*Original Article*

# The effects of exercise training on muscle atrophy in haemodialysis patients

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## Abstract

**Background.** Patients with end-stage renal disease on haemodialysis (HD) have limited work capacity. Many structural and functional alterations in skeletal muscles contribute to this disability.

**Methods.** To evaluate the effects of exercise training on uraemic myopathy, seven HD patients (mean age $44.1 \pm 17.2$ years) were studied. Open muscle biopsies were taken from their vastus lateralis muscle before and after a 6-month exercise rehabilitation programme and examined by routine light- and transmission electron-microscopy. Histochemical stainings of frozen sections were performed and morphometric analysis was also applied to estimate the proportion of each fibre type and the muscle fibre area. Spiroergometric and neurophysiological testing and peak extension forces of the lower limbs were measured before and after exercise training.

**Results.** All patients showed impaired exercise capacity, which was associated with marked muscular atrophy (mean area $2548 \pm 463$ μm$^2$) and reduction in muscle strength and nerve conduction velocity. All types of fibres were atrophied, but type II were more affected. The ultrastructural study showed severe degenerative changes in skeletal muscle fibres, mitochondria, and capillaries. Exercise training had an impressive effect on muscular atrophy; in particular the proportion of type II fibres increased by 51% and mean muscle fibre area by 29%. Favourable changes were also seen on the structure and number of capillaries and mitochondria. These results were confirmed by a 48% increase in VO$_2$ peak and a 29% in exercise time, as well as an improvement in the peak muscle strength of the lower limbs and in nerve conduction velocity.

**Conclusions.** Skeletal muscle atrophy in HD patients contribute to their poor exercise tolerance. The application of an exercise training rehabilitation programme improved muscle atrophy markedly, and therefore had beneficial effects in overall work performance.

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## Introduction

Patients with end-stage renal disease have limited physical fitness and many factors, as anaemia, cardiac dysfunction, muscle abnormalities, depression, etc. are attributed to it [1–4]. Furthermore, muscular weakness and fatigue, myoclonus and cramps, that predominantly involve the lower limbs, are some of the daily symptoms in HD patients, that limited their work-related or other pleasant activities dramatically [5–7].

Muscular atrophy of uraemic patients had been described either as a consequence of 'uraemic neuropathy' caused by primary axonal degeneration with segmental demyelination, or as 'uraemic myopathy' due to abnormal structure and function of muscle fibres of these patients. Effective haemodialysis (HD) fails to correct these disorders [1,4,8]; the uraemic neuropathy is improved with adequate dialysis and it is resulted by the accumulation of dialysable metabolites [9,10], but the muscular atrophy persists. On the other hand, dialysis can cause other disturbances that affect separately or in combination primary the skeletal muscles. The muscle fibres of patients on HD have many abnormalities, possibly due to adaptations of these cells to the altered internal environment. These abnormalities include changes in capillaries, enzymes, contractile proteins etc. [11]. Myopathy often occurs in uraemic patients as a consequence of high serum calcium level, azotaemia, acidaemia, low level of carnitine, and/or secondary hyperparathyroidism [12–14].

The extremely limited work capacity of uraemic patients improves after human erythropoietin treatment as well as after exercise training. It is reported that exercise affects anaemia, cardiovascular function, aerobic capacity, depression, and quality of life of these patients beneficially [2–4,15]. Studies associated with improvement of the cardiopulmonary functions of HD patients following physical training include

aerobic capacity, heart rate, and stroke volume, factors that determine oxygen extraction by skeletal muscle etc. Moore's studies on aerobic capacity of these patients showed that many patients increased $VO_2$ peak after training, but most improved exercise capacity, showing that oxygen delivery is not always the limited factor [16]. Other reports described muscle atrophy in HD patients or studied potential peripheral adaptations that increased oxidative capacity, capillary to fibre ratio and consequently contractile function. However, there remains the question of the mechanisms, central or peripheral, involved during adaptation to exercise training in patients on maintenance HD.

The present study was designed to evaluate morphologically and morphometrically the lower limbs' muscle fibre profile of patients on maintenance HD with no other systemic disease before and after 6-months exercise training, with concurrent testing of muscle strength, nerve conduction velocity and aerobic capacity.

## Subjects and methods

### Study patients

Seven patients (5 men and 2 women; mean age $44.1 \pm 17.2$ years) with end-stage renal failure on maintenance haemodialysis treatment (mean years on HD $4.6 \pm 4.1$) were entered into this study. All had been dialysed 3 days per week for an average of 4 h/session, for at least 1 year prior to the study. None of the patients had diabetes mellitus, clinical evidence of hyperparathyroidism, severe peripheral neuropathy, orthopaedic limitations, symptomatic cardiovascular disease, or any other medical problem that contraindicated participation in an exercise training programme. Four of the patients had arterial hypertension. One patient was anephric. Almost all of them had complained of muscular weakness, as well as cramps and aches. None was under medication that could cause myopathy. The subjects remained in a stable medication regimen, diet, and dialysis schedule during the study. The dialysis prescription as well as the level of haematocrit were also planned to remain constant for all patients. Six of the patients were receiving erythropoietin therapy; the dose was only changed as required by the haematocrit. Haematocrit, urea, creatinine, glucose, electrolytes, calcium, phosphate, alkaline phosphatase, and parathyroid hormone were estimated by standard techniques at regular intervals. Informed consent was obtained from all patients according to guidelines approved by the Human Subjects Committee.

### Exercise testing

Before entering the rehabilitation programme and soon after its end all patients underwent a full medical examination and an exercise test on a non-dialysis day. The symptom-limited exercise test was performed on a motor-driven treadmill according to a multistage modified Bruce protocol. Patients were encouraged to exercise until subjective exhaustion. Blood pressure was measured at the end of each 3-min stage, while the electrocardiogram was monitored continuously. To assess aerobic exercise capacity oxygen consumption ($VO_2$) was calculated every 30 s using a telemetric spiroergometric system ($K_2$ Cosmed). Peak oxygen consumption ($VO_2$ peak) was defined as the highest $VO_2$ attained during the exercise test. Blood samples for lactate concentrations were collected and analysed before and after the testing.

### Muscle strength estimation

Peak extension forces of the lower limbs of all patients were measured by a dynamometer before and soon after the programme. This dynamometer consisted of a long beam with a seat on it, that could be moved backward and forward according to the height of the subjects, and two force transducers, one for each leg, connected to a PC *via* an A/D card. The force transducers were linear in the range of 50–3000 Newtons with an accuracy of $\pm 1.2$ Newton. All subjects were tested with a knee angle of approximately 120° and a leg to foot angle of approximately 90°. Thighs to trunk angle was approximately 110°. Peak muscle strength in both knee extensor muscles was recorded as the highest force during maximal voluntary knee extensions with constant angular velocity. The subject was positioned in the tensiometer chair and secured in place with a seat belt placed around the pelvis. During each effort, each subject was required to maintain contact with the back rest and to hold onto hand grips located crossing in front of his chest. Each testing session began with the patient performing a warmup set of three to four repetitions with a light effort. Two minutes of rest were given between the warm-up and the start of the bilateral knee peak extension strength test. The patient pushed the pedal transducers with the maximal effort for approximately 1.5 s. Data were analysed by a program and the force–time curves were printed. Peak force was defined as the highest value.

### Neurophysiological testing

Conduction velocity of the peroneal nerve and residual time were measured before and after the exercise training programme on a Neuropack 4 mini model MEB–5304 K system. These assessments were performed on both legs by percutaneous stimulation of the corresponding nerves. Before the tests, the muscular strength, the tendon reflexes and the cutaneous and deep sensation of each patient were examined clinically.

