) be estimated to be 2–5%, whereas the decrease in the unstable post-polio subjects was on average 16%and 22%, respectively, for knee-extension at 60 and 180°/sec and 18% for isometric knee-extension, but only 4, 0 and 10% in the stable subjects. Thus, overall, unstable subjects reporting new or increased muscle weakness during the 4–5 years showed a more marked reduction of muscle strength as measured by dynamometer. Our findings also emphasize the validity of the perception of increased muscle weakness by the post-polio subject and of using that information in further analyses even if in individual cases new muscle weakness was not perceived in spite of a recorded reduction at dynamometer measurements. It is also to note that our subjects were weight stable; a weight increase may otherwise have increased the perception of muscle weakness. Reduction in dynamometer-measured muscle strength during the observation period was observed in subjects with rather well preserved as well as in those with weaker muscles, and there was no significant difference in the initial strength values between the unstable and stable groups. A good reliability of repeated dynamometer measurements of knee-extensor and flexor strength within a short time interval in post-polio subjects is reported in the present study as well as in a recent study by Kilfoil and St. Pierre (17).

The compensatory processes show variable

patterns, as demonstrated in the present study, and depend both on muscle fiber hypertrophy and reinnervation by collateral sprouting. In a subsequent paper (preliminarily reported in ref. [13]) it will be demonstrated that most subjects, unstable as well as stable, show signs of an ongoing reinnervation/denervation with an increase in number of muscle fibers within the motor units. However, there is also indirect evidence of quite a large reduction in the number of motor units during the observation period. Why certain subjects can still use compensatory processes fully and others cannot is still unclear, but there may be some upper limits for muscle fiber hypertrophy as well as reinnervation. The upper limit for hypertrophy may well be reached before that of reinnervation.

The hypertrophic fibers have been interpreted to be the result of extreme use of remaining motor units in weight-bearing activities (12), which is also evident in the anterior tibialis muscle in still mobile subjects with motor unit overuse (4). In the present study the subjects being reexamined after 4–5 years, those with the largest muscle fibers in the vastus lateralis muscle showed a reduction in muscle fibre area, although those areas remained far beyond the normal range in several cases. The question then arises whether they had reached an optimal size for maintained fiber hypertrophy, a suggestion already made on the basis of a high-resistance training study in post-polio subjects, in which the 2 subjects with the highest fiber areas showed a reduction (9). It might be that muscle fibers which hypertrophy, when exposed to a high degree of resistance, either split or start to hypotrophy after reaching a certain size. However, splitting was only seen in a few fibers in half of the subjects. There may also in a few subjects have been major changes in the physical activity pattern with reduced resistance load, which caused the decrease in fiber size exemplified below. Reduction in fiber size could be seen as well as type I as in type II fibers. Other subjects, on the other hand, showed an increase in fiber size during the follow-up period. This finding can be interpreted as on-going compensation, although no functionally complete, for the continuous loss of muscle fibers, which is assumed from electrophysiological and histopathological data (10,13). The on-going process of denervation and reinnervation in the muscles affected by polio is indicated by increased jitter, blocking, high fiber density and further increase in the large macro motor unit potentials (13), which already initially were 7.5 times larger than in the reference population (10). It might then have been assumed that an increasing frequency of atrophic muscle fibers would be noted in the biopsies. This was not the case, the main explanation being the relatively slow process and the fact that some atrophic fibers had already been recorded in the previous examination. It should be pointed out that a large variation in fiber size was noted both at the first and at the second examination, as illustrated in the case reports with large recorded quartile ranges (Fig. 4).

As has been previously reported (7,10), it has not yet been possible to distinguish between stable patients with prior poliomyelitis and those with new weakness from electromyographic or muscle biopsy findings. No clear distinctions were brought out in the present study either, since subjects with new or increased muscle weakness and those without new symptoms could not be clearly separated with respect to changes in fiber areas (Figs. 3 and 5). However, if functional deterioration causes a subject to change his activity pattern markedly, such as changing from walking to using a wheelchair, further loss of strength and fiber areas can be assumed, since the daily resistance training in weight-bearing activities ceases. In the present study, this is illustrated in the subject with the largest decrease in the area of type I fibers (from a median value of 12.7 to 3.4 $\mu m^2 \times 10^3$) and also in type II fibers (from 7.9 to 6.4 $\mu m^2 \times 10^3$) (patient no. 1018 in Fig. 4D, see also Fig. 5B). It should also be noted that the variation coefficients for fiber areas were very large, specially at the first examination (type I 61%, type II 72%, compared to an average of 38% and 47% respectively, for the whole group), probably indicating on-going changes in fiber size. This patient's muscle strength was reduced by 30–40% during the same period, as evident from the dynamometer readings, and he had used his wheelchair more or less constantly during the last 2 years.

Some evidence of reduced capillarization was noted at the first examination, a finding similar to that reported from the anterior tibial muscle (5) and an overall reduction in the concentration of an oxidative enzyme, citrate synthase (12), was also seen. Some subjects with rather normal values showed reduction in the enzymatic activity values during the observation period, which might be due to some reduced level of physical activity during that

period. Several factors may contribute to the complaints of muscle fatigue; reduced capillarization and oxidative enzyme activities are possible explanations. The increased fatigability in the endurance test in the stable, but not in the unstable, subjects points to divergent patterns for the increase in muscle weakness and in fatigue. Unfortunately, the number of subjects with biopsies for enzyme measurements was too low to allow separate analyses to be made for unstable and stable patients, and measurements of capillarization gave no conclusive evidence of changes over time.

