Long lasting muscle trophism in complete upper motor neuron lesion paraplegia

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Abstract

Subjects with complete lesion of the spinal cord (T3/4 – T11 level) develop spastic paraplegia. In these patients muscles of the lower limb are still innervated by motor neurons that, however, have lost connection with the central nervous system (CNS). In this study we have determined the progression of muscle atrophy in spastic patients comparing force, CT scan imaging, histological and ultrastructural findings in short-term (2 patients, 0.9 years from injury), in mid term (2 patients, 2.0 and 2.4 years from injury) and long-term (2 patients, 17.0 and 20.3 years from injury) paraplegic subjects. In comparison to the short term patients, in the mid term group there are not significant difference in muscle size (CT Scan) and histological (fiber size and tissue composition) and significant difference is present in torque of knee extension (31.53 ± 5.42 vs 13.82 ± 10.00Nm, p=0.03) while in long term group there are significant differences in torque of knee extension 31.53 ± 5.42 vs 19.23 ± 1.63Nm, p=0.02) and in fiber size (30.59 ± 14.39 vs 40.42 ± 17.01μm, p<0.001). In comparison to the mid term patients, in the long term group there are not significant differences of either functional (torque of knee extension) and muscle size (CT Scan), while there is a significant increase in fiber size (30.75 ± 23.55 vs 40.42 ± 17.01μm, p<0.001). Electron Microscopy (EM) indicates that even after long periods of paralysis, muscle fibers maintain a quite organized contractile apparatus, with distinct myofibrils and sarcomeres, while the major alteration appear to be a misplacement of the activating (EC coupling) and metabolic (mitochondria) machinery. Ultra structural characteristics of some myofibers confirm that minor lower motoneuron denervation is present in all groups and may contribute to the functional outcomes of the thigh muscles. These findings indicates that, in spastic patients, the initial rapid loss of muscle mass occurring in the first months after the injury is followed by a prolonged steady state in which muscle maintains its mass, composition, and performance for extended periods of time, i.e. up to 20 years. The maintenance of muscle suggests that there are not upper-time limits to start successfully a training program to recover muscle mass and function in these patients by body weight-supported treadmill training (BWSTT), electrically induced cycle training (EICT) or Functional Electrical Stimulation (FES).

Key words: human, muscle, paraplegia, upper motor neuron lesion, CT scan, biopsy, histology, electron microscopy, spinal-cord injury.

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Lesion of the spinal cord at the level of T3/4 – T11 interrupts connections between the central nervous system and the motor neurons that innervates peripheral area of the body. Muscles in the lower extremities being innervated by intact lower motor neurons develop spastic paraplegia, characterized by uncontrolled movements

of the affected areas, and spasms generated by reflex circuits [11-18].

Beside the uncontrolled muscle activity, the other effect of these type of spinal cord lesions is an evident loss of muscle mass. The time course of this muscle atrophy is quite fast immediately after the injury, but slows down significantly with longer time elapsed from the injury event. It has been reported that within the first five days after injury, the thickness of the muscle bulk measured by ultrasound is already decreased of about 16% of the initial value, and this loss of muscle mass progresses quite quickly to about 40% at day 20, to remain then more or less constant at 40 days [28-29].

The steady-state of muscle atrophy in spastic paralysis has been documented [12,24] by Computer Tomography (CT) in groups of patients trained with either functional electrical stimulation (FES), i.e. electrically induced cycle training (EICT) and/or body weight-supported treadmill training (BWSTT). These studies have shown that, before exercise, the degree of atrophy in the individual muscle fibers was more variable than after periods of FES or BWSTT training [24]. Other studies on bioptic samples also support the idea that the atrophy in spastic subjects is reversible. However, most of these studies, especially for the pre-training phase, were performed during the early stage of spastic paralysis, the end point being typically within 24 months after injury [1-3,11,16,21,27]. In all the studies the positive effect of increased exercise by FES is confirmed by histochemical or electrophoretic analyses of isomyosins in tissue sample and/or individual myofibers to correlate function (muscle power and endurance) to structure (myofiber size by light microscopy morphometry) or muscle size by CT Cross Scan Area. However, little information is provided on the influence of other muscle-related factors, beside myofiber size and typing, e.g., ratio among myofibers and interstitial tissue, percentage of lower motoneuron denervated myofibers, and subtle changes at the ultra structural levels.

In the present study we investigated using CT scan, functional testing (knee extension torque), and structural approaches (light and electron microscopy of muscle biopsies from the thigh muscles) paraplegic patients before starting a FES training program, to study the progression of atrophy. Furthermore, we analyzed the extent of the eventual myofiber peripheral denervation by morphometry and N-CAM staining [9,15,31].

Our results indicate no progression of the muscle atrophy in long-term paraplegic patients. Therefore, FES treatment of these patients can be started at any time, i.e., even twenty years after SCI, to recover muscle mass and function by EICT, BWSTT or FES.