### Muscle biopsy

Two biopsy samples were taken from each patient's left leg vastus lateralis at the start and at the end of the study. The biopsies were obtained with open surgery using local anaesthesia. The skin was prepared with antiseptic and draped. The skin and subcutaneous tissue down to the muscle sheath were injected with 1% lignocaine, and a small incision was made down to the muscle sheath, at approximately midthigh level in the midline. A small sample of muscle fibres, approximately $15 \times 5 \times 5$ mm$^3$, was separated with round-end forceps and sutures were made at each end before cutting. After completion of the biopsy, finger pressure was applied to the site with a swab and the skin was sutured.

The biopsy specimen was kept moist on a piece of gauze moistened with normal saline, and was cut into two parts across the muscle fibres, one for the histology–histochemistry and the other for the ultrastructural study. The second incision was located 5 mm proximal to the site of the initial biopsy.

## Histological and histochemical study

Half of the biopsy material was snap frozen in isopentane ($-160°$C), cooled in liquid nitrogen, and stored at $-70°$C. Then the samples were cut into transverse sections 10 μm each in a cryostat (Bright) at $-20°$C and processed for histological stainings: H & E, haematoxylin–van Gieson, and for histochemical stainings, myofibrillar ATPase (preincubation at pH 4.3 and 9.4) to demonstrate fibre types, succinate dehydrogenase (SDH) and NADH–trichrome reductase to show the oxidative fibres and periodic acid–Schiff reagent (PAS) staining for glycogen.

A morphometric analysis was applied with a camera lucida connected to a light-microscope and a digitizer connected to a computer. The transverse areas from each sample were examined, and the fibre type distributions were determined from sections stained for ATPase and NADH-trichrome reductase. A total number of 300 fibres at least was studied from each sample. The fibre diameter and area of each muscle fibre, as well as the percentage of each fibre type was estimated. The sizes and distribution of the two main fibre types of each sample were demonstrated on histograms.

## Ultrastructural study

The other part of the vastus lateralis was prepared for the electron-microscope study. The specimens were fixed in 2.5% glutaraldehyde in phospate buffer at pH 7.4 for 1 h at 4°C, washed in the same buffer and postfixed for another hour in 1% osmium tetroxide in pH 7.4 buffer, dehydrated in ascending ethyl alcohol and embedded in Epon 812 resin [17].

Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron-microscope (Zeiss EM 9S–2).

## Exercise training rehabilitation programme

The exercise training rehabilitation programme in all HD patients comprised 90-min sessions, which were held indoors three times per week (on non-dialysis days) for 6 months, under the continuous supervision of a physician, an exercise physiologist, and a trainer. The training routine consisted of 10 min warm-up on cycle ergometry or treadmill, followed by 50 min either aerobic exercises, as callisthenics and steps, swimming or ball games, 10 min of low-weight resistance exercises, 10 min stretching exercises and 10 min cool-down. The intensity of exercise was estimated on the basis of each patient's maximal aerobic capacity, heart rate, and blood pressure response during the exercise treadmill stress test and progressed gradually. None of the patients had any complication attributable to the exercise training.

## Statistical analysis

Continuous data are reported as mean value $\pm$ SD and categorical data as percentages. The paired Student's $t$ test was used for comparing differences between the mean values before and after exercise training. Pearson product moment correlation was used for correlating type I, type II, mean muscle fibre area, and peak strength before and after exercise training. Differences were considered significant at $P$ values $<0.05$.

## Results

## Before exercise training

### Clinical data

The baseline characteristics of the seven patients are shown in Table 1 and their clinical data in Table 2. Symptoms of peripheral neuropathy such as cramps and restless legs syndrome were reported in six patients. Muscle atrophy and objective neurological findings were present in all patients. Impaired vibration sense and abnormal reflexes were found in five patients. The exercise testing was terminated because of leg fatigue (5/7) and general fatigue (2/7). Mean duration of treadmill exercise test was $16.3 \pm 3.2$ min, while the $VO_2$ peak was $17.7 \pm 5.0$ ml/kg/min and blood lactate concentration was $9.4 \pm 1.7$ mmol/l. The peak isometric force of the lower limbs was $271 \pm 102$ N for the right leg and $296 \pm 129$ N for the left leg. The mean nerve conduction velocity was $40.3 \pm 3.0$ m/s and the distal time was $4.4 \pm 0.3$ ms in the left leg, while almost similar results were found in the right leg ($40.8 \pm 3.8$ m/s and $4.6 \pm 0.2$ ms correspondingly).

### Morphometry

Results are summarized in Table 3. All patients showed impressive fibre atrophy, the mean muscle fibre area being reduced to $2548 \pm 463$ μm$^2$ when the muscle sections were stained for NADH-TR or to $2776 \pm 817$ μm$^2$ when they were stained for ATPase, compared to $4150 \pm 246$ μm$^2$ reported value for the same muscle of sedentary age-matched healthy individuals [18,19]. The mean fibre cross-sectional areas when the sections were stained for NADH-TR were a little smaller than those of the same muscle sample when stained for ATPase, because of the different staining procedure [20]. Muscle fibres of both types were atrophied but type II fibres were the more affected. Type I fibres covered the $54.6 \pm 18.9\%$ of the whole sample fibres, their mean area being $2831 \pm 846$ μm$^2$, and type II fibres covered the $45.4 \pm 18.9\%$, their mean fibre area being $2683 \pm 763$ μm$^2$.

### Histology and histochemistry

The biopsies of the vastus lateralis muscle showed pathological atrophied muscle fibres, with random variation in fibre size, numerous small angulated fibres in clusters or sparse among normal or hypertrophied ones (Figure 1). The nuclei were normally located at the periphery of the fibre; There were neither internal nuclei seen in proportion $>3\%$, nor extensive cellular reactions. In a few cases, local infiltration and muscle-fibre degeneration was observed, but the cellular type of the leukocyte infiltration was difficult to be

**Table 1.** Characteristics of the HD patients

| Patient | Sex | Age (years) | Weight (kg) | BSA (m$^2$) | Years on dialysis | Years on renal disease | Renal disease |
|---|---|---|---|---|---|---|---|
| 1 | M | 25 | 82 | 2.05 | 1.0 | 2.0 | Chronic glomerulonephritis |
| 2 | M | 33 | 65 | 1.76 | 7.0 | 13.0 | Nephrosclerosis |
| 3 | M | 59 | 83 | 1.99 | 5.0 | 8.0 | Chronic glomerulonephritis |
| 4 | F | 53 | 56 | 1.44 | 12.0 | 16.0 | Chronic pyelonephritis |
| 5 | F | 58 | 49 | 1.45 | 1.0 | 7.0 | Polycystic kidneys |
| 6 | M | 21 | 50 | 1.51 | 5.0 | 9.0 | Vesicoureteral reflux |
| 7 | M | 60 | 84 | 1.90 | 1.0 | 3.0 | Obstructive nephropathy |
| Mean | | 44.1 | 67.0 | 1.73 | 4.6 | 8.3 | |
| SD | | 17.2 | 15.9 | 0.26 | 4.1 | 5.0 | |

