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Muscle biopsies show that FES of denervated muscles reverses human muscle degeneration from permanent spinal motoneuron lesion

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Abstract—This paper presents biopsy analyses in support of the clinical evidence of muscle recovery induced by a new system of life-long functional-electrical-stimulation (FES) training in permanent spinal-motoneuron-denervated human muscle. Not earlier than 1 year after subjects experienced complete *conus cauda* lesion, their thigh muscles were electrically stimulated at home for several years with large skin surface electrodes and an expressly designed stimulator that delivered much longer impulses than those presently available for clinical use. The poor excitability of long-term denervated muscles was first improved by several months of twitch-contraction training. Then, the muscles were tetanically stimulated against progressively increased loads. Needle biopsies of *vastus lateralis* from long-term denervated subjects showed severe myofiber atrophy or lipodystrophy beginning 2 years after spinal cord injury (SCI). Muscle biopsies from a group of 3.6- to 13.5-year denervated subjects, who underwent 2.4 to 9.3 years of FES, show that this progressive training almost reverted long-term muscle atrophy/degeneration.

Key words: FES, functional electrical stimulation, human, long-duration impulses, long-term denervation, muscle, muscle degeneration, muscle recovery, myofiber regeneration, permanent denervation, spinal cord injury, spinal motoneuron lesion.

INTRODUCTION

Spinal cord injury (SCI) causes a rapid loss of muscle mass, which is especially severe when the injury involves spinal motoneurons. While much interest has been shown in the use of functional electrical stimulation (FES) to restore movement of the limbs of subjects paralyzed by upper motoneuron lesions [1–2], very few clinicians hope to recover permanent spinal-motoneuron-denervated muscles by means of electrical stimulation training. Indeed, atrophy is especially severe when the injury involves spinal motoneurons, in which long-term

Abbreviations: BMCA = Brain Motor Control Assessment, CT = computerized tomography, DDM = denervated and degenerated muscle, EMG = electromyography, EU = European Union, FES = functional electrical stimulation, H-E = hematoxylin-eosin, LMS = lumbosacral magnetic stimulation, SCI = spinal cord injury, TMS = transcranial magnetic stimulation.

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irreversible denervation results in fat substitution and muscle fibrosis. Although early denervation has been widely studied both in animal models and humans, the long-term effects of denervation have attracted much less attention since the general belief is that all myofibers disappear within several months of denervation [3–5]. In rats, myofibers exhibit a net loss of nuclear domains for 7 months, followed by nuclear groupings, a specific morphologic marker of long-term severe muscle atrophy [6]. Furthermore, permanent spinal motoneuron denervation has also demonstrated to be accompanied by a continuous production of new myofibers [7–12]. Activated satellite cells, myotubes, and regenerated myofibers are consistently present in atrophic rat muscles, even after year-long permanent denervation in both hemidiaphragm and leg muscles [7–9]. Interestingly, we have recently extended these observations to human muscle [13]. All these events can prolong the period during which denervated tissue may be recovered through reinnervation or FES training.

In permanent, complete *conus cauda* lesions, SCI results in irreversible loss of the nerve supply to some or all the muscles of the affected limbs, making the marked atrophy of the denervated muscle and the severe secondary medical problems of skin and bone more difficult to treat successfully (e.g., pressure sores and bone demineralization and fracture). Without functional motor nerve fibers, activating a sufficient population of myofibers is more problematic when electric currents are provided by surface electrodes; 6 months after SCI, long-term spinal-motoneuron-denervated muscle is poorly excitable by standard clinically approved electrodes and stimulation devices. Despite these difficulties, pilot studies on FES of long-term human denervated and degenerated muscles (DDM) have been published [5]. Especially encouraging are some of our previous results, which have shown that a new electrical-stimulation training strategy is effective in restoring a certain degree of muscle mass and force production even after long-standing complete spinal-motoneuron lesions [14–17]. These clinical works strongly supported the idea that FES training of denervated muscles could restore DDM, a fact that had not previously been recognized, principally because of the lack of customized technology that can meet demands completely different from those required for motor-nerve stimulation. On the other hand, a stimulator delivering biphasic long-duration impulses with a pulse width between 10 ms and 200 ms and amplitudes of up to ±80 V and ±200 mA is able to elicit DDM twitch contractions via surface electrodes [16–17]. Within a few months, such training increases myofiber excitability of the quadriceps muscle to a level that allows tetanic contractions. Finally, the structural and metabolic characteristics of the tetanically stimulated muscles are restored to values that allow electrically supported standing up and standing. Recently, clinical results by computerized tomography (CT) scan and force measurements during tetanic stimulation of the thigh muscles were promising enough to convince ethical committees and funding agencies to accept our proposal for a European Union (EU) Trial ("RISE") for restoration of DDM in *conus cauda* syndrome by FES training. With the support of the EU RISE project, we are using light and electron microscopy to analyze in detail biopsies of human long-term DDM before and after FES.