Materials and Methods

Characteristics of Patients

The six subjects used in this study (age 20-48, 1 female and 5 male) all had experienced traumatic spinal cord injury [15]. The causes for paraplegia were three motor cycle accidents, two car accidents, and one sport accident with a bobsleigh (Table 1). All subjects were volunteers and were enrolled into the project with a detailed information before signing an informed consent. All these volunteers were highly motivated to start FES and to perform bilateral muscle biopsy of the paretic legs. In the "short-term" group 2 paraplegic patients (1 male and 1 female) with upper motor neuron lesion were included (Table 1). The time after injury was 0,9 years, the age was 43 and 26 years, the body weights were 55 and 68 kg, and the heights were 172 and 180 cm. The level of lesion was Th4 and Th7. The "mid-term" group consisted of 2 paraplegic men. The time after injury was 2 and 2,4 years, , the age was 48 and 28 years, the body weights were 86 and 80 kg, and the heights were 181 and 170 cm.. The level of lesion was Th4-5 and Th6-7. The "long-term" group consisted of 2 paralyzed male paralyzed for 17.0 to 20.3 years , the age was 39 and 40 years, the body weights were 62 and 70 kg, and the heights were 180 and 178 cm.. The level of lesion was between Th4 and Th6. Muscle biopsies of both thigh muscles (vastus lateralis) were taken.

Clinical and functional assessments, follow-up and muscle biopsies were performed at the Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Vienna, Austria. Light microscopy analyses were performed at the C.N.R. Institute of Neuroscience, Laboratory of Applied Myology of the Department of Biomedical Sciences, Interdepartmental Research Center of Myology, University of Padova Medical School, Padova, Italy. Electron microscopy was performed at the IIM, Interuniversitary Institute of Miology and CeSI, Center for Research on Ageing, University G. d'Annunzio, Chieti, Italy.

Computer Tomography (CT) scan

All patients were lined supine on the CT table parallel to the table axis (in feet-first position). We used the top of the trochanteres maiori as reference point, which is determined by CT scan, and the first body section was taken at this point. The CT-cut plane ought to be well defined, so that results could be compared between different patients. Additional thigh sections are performed distally every 100 mm. To clearly distinguish fat from skeletal muscle tissue a soft window frame (window 350, center 50) was used. Beside complete cross sectional area of the upper thigh, the cross sectional areas of gluteus m., quadriceps m. and hamstrings, as well as their density (measured in Hounsfield units, HU), were determined as described in [23].

Force-measurement knee extension torque

The force measurement was performed in sitting position using a purpose-designed chair where subjects sit with the legs in 90° knee flexion position. A dynamometer fixed between the chair and the leg measures force of the quadriceps m. during electrical

Patient status								
	Time of paralysis to biopsy	**Biopsy No.**	**Age**	**Sex**	**Height**	**Weight**	**Level of lesion**	**Cause of paraplegia**
	years		**years**		**cm**	**kg**		
Short term group:								
S.T. 1	0,9	F 12	43	F	172	55	Th 7	Car accident
S.T. 2	0,9	F 10	26	M	180	68	Th 4	Car accident
Mid term group:								
M.T. 1	2,0	F 02	48	M	181	86	Th 6-7	Accident with bobsleigh
M.T. 2	2,4	F 01	28	M	170	80	Th 4-5	Motorcycle accident
Long term group:								
l.T. 1	17,0	F 06	39	M	180	62	Th 4-5	Motorcycle accident
l.T. 2	20,3	F 09	40	M	178	70	Th 5-6	Motorcycle accident

Table 1. Demographic data of the short, mid and long term paraplegic subjects. All subjects had traumatic spinal cord injury with complete paralysis of the upper limbs

force of the quadriceps m. during electrical stimulation. Force of the thigh muscles was measured as extension torque and expressed in Nm, using increasing stimulation amplitudes from 0 to 120 Vpp in 10 V steps.

Human Muscle Biopsy

Through a small skin incision (6 mm diameter), needle muscle biopsies were taken from the right and left vastus lateralis muscle at a single time point for each patient, as described in [16]. The resulting specimens were then prepared for light and electron microscopy and analyzed.

Hematoxilin-eosin

Cryosections (10 µm thick) of frozen biopsies were stained with hematoxilin-eosin (H&E), using conventional techniques. Morphometric analysis. Images were acquired using a Zeiss microscope connected to a Leica DC 300F camera at low magnitude under the same conditions that were used to acquire a reference ruler. Morphometric analysis reported in Table 3 was performed with Scion Image for Windows version Beta 4.0.2 (by 2000 Scion Corporation), free software downloaded from the web site: www.scioncorp.com, as decribed in [16].