**Table 2.** Clinical data of the patients before and after the 6-month exercise rehabilitation programme (mean value $\pm$ SD)

| | Before | (Range) | After | (Range) | $P$ |
|---|---|---|---|---|---|
| Hct (%) | 30.9 $\pm$ 4.2 | (26.5–38.0) | 30.4 $\pm$ 4.5 | (25.5–37.5) | NS |
| Urea (mg%) | 196 $\pm$ 30.8 | (159–241) | 199 $\pm$ 33.3 | (159–236) | NS |
| Creatinine (mg%) | 13.2 $\pm$ 4.0 | (8.6–20.8) | 13.2 $\pm$ 3.8 | (8.6–20.7) | NS |
| K (mEq/l) | 5.6 $\pm$ 0.5 | (5.1–6.4) | 5.7 $\pm$ 0.6 | (5.0–6.6) | NS |
| Na (mEq/l) | 140.3 $\pm$ 2.0 | (138–144) | 139.7 $\pm$ 3.1 | (134–144) | NS |
| Ca (mg %) | 8.6 $\pm$ 0.6 | (7.4–9.2) | 8.8 $\pm$ 0.7 | (7.6–9.7) | NS |
| P (mg %) | 6.5 $\pm$ 0.6 | (5.7–7.1) | 6.4 $\pm$ 1.6 | (4.4–8.8) | NS |
| i-PTH (pg/ml) | 181.3 $\pm$ 41.9 | (129–226) | 179.2 $\pm$ 40.9 | (132–228) | NS |
| Alkaline phosphatase (U/l) | 276.4 $\pm$ 97.9 | (104–402) | 269.4 $\pm$ 47.5 | (196–332) | NS |
| Isometric force max | | | | | |
|   Left leg (N) | 271 $\pm$ 102 | (100–433) | 422 $\pm$ 106 | (285–577) | <0.05 |
|   Right leg (N) | 296 $\pm$ 129 | (165–550) | 415 $\pm$ 138 | (229–663) | <0.05 |
| Conduction velocity | | | | | |
|   Left leg (m/s) | 40.3 $\pm$ 3.0 | (34.3–42.8) | 45.4 $\pm$ 3.2 | (39.1–48.3) | <0.05 |
|   Right leg (m/s) | 40.8 $\pm$ 3.8 | (35.1–44.1) | 45.8 $\pm$ 3.6 | (40.2–48.5) | <0.05 |
| Distal time | | | | | |
|   Left leg (ms) | 4.4 $\pm$ 0.3 | (4.0–5.0) | 4.0 $\pm$ 0.1 | (3.9–4.1) | <0.05 |
|   Right leg (ms) | 4.6 $\pm$ 0.2 | (4.2–5.3) | 4.1 $\pm$ 0.3 | (3.9–4.4) | <0.05 |
| Peak exercise performance | | | | | |
|   Exercise time (min) | 16.3 $\pm$ 3.2 | (12.7–21.0) | 21.0 $\pm$ 3.5 | (15.9–26.8) | <0.05 |
|   VO$_2$ peak (ml/kg/min) | 17.7 $\pm$ 5.0 | (9.1–23.3) | 26.2 $\pm$ 8.0 | (16.8–38.3) | <0.05 |
|   Blood lactate (mmol/l) | 9.4 $\pm$ 1.7 | (6.2–11.3) | 7.9 $\pm$ 2.9 | (3.2–10.6) | <0.05 |

**Table 3.** Morphometric and histochemical data of the patients at the beginning and at the end of the 6-month exercise rehabilitation programme (mean value $\pm$ SD)

| | Before | (Range) | After | (Range) | $P$ |
|---|---|---|---|---|---|
| Type I | | | | | |
|   Mean area (μm$^2$) | 2831 $\pm$ 846 | (1487–3950) | 3565 $\pm$ 764 | (2466–4605) | <0.05 |
|   % Type I | 54.6 $\pm$ 18.9 | | 31.6 $\pm$ 11.9 | | <0.05 |
| Type II | | | | | |
|   Mean area (μm$^2$) | 2683 $\pm$ 763 | (1577–3515) | 3319 $\pm$ 1049 | (1512–3935) | <0.05 |
|   % Type II | 45.4 $\pm$ 18.9 | | 68.4 $\pm$ 11.9 | | <0.05 |
| Mean muscle fibre area- | | | | | |
|   ATPase (μm$^2$) | 2776 $\pm$ 818 | (1504–3760) | 3381 $\pm$ 984 | (1715–4697) | <0.05 |
| Oxidative | | | | | |
|   Mean area (μm$^2$) | 2738 $\pm$ 560 | (2175–3670) | 3514 $\pm$ 323 | (3125–4010) | <0.05 |
|   % Oxidative | 33.4 $\pm$ 15.6 | | 53.4 $\pm$ 7.1 | | <0.05 |
| Non-oxidative | | | | | |
|   Mean area (μm$^2$) | 2533 $\pm$ 566 | (1366–3102) | 3067 $\pm$ 717 | (2117–4089) | <0.05 |
|   % Non-oxidative | 66.6 $\pm$ 15.6 | | 46.6 $\pm$ 7.1 | | <0.05 |
| Mean muscle fibre area- | | | | | |
|   NADH (μm$^2$) | 2548 $\pm$ 463 | (1594–2985) | 3284 $\pm$ 514 | (2610–4043) | <0.05 |
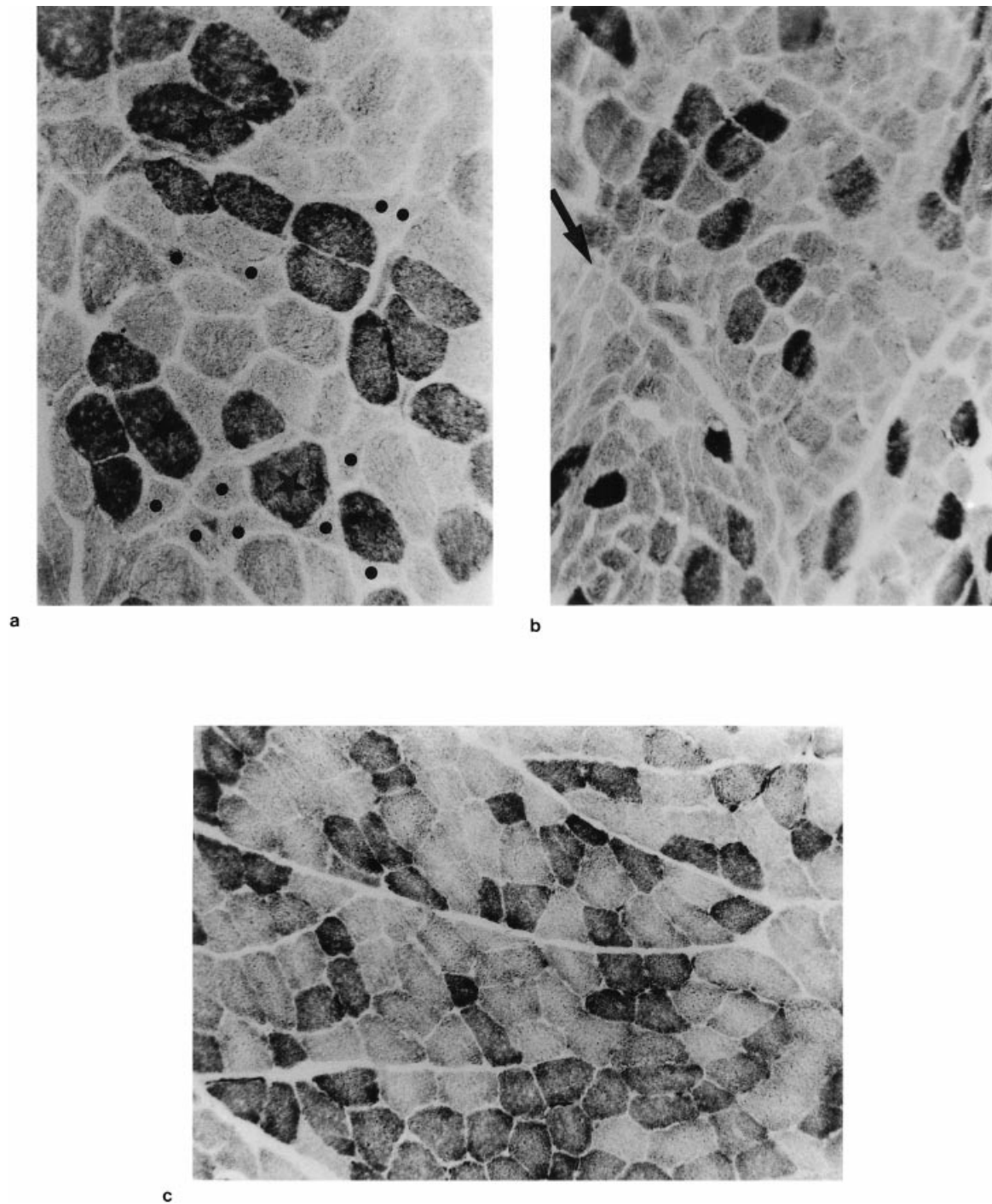
**Fig. 1a–c.** Biopsies from the vastus lateralis of the same patient before (a,b) and after (c) the training. (**a**) Variability in fibre size; the atrophic fibres (•) are non-oxidative. Small groups of darkly stained hypertrophied fibres (*); NADH-TR ($\times$100). (**b**) Large-group atrophy ($\rightarrow$) mainly consisted of non-oxidative fibres; NADH-TR ($\times$50). (**c**) After training, essentially normal appearance; NADH-TR ($\times$100).

identified, because the sections were frozen (Figure 2). In other cases mild perifascicular infiltration was seen. Connective tissue development was almost normal. Both type I and type II fibres were reduced in size, even though type II fibres were more affected (Figure 3), as was shown by the morphometric analysis. The variability of the fibre type size was not within normal limits, since type II fibres which were normally larger than type I, became almost equal to type I fibres.