We present the preliminary results of morphometric analyses of DDM in a group of subjects enrolled in the RISE Trial, who were biopsied before FES. We compare these subjects with a previous group of subjects who had undergone FES training of denervated muscles and were biopsied after 2.4 to 9.3 years of FES training that was initiated between 1.2 and 8.7 years after SCI. Our purpose was to present biopsy analyses in support of the clinical evidence of muscle recovery induced by the new life-long FES training in permanent spinal-motoneuron-denervated human muscle. Microscopic muscle analyses from these two independent groups of subjects support our previous clinical observations [14–15,18]. Besides recovery of the atrophic muscle fibers, sustained regeneration of new myofibers may explain the observations that in the FES-treated muscle, the fibers are larger than the fibers in untreated muscles of the group of DDM subjects from the EU RISE Trial [19].

MATERIALS AND METHODS

Characteristics of Subjects

Nine subjects (one female and eight male, aged 20 to 49), who had experienced traumatic *conus cauda* lesions, were assigned to two groups. The first group included five DDM subjects who were recently enrolled in the EU RISE Trial and were biopsied pre-FES training. Although pre- and post-FES-training biopsies of the RISE subjects were approved by ethical committees and accepted by subjects, the follow-up was too brief for us to perform posttraining bioptic analyses on this group (**Table 1**).

The second group of four subjects included the first and only worldwide analyses of spinal-motoneuron-denervated FES-trained subjects who demonstrated clear clinical signs of *quadriceps femoris* muscle mass and force improvements after several years of daily training (see "Training Strategy"). We assessed complete spinal motoneuron denervation of the quadriceps muscle by electrophysiological testing, i.e., by chronaxie measurements, needle electromyography (EMG), Brain Motor Control Assessment (BMCA), and transcranial and lumbosacral magnetic stimulation (TMS and LMS, respectively). Rheobase and chronaxie measurements [20–22] were performed with the use of a constant-current stimulator and two touch electrode probes placed successively to three different sites of the quadriceps muscle. Chronaxie values, which in normal innervated muscles are 0.3 to 0.7 ms, were over 20 to 30 ms in the denervated group. Excitability of the FES-trained denervated muscles increased (chronaxie of 5 to 10 ms), but never reached normal values.

Needle EMG of the quadriceps muscle was according to Dumitri and Zwarts [23]. The needle was inserted into the *m. rectus femoris*, which we examined at different depths and in four directions. When necessary, we performed additional needle EMG analyses in the *vastus lateralis* and *medialis* muscles. We looked for insertion activity, spontaneous activity during muscle relaxation (fibrillation potentials, positive sharp waves, and fasciculations), and volitional activity. Needle EMG examination was completed by the stimulation of the femoral nerve at the groin with a supramaximal single pulse to elicit an M-wave. In both denervated and FES-trained denervated muscles, no EMG responses occurred during volitional activation.

We conducted BMCA to rule out any voluntary or reflex innervation of the quadriceps muscle. The BMCA protocol is a comprehensive multichannel-surface EMG recording of the limb muscles used to characterize motor-control features in persons with upper and/or spinal motoneuron dysfunction [24]. Key information is contained in the overall temporal pattern of motor-unit activity, observed in the EMG envelope. In both denervated and FES-trained denervated muscles, no motor outputs were recorded during the entire BMCA protocol.