Immunohistochemistry

Cryosections were labeled with antibodies anti-N-CAM (from Chemicon International, diluted 1:200) for 1 hour at room temperature. (van der Meulen et al., 2003). The slides were then washed twice with TBS (5 min each) and incubated with FITC-conjugated anti-mouse Ig (from Sigma, F-2266 diluted 1:200) for 1h at room temperature. This was followed by a second 5 minute washing of the slides with TBS and nuclei counter-stained by Hoechst 33258. In the negative controls, the primary antibody was omitted.

Preparation of muscle specimens for transmission EM

The samples obtained via biopsies were fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 2h followed by buffer rinse and fixation for 1h in 1% osmium tetroxide. The specimens were dehydrated in a graded series of ethanol solutions and embedded in epoxy resin. Ultrathin sections (about 30-40 nm) were cut in Leica Ultracut R (Leica Microsystem, Austria) using a Diatome diamond knife (DiatomeLtd. CH-2501 Biel, Switzerland) and stained in 4% uranyl acetate and lead citrate. Sections were examined with a FP 505 Morgagni Series 268D electron microscope (Philips), equipped with Megaview III digital camera and Soft Imaging System (Germany).

Classification of muscle fibers by ultrastructural criteria

Muscle fibers were analysed from the qualitative point of view observing every single fiber at low, intermediate, and high magnification. For every fiber we analysed over the whole visible fiber interior before classifying it in one of the categories included in Table 4. Muscle fibers were classified according to the following parameters: a) organization of the contractile apparatus, i.e. clear cross striation visible in the all fiber interior; these

Cross Sectional Area and density of *quadriceps m.*				Force at 120Vss
		Area	Density	Force
		cm²	HU	Nm
Short term group:				
S.T. 1	left	33,21	41,00	27,00
	right	32,71	39,00	26,90
S.T. 2	left	40,70	52,00	37,60
	right	40,46	51,00	34,60
mean		**36,77**	**45,75**	**31,53**
SD		**4,41**	**6,70**	**5,42**
Mid term group:				
M.T. 1	left	26,66	36,00	7,70
	right	26,50	33,00	3,40
M.T. 2	left	47,85	43,81	19,20
	right	47,52	42,17	25,00
mean		**37,13**	**38,74**	**13,82**
SD		**12,19**	**5,10**	**10,00**
Long term group:				
L.T. 1	left	29,79	36,00	18,30
	right	29,77	37,00	20,00
L.T. 2	left	36,61	39,00	17,50
	right	37,17	38,00	21,10
mean		**33,34**	**37,50**	**19,23**
SD		**4,11**	**1,29**	**1,63**

Table 2. CT Scan and knee extension torque of quadriceps m. in short-, mid- and long-term upper motoneuron complete SCI. Results show that cross sectional area and quality of the muscles are very similar in left and right legs of all the patients. Furthermore no significant decrease of CT Scan values is present comparing all groups. Force of the quadriceps m. was assessed during stimulation-induced isometric knee extension. The inter-leg values are in reasonable agreement. Significant changes are present comparing short- vs mid- and long-term groups (t- student short vs mid p= 0.03; short vs long p=0.02). No significant difference are present mid- vs long-term. On the other hands the opposite results is obtained comparing mean fiber size (see table 3)

fibers were classified as striated fiber (Table 4, column B) or b) presence of disorganization of the contractile apparatus, thickening of the Z line, disarrangement of triads, mitochondria grouping, etc. (degeneration). Fibers presenting degeneration traits were classified as severely atrophic fibers (Table 4, column C). Figures were mounted and labeled using Adobe Photoshop® v7.0.

Statistical analysis.

Student's t-test was used to compare results. A p value less than 0.05 is considered significant. Laboratory data are expressed as mean + SD of four values in each group.

Results

CT-scan

Examination of patients by CT scan demonstrates minimal differences in thigh muscles between the three

Mean Muscle Fiber Diameter			
		diameter	SD
Short term group:			
S.T. 1	left	28,20	10,00
	right	26,30	14,60
S.T. 2	left	35,00	16,00
	right	38,40	15,40
Mean diameter		**30,59**	**14,39**
Mid term group:			
M.T. 1	left	22,08	8,90
	right	23,30	15,20
M.T. 2	left	30,80	23,40
	right	28,30	20,90
Mean diameter		**30,75**	**23,55**
Long term group:			
L.T. 1	left	48,50	18,00
	right	35,60	15,40
L.T. 2	left	38,90	17,60
	right	41,50	15,30
Mean diameter		**40,42**	**17,01**

Table 3. Minimum transverse diameter of myofibers of vastus lateralis muscle in short- mid- and long-term upper motoneuron complete SCI. Size of myofibers are very similar in left and right legs of all the patients. The increased diameter of the long term group is statistically significant vs both short and mid term groups. No statistically significant difference is present between short and mid term groups.