The atrophic fibres were seen in the biopsies usually as small group of 3–10 fibres surrounded by relatively normal-sized fibres, a finding characteristic of denervation atrophy (Figures 1,2). In a couple of cases though, the atrophic fibres were numerous covering an extended area, almost a whole fascicle. The atrophy observed in
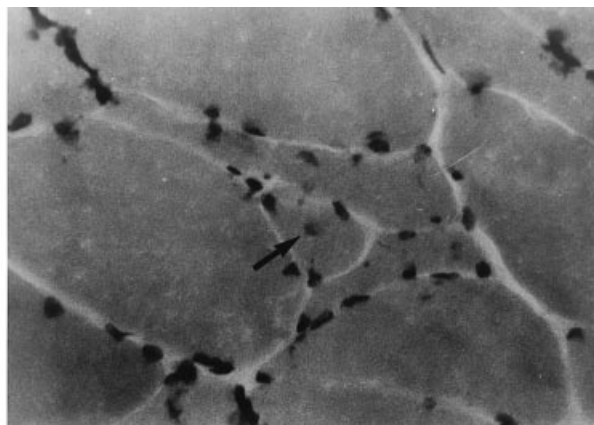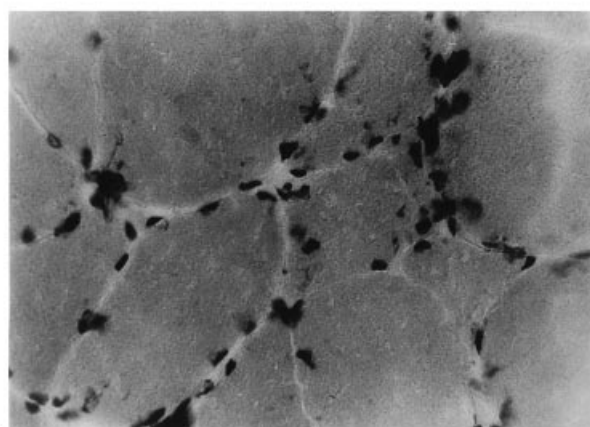
**Fig. 2a,b.** Biopsies of the vastus lateralis before the training. (**a**) Small-group atrophy. Arrow shows central nucleus. H & E (×400). (**b**) Necrotic fibres undergoing phagocytosis. H & E (×400).

either cases of small- or large-group atrophy, seems to be rather selective of type II fibres, involved mainly type II fibres. Hypertrophy of some fibres was also seen (Figure 1), probably as a compensation for the loss of function in smaller atrophic fibres. Type-specific hypertrophy was not seen.

The normal mosaic of the different histochemical fibre types seen normally became disturbed (Figures 2,3). The biopsies showed small or large clusters of one fibre type next to clusters of another fibre type; this fibre grouping was usually associated with reinnervation.

The usual ratio of type I to type II, analysed for vastus lateralis as normally 1:2, was changed to approximately 1:1, as was estimated by the morphometric analysis. Type I fibres predominated, or otherwise type II fibres were more affected.

An interesting finding in the biopsies of these patients was excessive glycogen deposition in well-defined rows, seen with PAS–Schiff reagent staining, and it was more prominent in the non-oxidative fibres (Figure 4). This sarcoplasmic derangement morphologically resembled glycogenosis.

These changes that characterized the muscle biopsies of the HD patients were non-specific, since most of them, like the small-group atrophy and the fibre type grouping, implicated neurogenic atrophy, whereas other findings, like large-group atrophy and type I predominance tends to be associated to myopathic conditions.

### Ultrastructural findings

The ultrastructural study of the pre-exercised specimens showed severe non-specific degenerative changes in skeletal muscle fibres, mitochondria, and capillaries that seem to be characteristics of atrophy of neuropathic origin. All fibres were atrophied, but more extensive atrophy was found in type II fibres. There was a peripheral loss of subcellular elements and focal undulations of the sarcolemma noticed. The external lamina of fibres' sarcolemma and the basal lamina of capillaries remained after their necrosis. Mitochondria also showed a spectrum of changes. Frequently they were swollen, their cristae had disappeared, and the matrix had lost its normal density (Figure 5).

Central nuclei with many invaginations were found, as well as disorganization of sarcomeres with Z-disk streaming and loss of A and I bands in many fibres. Subsarcolemmal concentration of cylindrical spirals was found in type II fibres. Finally, there was a deposition of glycogen in most of the muscle fibres (Figure 6).

## After exercise training

### Clinical data

The clinical data of the seven patients at the end of the exercise training rehabilitation programme are shown in Table 2. Physical training was associated by a significant improvement of exercise capacity in all patients. The mean exercise time in the treadmill test was by 28.8% increased and the $VO_2$ peak by 48%, while blood lactate concentration was decreased by 16%. Muscle strength of the lower limbs was increased by 40.2% in the right leg and by 55.7% in the left leg. Motor conduction velocity was also increased by 12.7% and distal time decreased by 9.1% in the left leg and by 12.3% and 10.9% respectively in the right leg. Symptoms of peripheral neuropathy were improved in three patients, but no difference was found in the clinical signs of the neuropathy.