We recorded stimulus-induced activities of the limb muscles with multichannel surface EMG according to Dimitrijevic et al. [25]. Transcranial stimulation was conducted with a double-cone coil (MAGSTIM 200, Magstim Company Ltd, Wales, UK) placed over the medial caput. We applied lumbosacral stimulation at vertebral levels T12, L2, and L4 with the use of a circular coil. We increased the amplitude of the magnetic stimulation from 0.4 T to 4 T in steps of 10 percent. In both the denervated and the FES-trained denervated muscle groups reported here, no responses occurred to either TMS or LMS. In the few cases in which LMS showed some incomplete spinal-motoneuron denervation, the results of the microscopy of the quadriceps muscle were not included in this paper.

Table 1.
Time intervals between spinal cord injury (SCI) and muscle biopsy, between SCI and onset of func
tional electrical stimulation (FES), and between start of FES and biopsy in long-term spinal-motoneuron denervation of human *vastus lateralis* muscle; biopsies taken uni- or bilaterally.

Subject	Age/Sex	Time Intervals (yr) Between		
		SCI and Muscle Biopsy	SCI and FESdm Onset	FESdm Onset and Biopsy
Den1&2	20/Male	0.8	—	—
Den3	37/Male	1.3	—	—
Den4&5	45/Female	2.9	—	—
Den6	49/Male	3.3	—	—
Den7&8	33/Male	19.0	—	—
FESdm1&2	48/Male	3.6	1.2	2.4
FESdm3&4	42/Male	6.3	2.0	4.3
FESdm5&6	35/Male	10.6	1.3	9.3
FESdm7	30/Male	13.5	8.7	4.8

Den = denervated muscle/myofibers, FESdm = functional electrical stimulation of denervated muscle.

Training Strategy

The contractile response of denervated muscle to electrical stimulation depends on the stage of postdenervation muscle atrophy/degeneration, which in turn depends on the time between the denervation event and the onset of stimulation [15,18,21]. In subjects who had been injured for not less than 1 year, we applied biphasic stimulation impulses of very long duration and high intensity at the beginning of treatment [16]. We then adjusted the stimulation parameters according to the increasing excitability induced by FES training. In short, the several-year-long stimulation strategy may be divided in four phases (see "Phases" 1 to 4). A physiotherapist evaluated subjects every 6 to 8 weeks and progressively modified the stimulation parameters to be used at home 5 days a week. The subjects themselves applied the electrode above their thigh muscles, always in the same positions, and activated the preprogrammed stimulator. The surface electrodes made of conductive silicone rubber were applied directly to the skin via a wet sponge cloth (early training) or gel (later on, when their skin had adapted to the high electrical current). Flexible electrodes that fit closely to uneven skin surface provided homogeneous distribution of the electrical field in the stimulated thighs. Every 9 to 12 months, we clinically assessed the subjects.

Phase 1: Early Twitch Stimulation

At least 1.2 years after SCI (and after appropriate instructions to avoid skin burns related to the high current levels to be used), the subjects began a home training program. Anatomically shaped electrodes of a large surface area were strapped to the anterior aspect of subjects' thighs in proximal and distal positions. First, we assessed the severely reduced excitability of long-term denervated myofibers (long-term complete spinal-motoneuron denervation) by delivering very long biphasic rectangular impulses, which, however, yielded only twitch contractions of the thigh muscles. To activate fibers throughout the quadriceps femoris muscles in complete *conus cauda* syndrome, we delivered biphasic rectangular current pulses of 150 to 200 ms duration, up to ±200 mA amplitude, representing an impulse energy of up to 3.2 J. Since no stimulator on the market could deliver such a high current intensity, we developed a generator of long, high-strength stimuli [16–17]. Training was initiated with stimulation at 2 Hz, and delivered for 15 min per day (a series of 4 s "on," 2 s "off"), 5 days a week. The duration of these impulses was approximately 1,500 times longer than that of the impulses used in subjects with upper motoneuron lesions. With an interpulse interval of about 400 ms, the resulting stimulation frequency was slightly less than 2 Hz (single twitches elicited every half second). During the next few months, the progressively increasing muscle excitability permitted an increase of the twitch stimulation to series of 5 s "on," 1 s "off," 3 to 5 min of stimulation with 1 to 2 min of rest.