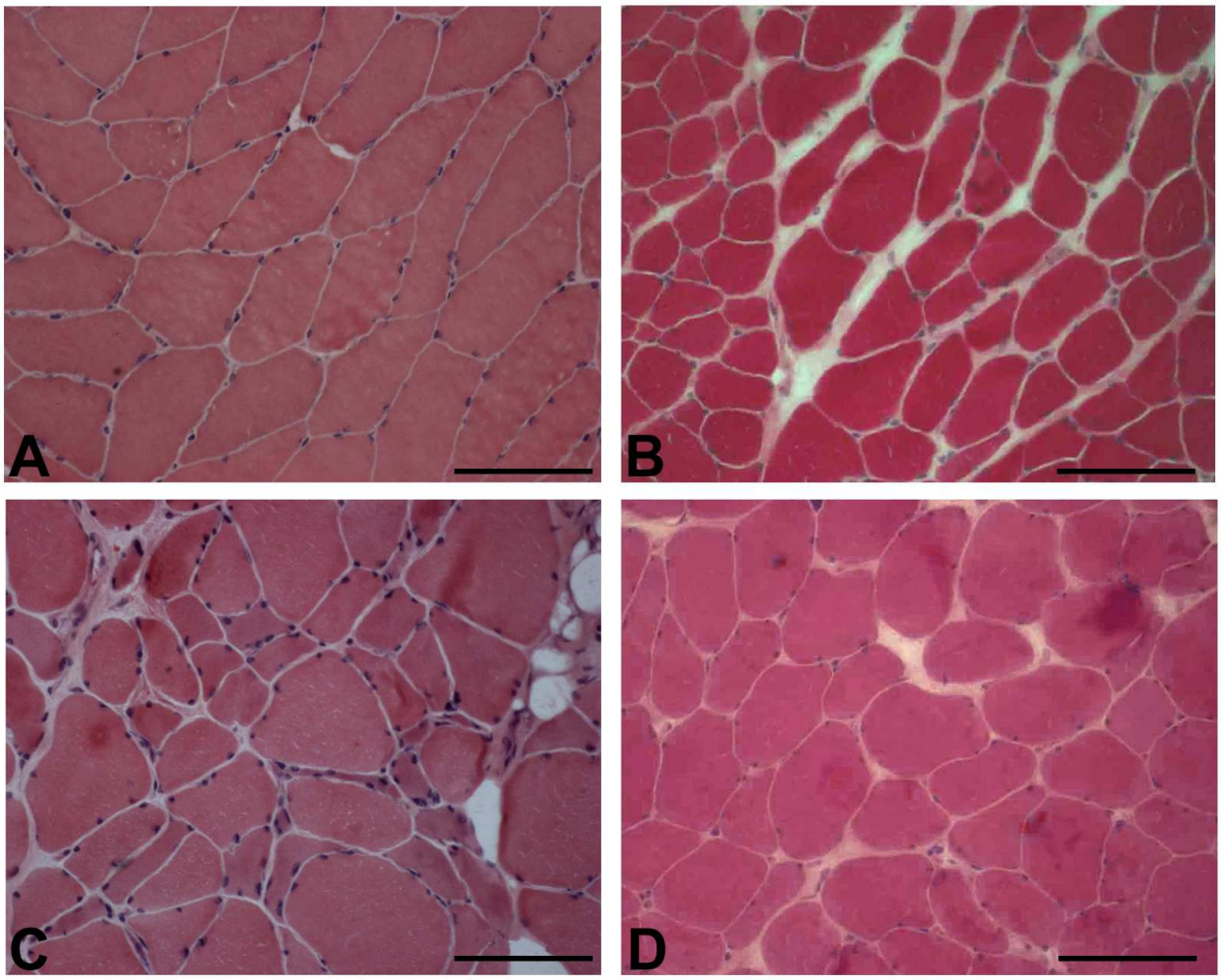

Figure 1. Morphological appearance of normal, and short- mid- and long-term paralyzed muscles tissue in cross sections H-E Stained. A, Normal adult human muscle; B, Short-term, C, Mid-term and D, Long-term paralyzed muscle with upper motor neuron lesion. Myofiber profiles in normal muscle are separated by minimal interstitial tissue, fibers have a large diameter and appear closely packed. In short- mid- and long-term groups the interstitial space between fibers is slightly enlarged. Fiber size is also more variable than in normal muscle with some fibers appearing unusually large and a small percentage of them presenting an extremely small diameters. Bars: 100µm

groups of patients, both for the cross sectional area and for the mean tissue density. In the short-term group the average cross sectional area of quadriceps m. measured at 20 cm proximal to the knee joint /distal of the top of the trochanter major is 36.77 ± 4.41 cm², (with a mean tissue density of 45.75 ± 6.70 HU) whereas in mid term paralyzed patients the cross sectional area is 37.13 ± 12.19 cm² (mean tissue density of 38.74 ± 5.10 HU) and in long term paralyzed patients the cross sectional area is 33.34 ± 4.11 cm² (mean tissue density of 37.50 ± 1.29 HU). Results of CT examination are reported in detail in Table 2. Cross sectional area and quality of the muscles are very similar in the left and right legs of all the patients. No significant changes are present comparing short- mid- and long-term groups.