### Morphometry

The morphometric study of the patients showed a remarkable improvement of the muscular atrophy following exercise training. The mean area for type I fibres increased by 25.9% and type II fibres by 23.7% (Table 3). Despite this improvement, there was still muscle fibre atrophy of both types, which was more severe in type II fibres. Finally, the proportion of type II fibres was increased from 45.4 to 68.4%, whereas the non-oxidative fibres were decreased from 66.6 to
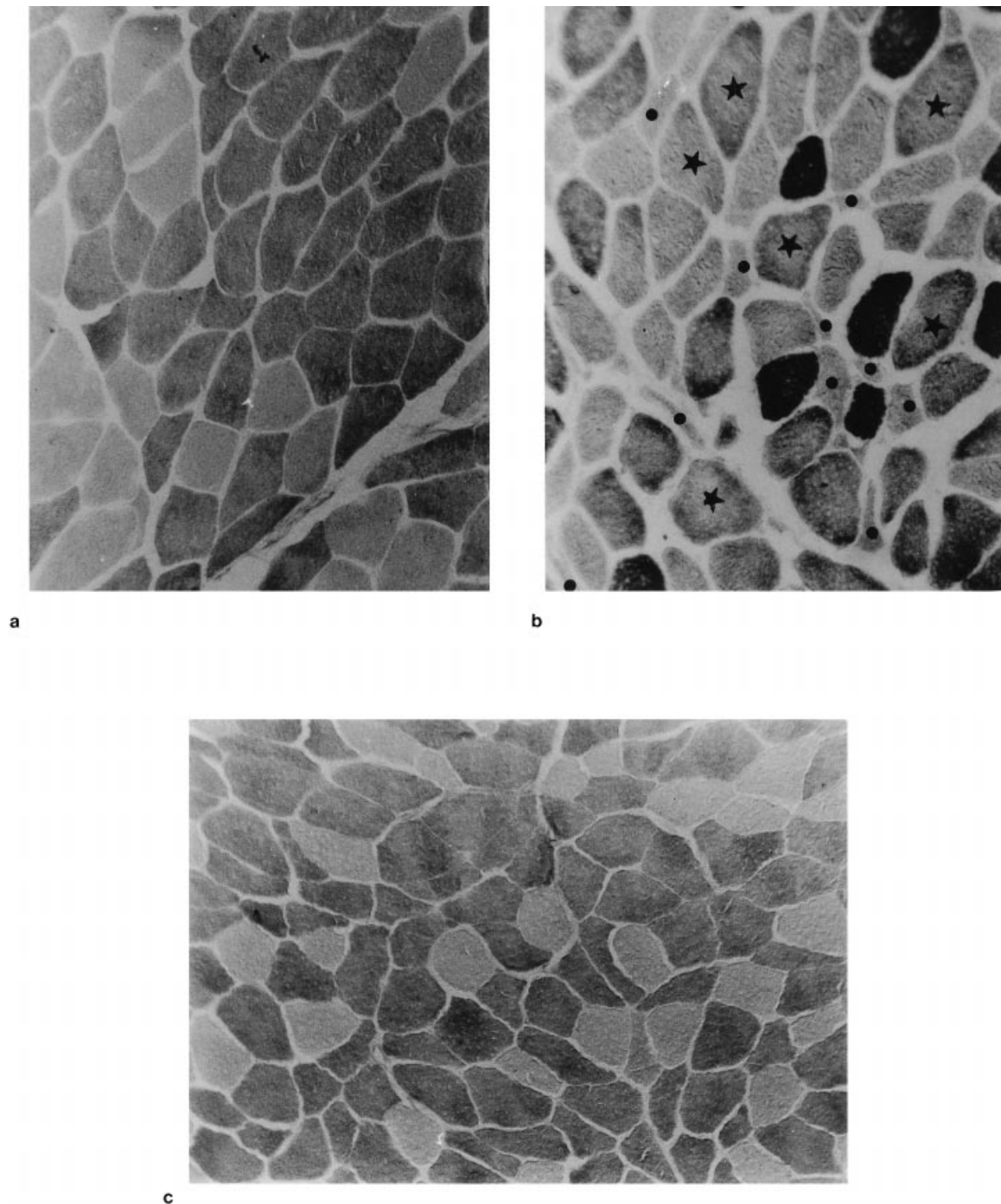
**Fig. 3a–c.** Muscle biopsies of the vastus lateralis of the same patient before (a,b) and after (c) the training. (**a**) Fibre grouping. ATPase preincubated at pH 9.4 ($\times$100). (**b**) Variation in the size of fibres with both hypertropic (*) and atrophic type II fibres (•) present. ATPase preincubated at pH 4.3 ($\times$100). (**c**) After the training. Normal mosaic pattern. ATPase preincubated at pH 9.4 ($\times$100).

46.6%. No significant relationships were found between any pair of variables, type I fibres, type II fibres, mean muscle fibre area and the muscle function estimated as peak strength before and after training.

*Histology and histochemistry*

The above morphometric quantitative improvement in muscle atrophy following exercise training was con-

firmed by the histological study. The overall architecture was better and the muscle looked reasonably normal. The muscle fibres were now of better shape, well organized into myofibrils, and the structural abnormalities were minimized (Figures 2,3). The cellular reactions were also reduced.

The muscle cell sizes of both fibre types were increased, especially in type II fibres. The hypertrophied fibres usually associated with exercise are type
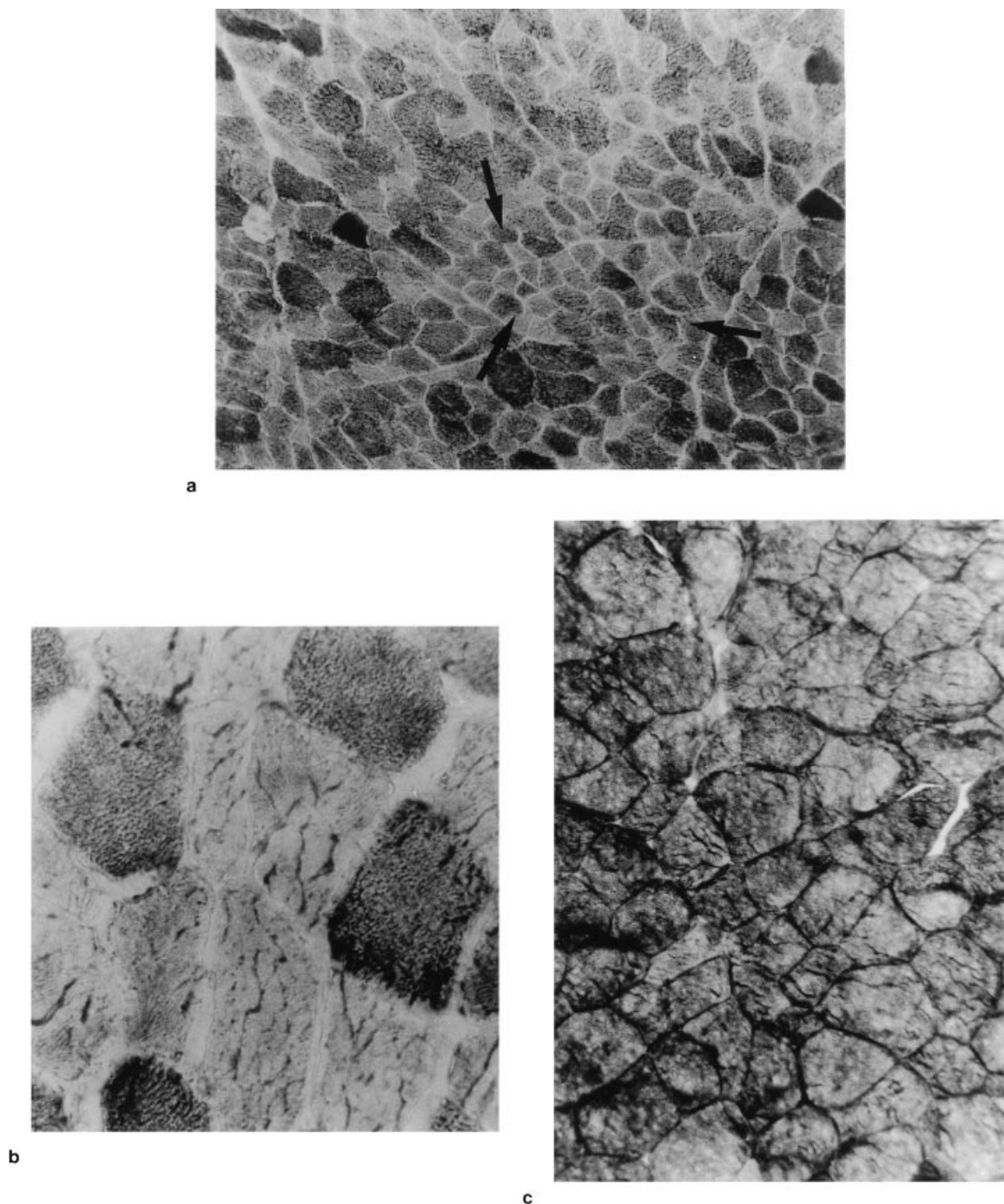
**Fig. 4a–c.** Muscle biopsies of vastus lateralis before the training. (**a**) Large-group atrophy (→); excessive glycogen deposition in rows. PAS (×50). (**b**) Excessive glycogen deposition in rows, but differentiation between fibre types was retained. PAS (×400). (**c**) Moderately strong overall stain, but the differentiation between fibre types was not retained (×100).