Phase 2: Late Twitch Stimulation

As the training proceeded, muscle excitability continued to increase. Thus, during the successive 3 months of training, the pulses could be shortened to 80 to 100 ms. After an additional 1 to 2 months, the protocol was changed to the following tetanic pattern (Phases 3 and 4).

Phase 3: Burst Stimulation for Long-Term Spinal-Motoneuron-Denervated Muscles

The tetanic pattern for DDM [14–16,18] consisted of a 40 ms pulse and a 10 ms pause delivered at 20 Hz for 2 s "on," 2 s "off," 3 to 5 min of stimulation with 1 min of rest (i.e., 3 to 5 times a session), twice a day, 5 days a week.

Phase 4: Force/Endurance Stimulation

Between the 9th and 12th month of FES training of denervated muscles, force-training sessions were introduced by tetanic contractions with 70 to 80 percent of maximum load, 8 to 12 repetitions, 4 to 6 sets, with 2 min of rest, twice a week. At first, the leg contracted to full knee extension without any ankle weight, and later, with an ankle weight of up to 5 kg, in 0.5 kg steps.

With this progressive FES training, the mass and force of thigh muscles increased to values that allowed electrical-stimulation-supported standing up and standing exercise [14–16,18].

Analyses of Human Muscle Biopsy

Using Bergström needles (UNIMED 5.00 × 100 mm, Ref. No. 23.601 500100), we biopsied the right and left *vastus lateralis* muscle through a small skin area (at the times stated in **Table 1**). We stained cryosections of frozen biopsies with hematoxylin-eosin (H-E). The details of fiber morphometry are reported by Rossini et al. [13]. In brief, we collected three 10 µm-thick sections on glass slides and stained them with H-E with the use of conventional techniques. We acquired images with a Zeiss microscope connected to a Leica DC 300F camera at low magnification under the same conditions that we used to acquire a reference ruler. The minimum transverse diameter of each

myofiber was measured against the reference ruler. We grouped the myofibers and plotted the relative percentile in 10 μm steps. We performed morphometric analysis with Scion Image for Windows, version Beta 4.0.2 (by 2000 Scion Corporation).*

RESULTS

Light Microscopy of Human Long-Term Denervated Muscle

Denervation of skeletal muscle causes rapid loss in contractile force, which is accompanied by several structural, biochemical, and physiological changes. Despite the severe atrophy of the thigh, **Figure 1** surprisingly displays that 1-year spinal-motoneuron-denervated human *vastus lateralis* muscles show more than 70 percent of the cryosection area being covered by atrophic myofiber profiles (**Figure 1(a)** and **(b)**). Only 3 years post-SCI, the biopsies show the expected degeneration of muscle tissue. Indeed, fat and loose connective tissue areas prevail on the myofiber area (**Figure 1(c)** and **(d)**). The microscope images on which **Figure 1** is based show that up to 1 year after SCI, the denervated myofibers underwent pure atrophy, while adipocytes and collagen sheets are scanty (**Figure 2(a)–(b)** and **(d)–(e)**, respectively; compare in the inset of **Figure 2(b)** the fiber size of normal human muscle). These results are unattended, at least to our knowledge. The severe lipodystrophy of lower motoneuron-denervated human muscles is shown 3.3 years and 19.0 years after SCI, as shown in **Figure 2(g)–(h)**, and **(j)–(k)**, respectively: Here, fat and/or loose and fibrous connective tissues prevail over the atrophic myofibers (3- to 19-year denervation percentile myofiber area decreases to 18.9 mean ± 9.9 standard deviation [SD] versus 76.1 ± 8.6 at 1-year denervation, $p < 0.001$, by student's *t*-test for independent groups). On the other hand, **Table 2** shows that the cumulative value for eight biopsies of denervated muscle minimum diameter is 16.8 ± 4.5 μm without significant differences between early and late denervation.