Force-measurement

We assessed the knee extension torque while activating the quadriceps m. by electrical stimulation to evaluate the thigh muscle function. The short-term paralysed group demonstrated a torque of 31.53 ± 5.42 Nm during stimulation-induced isometric knee extension. On the other hand, mid-term paralysed lower limbs had a mean torque of 13.82 ± 10.00 Nm and long-term paralysed lower limbs had a mean torque of 19.23 ± 1.63 Nm (Table 3). Significant changes are present comparing short- vs long- and mid- vs long-term group. But the relevance of the relations are doubtful, due to the inference of the antagonist muscles in the measurements.

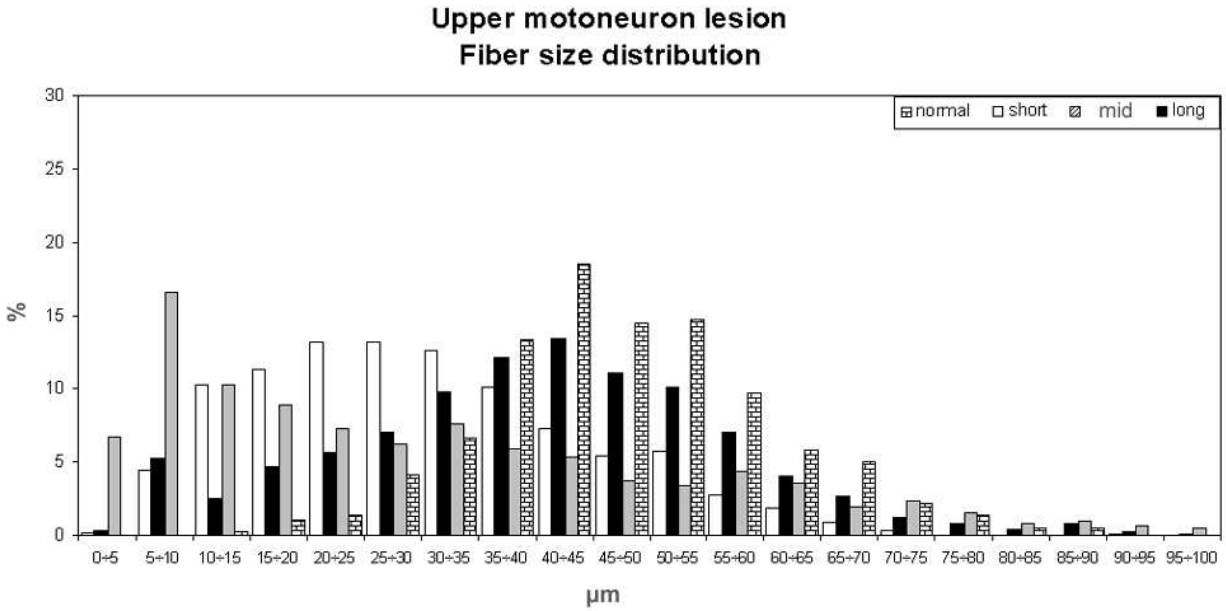

Figure 2. The cumulative diameter spectra of normal adult human muscle, short term (empty bar), mid (gray bar) and of the long term (black bar) groups. The short and mid term groups contain a larger percentage than long term group of myofibers with diameter in the range of 10 – 20 µm. Note that short term group has the Gaussian monomodal distribution of normal muscle fibers, while in mid- and long-term groups two population of myofibers are present (maximum at either 10 and 30 or 40 µm)

Histology

Figure 1 shows Hematoxilin-Eosin staining of transverse section of a normal human muscle with its well packed myofiber profiles of slightly different sizes and minimal interstitial tissue among them (Panel A). In panels B, C and D biopsies from representative short- mid- and long-term upper motor neuron lesion patients are shown. In all biopsies, the majority of muscle fibers present large and round profiles, with only a minority of them being small diameters and presenting the features of severe muscle atrophy. Interstitial spaces between myofibers are slightly enlarged in all the three groups in comparison to normal muscle.

The percentage of tissue types within the muscle biopsies of short-term paralysed subjects is: muscle fibers: 61.96 ± 8.49%; interstitial tissue: 31.98 ± 3.45%, in mid-term paralyzed thigh muscles is: muscle fibers: 65.11 ± 11.95%; interstitial tissue: 28.56 ± 10.30%, while in long-term paralyzed thigh muscles is: muscle fibers: 75.75 ± 6.27%; interstitial tissue: 21.00 ± 6.17%. The differences between the groups are not statistically significant. The percentage of interstitial tissue is significantly increased in all the three (short, mid and long term) groups of paralysed subjects in comparison to normal muscle ($p < 0.001$).