Further studies, including long-term follow-up, will elucidate positive and negative effects of the functional compensatory processes in the post-polio state. Such information will also be of interest for other conditions in which there is a successive reduction in the number of motor units, such as motor neuron disease and ageing, and will provide basic knowledge of biological processes in primary non-myopathic muscle fibres subjected to stress.

ACKNOWLEDGEMENTS

This study was supported by grants from King Gustaf V:80-years Foundation, the National Association against Traffic and Polio Victims, the Swedish Medical Research Council (project no. 03888) and IngaBritt and Arne Lundberg's Foundation.

REFERENCES

1. Andersen, P. & Henriksson J.: Capillary supply of the quadriceps femoris muscle in man: Adaptive response to exercise. J. Physiol (Lond) *270*: 677–690, 1977.
2. Agre, A. C. & Rodriguez, A. A.: Neuromuscular function in polio survivors at one-year follow-up. Arch Phys Med Rehabil *72*: 7–10, 1991.
3. Agre, J. C., Rodriquez, A. A. & Tafel, J. A.: Late effects of polio: critical review on neuromuscular function. Arch Phys Med Rehabil *72*:11–14, 1991.
4. Borg, K., Borg, J., Edström, L. & Grimby, L.: Effects of excessive use of remaining muscle fibers in prior polio and LV lesion. Muscle Nerve *11*: 1219–1230, 1988.
5. Borg K. & Henriksson, J.: Prior poliomyelitis-reduced capillary supply and metabolic enzyme content in hypertrophic slow-twitch (type I) muscle fibers. J Neurol Neurosurg Psych *54*: 236–240, 1991.
6. Borges, O.: Isometric and isokinetic knee-extension and flexion torque in men and women aged 20–70. Scand J Rehabil Med *16*: 45–53, 1989.
7. Cashman, N. R., Maselli, R., Wollman, R. L. et al: Late denervation in patients with antecedent paralytic poliomyelitis. N Engl J Med *317*: 7–12, 1987.
8. Dalakas, M. C., Elder, G. & Hallett, M. et al: A long-term follow-up study of patients with post-poliomyelitis neuromuscular symptoms. N Engl J Med *314*: 959–963, 1986.
9. Einarsson, G.: Muscle conditioning in late poliomyelitis. Arch Phys Med Rehabil *72*: 11–14, 1991.
10. Einarsson, G., Grimby, G. & Stålberg, E.: Electromyographic and morphological functional compensation in late poliomyelitis. Muscle Nerve *13*: 165–171, 1980.
11. Ernstoff, B., Wetterqvist, K., Kvist, H. & Grimby, G.: The effects of endurance training on individuals with post-poliomyelitis. Arch Phys Med Rehabil. 1994 (in press).
12. Grimby, G., Einarsson, G., Hedberg, M. & Aniansson, A.: Muscle adaptive changes in post-polio subjects. Scand J Rehabil Med *21*: 19–26, 1989.
13. Grimby, G., Stålberg, E. & Einarsson, G.: Muscle functional compensation in late polio. Arch Phys Med Rehabil *73*: 1000, 1992.
14. Grimby, G.: Physical activity and muscle training in the elderly. Acta Med Scand Suppl. 711: 233–237. 1986.
15. Halstead, L. S., Rossi, C. D.: New problems in old polio patients: results of a survey of 539 polio survivors. Orthopedics *8*: 845–853, 1985.
16. Henriksson, K.-G.: "Semi-open" muscle biopsy technique. A simple out-patient procedure. Acta Neurol Scand *59*: 317–323, 1979.
17. Kilfoil, M. & St. Pierre, D. M. M.: Reliability of Cybex II isokinetic evaluations of torque in post-poliomyelitis syndrome. Arch Phys Med Rehabil *74*: 730–735, 1993.
18. Larsson, L., Grimby, G., Karlsson, J.: Muscle strength and speed of movement in relation to age and muscle morphology. J Appl Physiol *46*: 271–281, 1979.
19. Lindh, M. H., Johansson, G. A., Hedberg, M. & Grimby, L. G.: Studies on maximal voluntary contraction in patients with fibromyalgia. Arch Phys Med Rehabil, 1994, in press.
20. Lowry, R. H. & Passoneau, J. V.: A flexible system of enzymatic analysis. Academic Press, New York, 1972.
21. Munin, M. C., Jaweed, M.M. & Staas, W.E. et al: Poliomyelitis weakness: a prospective study of quadriceps strength. Arch Phys Med Rehabil *72*: 729–733, 1991.
22. Munsat, T. L., Andres, P. & Thibideau, L.: Preliminary observations on long-term muscle force changes in the post-polio syndrome. In: Halstead, L. S. and Wiechers, D. (Eds.) Research and Clinical Aspects of the Late Effects of Poliomyelitis. Birth Defects: Orig. Series, vol 23, No. 4, 1987, pp 329–334.
23. Thorstensson, A. & Karlsson, J.: Fatigability and fibre composition of human skeletal muscle. Acta Physiol Scand *98*: 318–322, 1976.

Address for offprints:

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