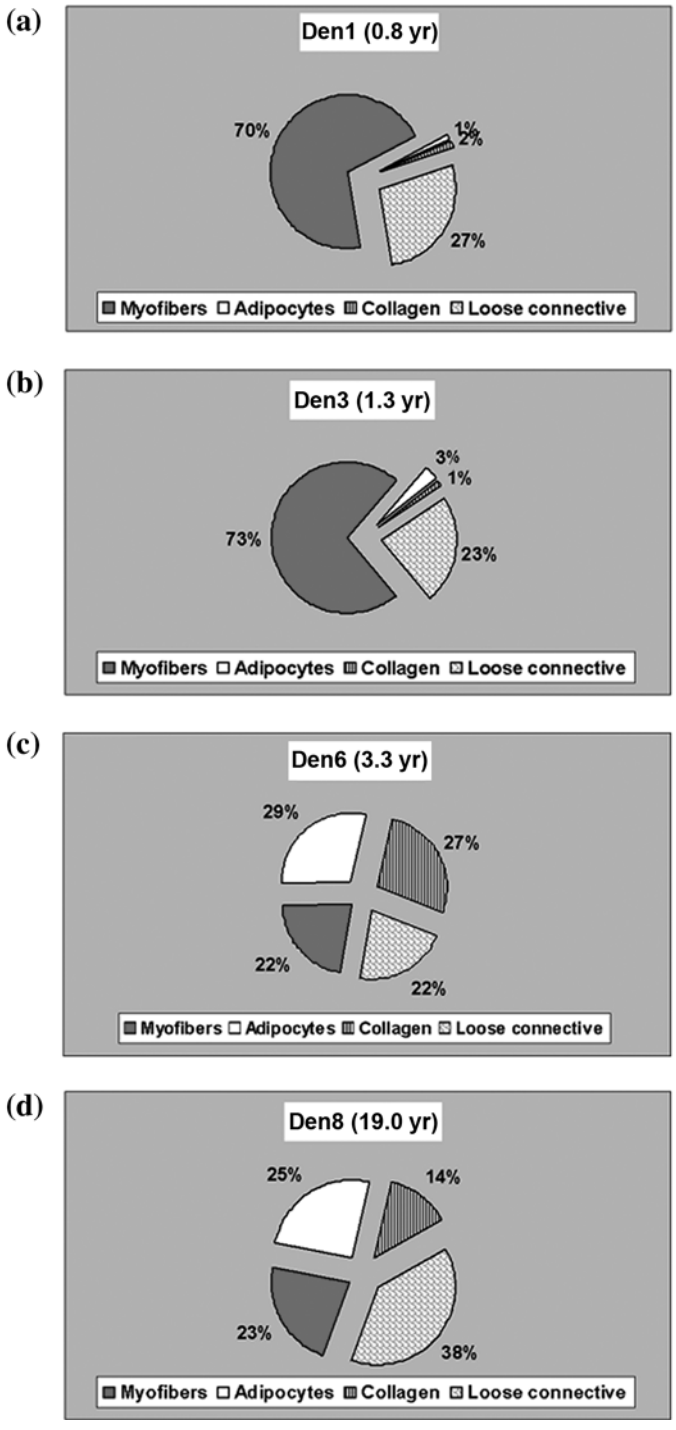

Figure 1.
Human long-term denervated *vastus lateralis* muscle. Percentile cryosection area covered by myofibers or connective tissues in long-term spinal-motoneuron denervation of human *vastus lateralis* muscle: **(a)** 0.8 yr denervation, **(b)** 1.3 yr denervation, **(c)** 3.3 yr denervation, and **(d)** 19.0 yr denervation. Heavy muscle degeneration is present after denervation periods of more than 2 years. (Den = denervated myofibers; numbers after "Den" identify biopsy taken.)