Plotting the fiber size spectra in an histogram allows to compare directly short-, mid- and long-term groups. The average diameter of the myofibers is 30.59 ± 14.39 µm (mean ± SD), 30.75 ± 23.55 and 40.42 ± 17.01 µm in short- mid- and long-term samples, respectively. The increase in mean fiber diameter in long-term patients may be explained with a lower number of fibers with smaller diameter. In fact, 26.38% and 40.64% of fibers in the short- and mid-term samples respectively present a fiber diameter smaller than 20 µm, whereas in the long-term groups this percentage is 12.93%. In the long term group it is more evident that two population of myofibers are present: very small myofibers of 10 mm median diameter and larger slightly atrophied myofibers

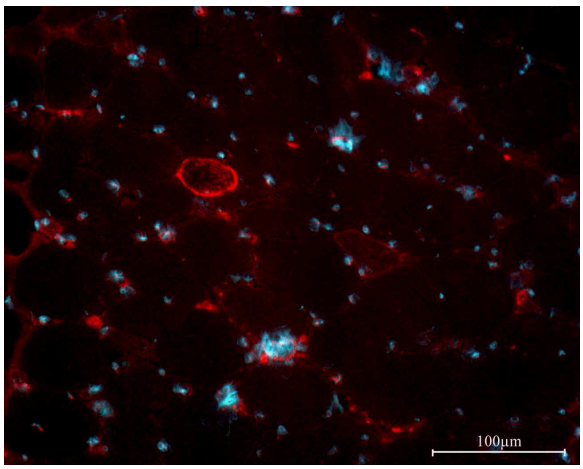

Figure 3. Denervated myofibers (red profiles) of different size and stain intensity are present in this 14-year upper motoneuron-complete paraplegic muscle after anti-NCAM monoclonal labeling. Blue nuclei are stained with Hoechst 33258. Bars: 100µm.

(median diameter 40 - 45 mm).

At least some of the small fibers are probably those that lost contact with the lower motoneuron, as suggested by their labeling with an anti-N-CAM antibody, a sound marker of myofiber peripheral denervation [3,8,15,26, 30]. Figure 3 shows that both very small and medium size myofibers are N-CAM positive.

Ultrastructural analysis of biopsies from long-term spastic patients

The most obvious feature found at the electron microscope analysis (Fig. 4, A) is the fact that most fibers in the biopsy from 17-year upper motor neuron lesion patients are quite large, confirming the histological observation (see Fig. 1, C). In addition, the EM analysis of