II fibres. The ratio of type I to type II fibres, which had been 54.6:45.4, became 31.6:68.4, which is almost 1:2, as reported for the normal vastus lateralis [21]. The normal mosaic pattern was visible in most cases, showing an almost normal two-fibre pattern on histochemical enzyme reactions, with good preservation of the fibre structure. Furthermore, the grouping was significantly reduced.
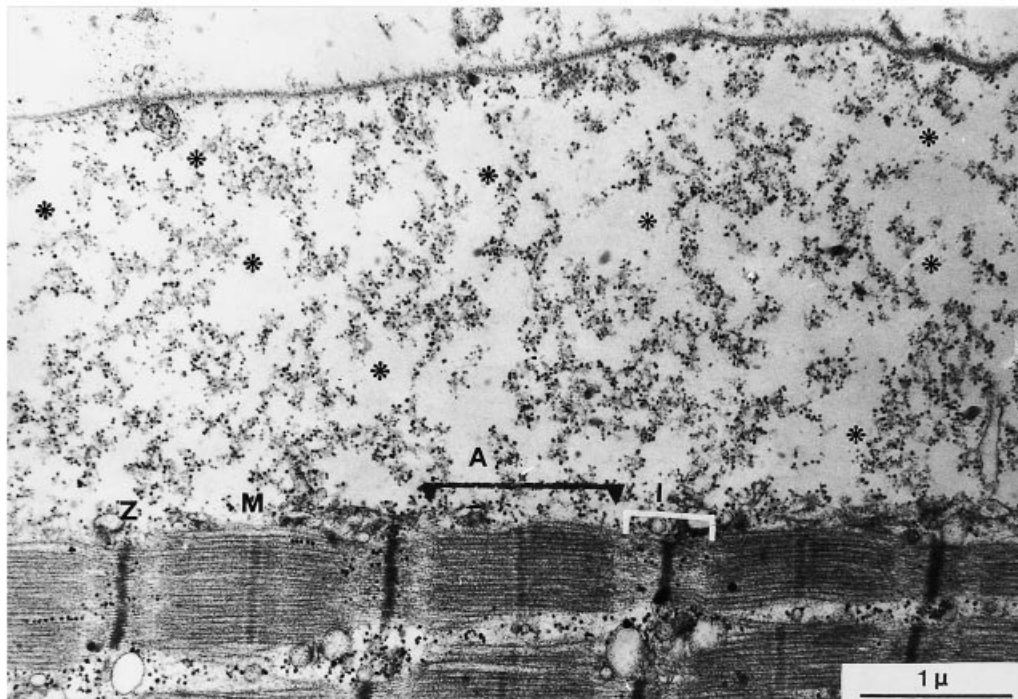
Although, the PAS stain showed a moderately strong overall stain, the differentiation between fibre types was clear. The multiple focal deposits of PAS stain in rows were still visible, even though they were of weaker intensity.

*Ultrastructural findings*

After the exercise training important favourable changes were noted on the size and structure of the
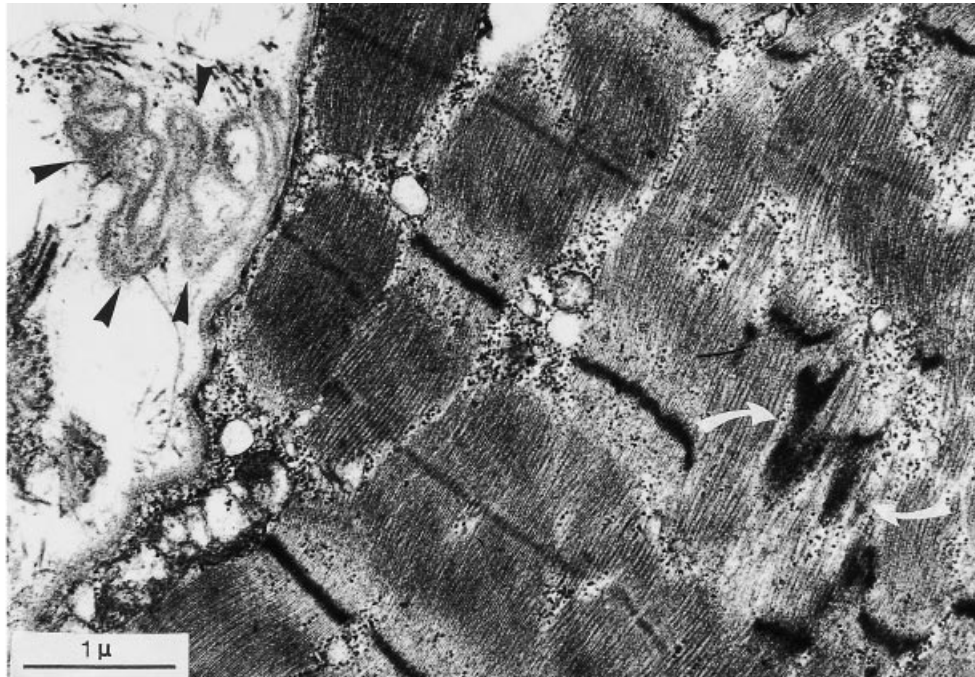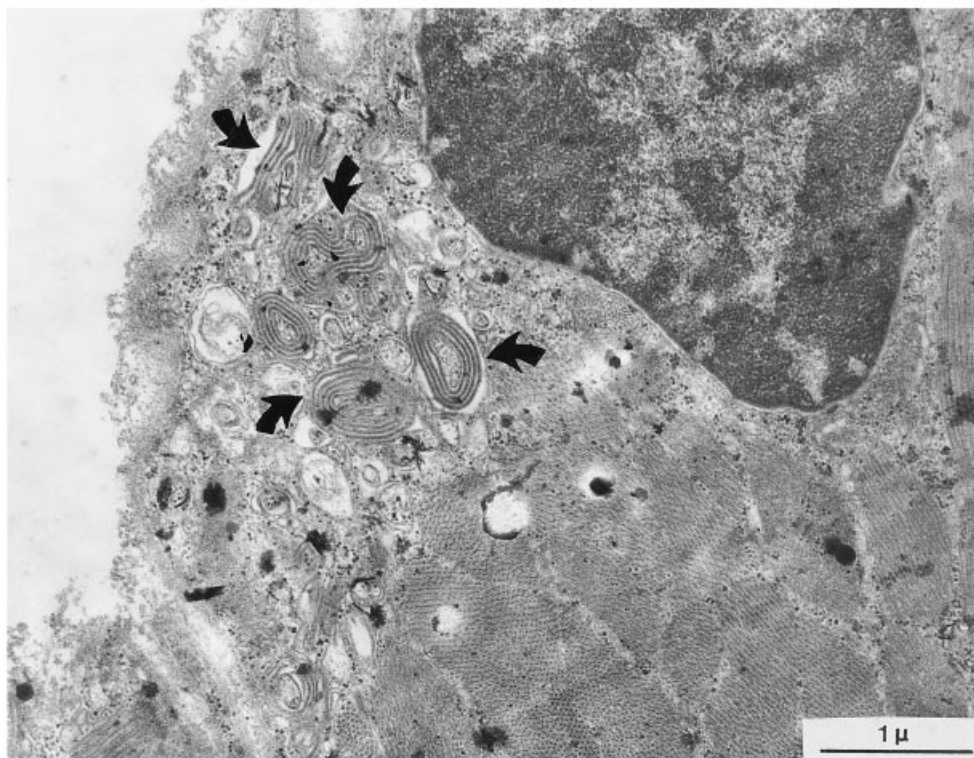
**Fig. 5a,b.** Electron-micrographs before the training. (**a**) Two skeletal muscle fibres (I and II). Type II (II) muscle fibre shows many degenerative changes as undulations of the sarcolemma (arrowheads), disorganization of sarcomeres, loss of A, I, and Z disk (*), and swollen and disrupted mitochondria (→). (**b**) An atrophic muscle fibre with peripheral loss of subcellular elements (*). A, A band; I, I band; Z, Z disk; M, M line.