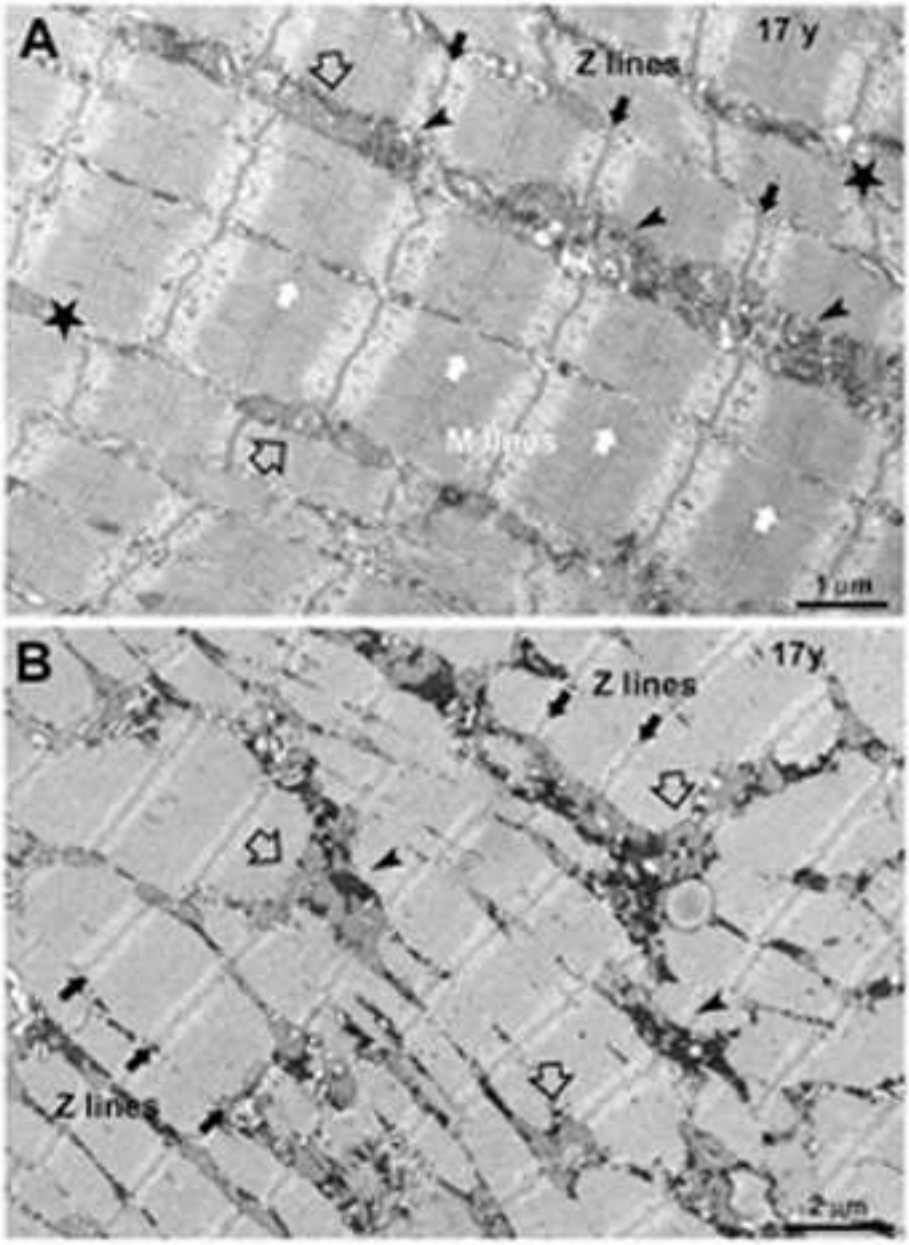

Figure4. Electron micrograph of longitudinal sections from vastus lateralis muscle fibers of spastic patient. Most of the analyzed muscle fibers in long-term spastic patients present an ultrastructure such as the one presented in A and B (77% or more, see Table 4). While these fibers are not as orderly as fibers in normal human muscle[4], their interior is filled with fairly well organized myofibrils that often are even well aligned with one another (A, black and white arrows points respectively at Z and M lines). In other fibers, or even in regions within the same fiber, there are areas in which myofibrils are a bit more disordered (B). Arrowheads and empty arrows in both panels points respectively at glycogen granules and mitochondria. Mitochondria are often misplaced and/or grouped in an abnormal fashion in these fibers.

longitudinal sections at higher magnification allows to

appreciate a second important feature: fiber interior presents the clear pale dark cross striation characteristic of skeletal muscle tissue. This observation indicates that the muscle disuse due to upper motor neuron injury did not induced the disarrangement of the contractile apparatus even 17 years after SCI. The EM analysis of the large and striated fibers indicates that, even if the ultra structure of myofibrils is quite well preserved (Fig. 4 A and B), there are features that differs from normal skeletal muscle fibers of humans [4]. The myofibrillar apparatus is very orderly and quite similar to that of normal muscle in some areas, whereas in others regions of the same fiber (and/or in other fibers) myofibrils are slightly less orderly (Fig. 4 B). In addition, even in those areas presenting a higher degree of organization, such as the one in Fig. 4 A, the fibers appear to be slightly less orderly that those of normally innervated muscle tissue. In fact, in normal muscle fibers, myofibrils are typically in register with one another in a way that Z and M lines of adjacent myofibrils are well aligned. This precise alignment is often lost in the samples analyzed in the present paper (Fig. 4 A, asterisk). The misalignment of the contractile apparatus is usually accompanied by the formation of abnormal areas filled with glycogen granules, mitochondria (empty arrows), and vesicles (Fig. 4, arrowheads). Mitochondria loses their specific association to the I band and are found often grouped in an abnormal fashion just below the sarcolemma (not shown) or between myofibrils (empty arrows), often forming longitudinally oriented columns. Misplacement of mitochondria is accompanied by significant alterations of the sarcotubular apparatus.

Electron microscopy			
	A	B	C
Patients	Analysed fibers	Striated fibers	Severely atrophic fibers
L.T.1	13	10 (77%)	3 (23%)
L.T.2	19	15 (79%)	4 (21%)

Table 4. Electron microscopy. In each patient at least 13 fibers were closely analyzed at the electron-microscopical level (column A). The majority of fibers (77% or more) presents a myoplasm filled with contractile material organized in myofibrils and sarcomeres (column B, see also Figure 5). Some fibers presenting classic features of denervation (i.e. disgregation of the myofibrillar structure) are present in these patients (4 to 23%, column C), and resemble fibers of patients that have lost their peripheral nerve endings [4,16].

Discussion

Over the recent years the interest of using FES to restore mobility of paralyzed patients had ups and downs [8,13,16,17,19,22,25]. Our recent results in the case of *conus cauda* complete injury are reopening the issue for both lower and upper motoneuron paraplegics. We shown that human skeletal muscle tissue undergoes three phases during long-term lower motor neuron denervation: i) Atrophy (up to 18 months post-injury); ii) Severe atrophy (2 – 5 year post-injury); iii) Lipodystrophy and Fibrosis (after 3-year post-injury) and that a new designed FES training substantially delays and reverses the severe atrophy of long-term denervated human muscles up to a level that allows the subjects to rise and to stand up [5,6,16,20,23].

Subjects with spinal cord injury at the level of T3/4 – T11 develop a so called spastic paraplegia. This means that the afferent and efferent nerve of the thigh muscles remain intact, but independent from the central control. The condition of the musculature in spastic paralyzed people (i.e., with an upper motor neuron lesion) shows overall clearly reduced values (40-50% in nearly all parameters) in comparison to the able-bodied control. These changes appear within 6 to 12 months reaching a steady state after 1 to 1.5 years (atrophic but relatively stable). The progression of the disuse atrophy is very fast at the onset, but slows down with increasing duration of the spastic paralysis. Within the first month the thickness of the muscle bulk measured by ultrasound decrease up to 40% [29]. It is reasonable to indicate this period (up to 3 months) the "early phase" of disuse paraplegia. Then a 50% steady-state atrophy in spastic incomplete paralysis is well documented up to two years after SCI. This time span up to one year is here labeled as the "short term" period [1,2,7,10,11,12,21,27], while the intermedial two patients (1.6 to 3.0 years, in this study) is labeled mid-term group. Information on the following periods, in particular later than 15 year post-SCI (to which pertains our "long-term" subjects) are poor, at best [24].

Within this study we compared short, mid and long term paraplegics with complete upper motor neuron lesions. The aim of this study was to test whether the steady-state muscle atrophy extends to the long term period of paralysis or there is a correlation between time after SCI and extent of muscle atrophy/dystrophy.

Despite the enormous difference in time of palsy, with the exception of force measurement, which are biased by co-contraction of antagonists muscles, the measured parameters did not show any statistically significant difference between the three groups. In some instances the results appear to be paradoxically better in the long-term group. In spite of non significant decreased cross area and density of CT scan and of the stimulation-induced isometric knee ex-tension force, the mean muscle fibre diameters is 30.59 ± 14.39 μm (mean ± SD) in the short-term, 30.75 ± 23.55 μm in the mid term and 40.42 ± 17.1 μm in the long term groups. As shown by the cumulative myofiber size spectrum (figure 2), this small increase in myofiber size in the long term patients is the results of a decreased content of small-size myofibers (diameter less than 20 mm). Labeling with an anti-

NCAM antibody and the electron microscopy characteristics suggest that the small fibers lost contact with their lower motoneuron and therefore were completely inactive since many years. Whether their decreased presence in the long term group is just the consequence of the low number of patient studied or is related to apoptosis/necrosis of lower motoneuron denervated myofibers or to long term reinnervation events remain to be determined. Anyhow, the three groups of paralysed leg muscles showed nearly an identical tissue distribution. There was only a very slight increase in the percentage of fat and decrease of loose connective tissue, both not statistically significant. Very long time of paralyses seems not have any significant influence on the condition of the musculature at the light microscopy level in muscle biopsies collected from 1 to 20 year post SCI. We have previously shown (16; Boncompagni et al., submitted for publication) how in patients affected by lower motor neuron lesion (no peripheral nerves) cross striation is severely disrupted and then followed by severe atrophy and finally fiber degeneration 3 – 5 years after SCI. On the other hand, in the patients selected for this study, which still have intact peripheral nerves, the pale-dark cross striation is present even tens of years after the injury (14-20 years) in the majority of the fibers analyzed (see Table 4, column B). Seldom fibers presenting classic denervation features, similar to those of lower motoneuron lesion patients, are present (Table 4, column C). The presence of these atrophic fibers, i.e. fibers that likely have lost their innervation, represent an important internal control that allows us to identify clearly which are the innervated fibers.

On the other hand, ultra structural analysis shows that in contrast to the normal muscle fibers, whose myofibrils are typically in register with one another in a way that Z and M lines of adjacent myofibrils are well aligned, this precise alignment is often lost in the samples analyzed in the present paper. This misalignment of the contractile apparatus is usually accompanied by the formation of abnormal areas filled with glycogen granules, mitochondria and vesicles. Misplacement of mitochondria, accompanied by significant alterations of the sarcotubular apparatus may explain the poor function of spastic muscles, that are weak and fatigable (endurance is poor) in spite of their large muscle bulk.

Therefore, FES for improving muscle trophism, blood circulation, body metabolism can be started at any time after palsy. People with long-term lower limb paralyses should consider functional electrical stimulation as a promising therapy, for improvement of the cardiovascular performances, for the amelioration of the body trophic conditions and for prevention of decubitus ulcers.

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