**Fig. 6a,b.** Electron-micrographs before the training. (**a**) Z disk streaming (white arrows) and retundant loops of basal lamina (arrowheads) in an atrophic muscle fibre. (**b**) Cylindrical spirals (arrows) in type IIb fibre, which forms subsarcolemmal clusters that extend parallel to the long fibre axis.

muscle fibres and capillaries, as well as on the number and structure of mitochondria in all patients. The fibre area of both type I and type II fibres was increased. There was a restoration of muscle fibres seen and normal distribution of myofilaments (Figure 7). There was also an activation of satellite cells found, proliferating myoblasts, which were fused to form multinucleated myotubes (Figure 8). Increased subsarcolemmal and intermyofibrillar glycogen accumulation, as well as restoration of mitochondria were also seen. Specifically, the mitochondria had normal shape, size, normal density, and good orientation of their cristae. Additionally, there were no cylindrical spirals in type II fibres. The endothelial cells of vessels were activated and the capillaries were open and hypertrophied with numerous pinocytic vesicles. Finally, increased numbers of leukocytes and natural killer cells were present.

## Discussion

The main findings of this study were the non-type-selective fibre atrophy of the vastus lateralis in chronic HD patients, and the striking beneficial effect of the exercise training on the recovery of the atrophic muscle fibres, and consequently on muscle strength and exercise performance. The former result is not surprising, and although some authors characterized the muscular atrophy of HD patients mainly as secondary myopathy, possibly of neuropathic origin, it clearly suggests in addition to neurogenic myopathy, a primary atrophic myopathy [12–14,22–24]. The latter finding, the beneficial effects of exercise training on muscle structural and functional abnormalities, may introduce a useful therapeutic method of treating uraemic myopathy, supporting the value of renal rehabilitation programmes for the management of patients undergoing haemodialysis.

Skeletal muscle dysfunction, in association with anaemia and/or impaired cardiovascular function, affects physical activity markedly in HD patients [1,2,6,15,25]. However, it was suggested that muscle metabolic and morphological abnormalities are the most important limiting factors for work capacity in these patients [25]. It should also be taken into consideration that one of the causes of muscular atrophy has been reported as the inactivity caused by the primary clinical problem [26,27].

In the present study, the morphometric analysis showed that both type I and II fibre areas were significantly reduced compared to normal reported values [18,19,26,28,29]. Our results are in agreement with previous studies that showed marked muscular atrophy in HD patients [30–32]. The fibre grouping, another characteristic finding, was observed in the specimens of all patients, both older and younger, so that it could not be taken as an indication of degenerative changes of old age. Our ultrastructural findings are similar to the results of Diesel et al. concerning Z-band degeneration and loss of myofilaments and mitochondrial changes in all patients [11]. These degenerative changes, in addition to evidence of muscle regeneration, indicated an ongoing process of damage and repair. However, we did not notice any paracrystalline or angular electron-dense intramitochondrial inclusions.

Uraemic peripheral neuropathy is considered as the reason for the development of muscle atrophy in HD patients [9,10,12]. Likewise, we found reduced nerve conduction velocity from the neurophysiological tests. Slowed nerve conduction as a frequent occurrence in uraemic patients has been known for a long time [7,9]. This occurs when creatinine clearance decreases below 10% of normal, and is evident throughout the peripheral nervous system, with slowing being greater in proximal segments. The late responses of H reflex and F response appears early in the course of renal failure [33].

Besides the findings that were suggestive of neurogenic damage, like small-group atrophy, fibre grouping, and in some cases perifascicular atrophy, there were also some myopathic elements like cellular reactions, and perifascicular development of connective tissue. Moreover, the increased deposition of glycogen into muscle fibres, reported in this study has also been found previously [5,11,34]. This alteration may be due to the impaired carbohydrate metabolism in uraemia [35]. This glycogen accumulation was associated with the low lactate response to exercise, caused by a block of glycolysis in muscles, and closely resembles glycogen storage diseases as McArdle's disease [6,35]. Diesel et al. also suggested that HD patients terminated maximum exercise test at low blood lactate concentrations, because of mitochondrial and myofilamentous abnormalities [11].

Muscle weakness and wasting, found in almost all patients, are frequently present in chronic uraemic patients undergoing haemodialysis [2,5,13]. The muscle wasting is due to the reduction in the cross-sectional area of individual muscle fibres, often affecting one fibre type more than another [24]. Muscle weakness and fatigue seems to be multifactorial, as a consequence of abnormalities at many levels [36,37]. It seems that the presence of sarcolemmal concentration of cylindrical spirals in type II fibres was one of the main causes for the muscle cramps that we noticed in six of the patients before physical training [38–40]. The toxicity of the parathyroid and other $Ca^{2+}$-related hormones as well as of several potential neurotoxins have also been connected with the myopathic changes in HD patients, and since dialysis did not improve polyneuropathy, the idea of middle-molecule substances as a cause of uraemic neuropathy and myopathy has been considered very attractive [9,10,12,31,32,41].

Exercise training programmes have been reported to be useful in modifying the morbidity and behaviour of chronic uraemic patients, causing physiological, metabolic, and psychological benefits [2–5,15,42,43]. Similarly in the present study, a 6-month exercise training, mainly of aerobic type, resulted in significant increases in exercise capacity and muscle strength of
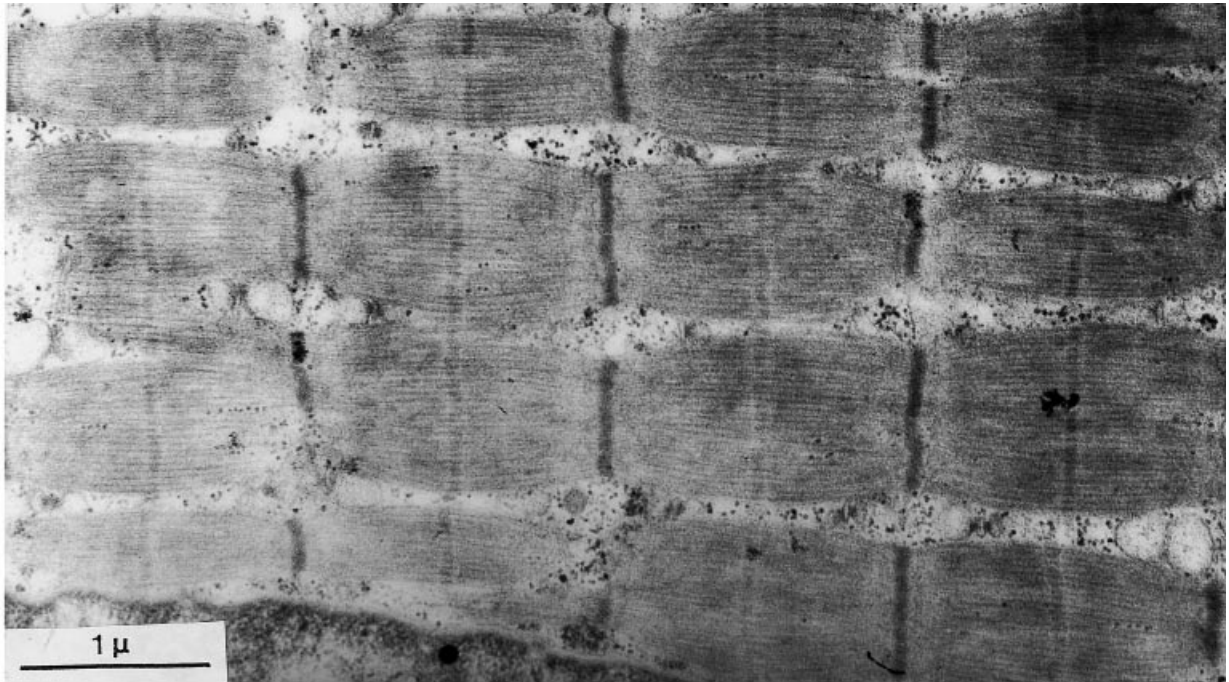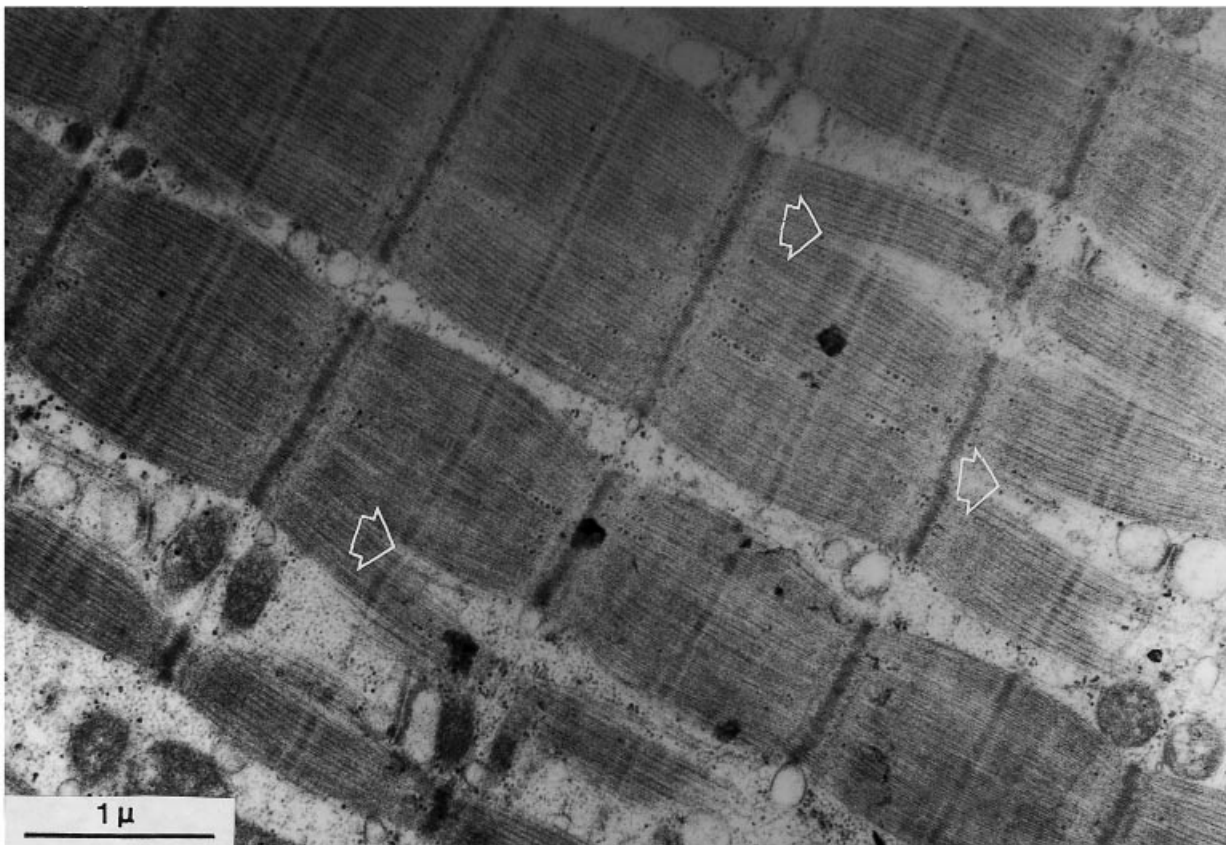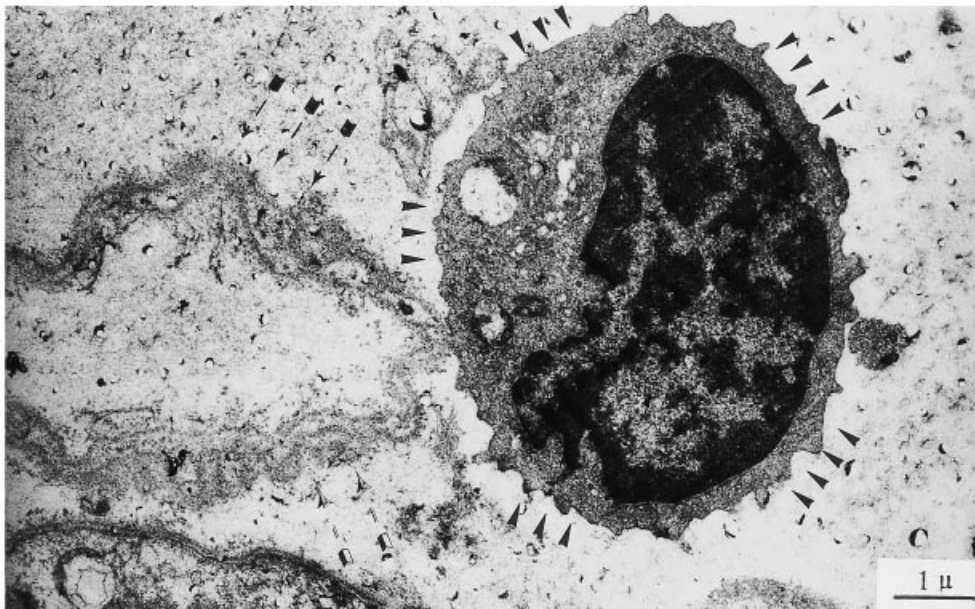
**Fig. 7a,b.** Electron-micrographs after the training. (**a**) Longitudinal section of a fibre with well-oriented sarcomeres. (b) Longitudinal section of a skeletal muscle fibre with splitting of myofibrils (arrows).

**Fig. 8a,b.** Electron-micrographs after the training. (**a**) Myoblast (black arrows) with central nucleus, irregular myofilament (white arrowheads) with immature and randomly oriented Z disk (white arrows). Pentads (P). (**b**) A macrophage with cytoplasmic pseudopodia (arrowheads) near a collapsed basal lamina of a disappeared capillary (black arrows).

HD patients. Although the mechanisms explaining the improvement in skeletal muscle abnormalities and exercise capacity are not clear, it is suggested that it may be primarily related to enhanced oxidative capacity and other muscular metabolic and functional energetic by exercise training [4,33,36,42].

The aerobic exercise that our patients had been trained for usually activated glycolytic as well as oxidative fibres, and hence may improve muscle strength and performance [4,8,18]. Indeed such changes were found in our patient group: the proportion of the oxidative fibres increased by 59%, and their fibre area by 28%. Following exercise training the lactate production with exercise was found to be even more diminished compared to the baseline levels. This may be due to an improvement in anaerobic mechanisms of skeletal muscles.

Muscle adaptations to exercise has been shown mainly by the dramatic recovery of the muscle fibre size and structure as shown by both light- and electron-microscope area. We found that after the 6-month exercise training the recovery of the atrophic fibres was impressive; the mean muscle cross-sectional area improved by 29%, type I fibre area was increased by 26%, and type II by 24%. Exercise affected also the proportions of fibre types and shifted the ratio of the fibre types to normal values. We found a 51% increase in the proportion of type II fibres and a 42% decrease in the proportion of type I fibres, so that the ratio of type I to type II became approximately 1:2, as was estimated for the normal vastus lateralis [21]. Formation of new muscle fibres, as well as regeneration of degenerated fibres in HD patients after exercise training were found in our ultrastructural study. Especially, a large number of myoblasts was observed, which was recognized by the presence of scanty myofilaments and irregular primitive Z bands. These beneficial changes of the muscle fibres were also associated by a restoration of the mitochondria. A contribution of peripheral neural adjustments to the metabolic changes after training cannot be excluded [44]. The increase of motor conduction velocity in our patients after exercise training confirms this suggestion.

The results of this study demonstrate that exercise training has beneficial effects on the restoration of the atrophic muscles in chronic haemodialysis patients. The parallel improvement in muscle strength and aerobic capacity supports the hypothesis that peripheral adaptations to exercise play a significant role in the overall augmentation of work capacity in HD patients. We therefore conclude that exercise training rehabilitation programmes may have a more universal application in the management of end-stage renal disease patients.

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