*Free software downloaded from the Web site: www.scioncorp.com

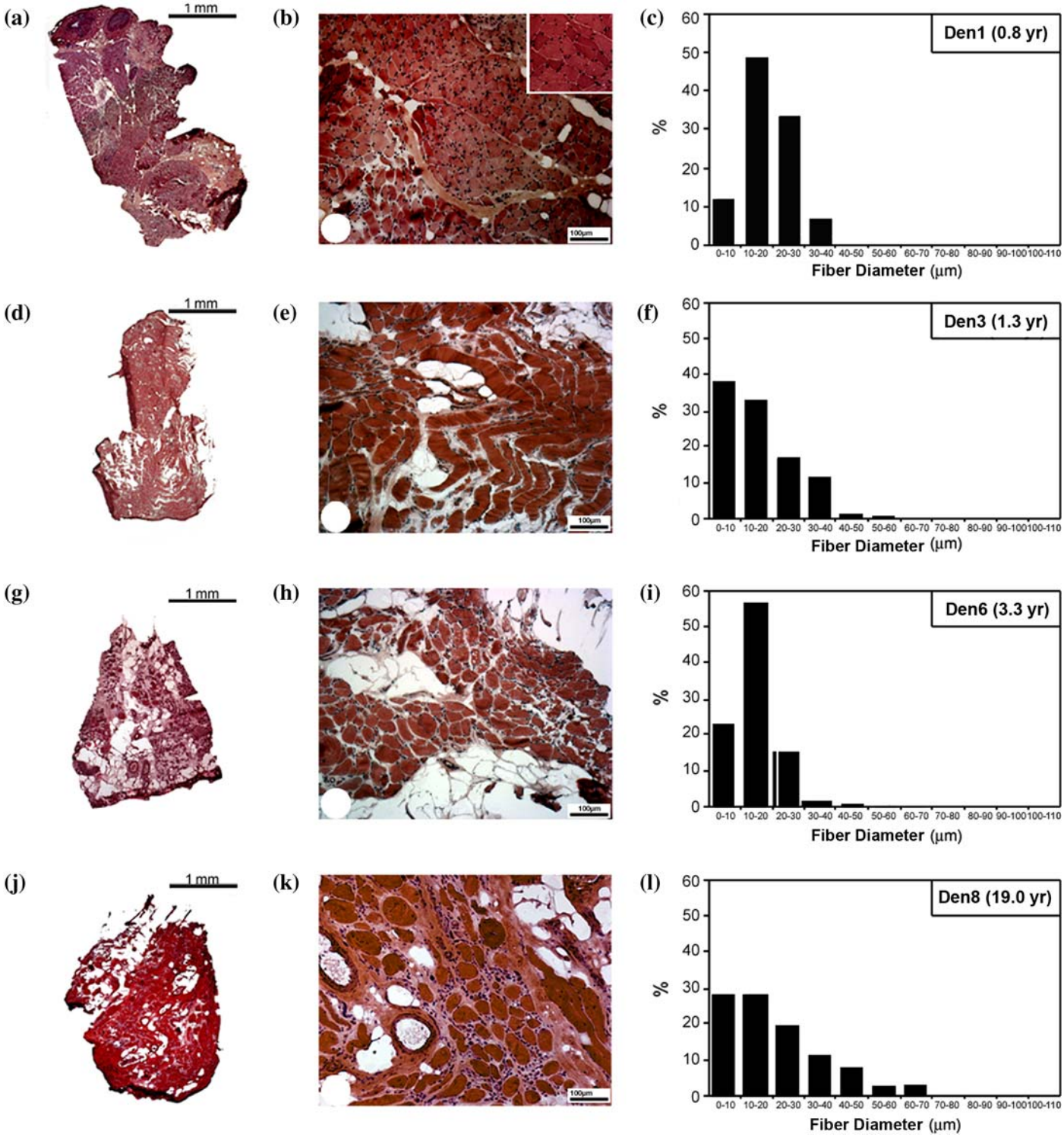

Figure 2.

Human long-term denervated *vastus lateralis* muscle: **(a)** to **(c)** 0.8 yr denervation, **(d)** to **(f)** 1.3 yr denervation, **(g)** to **(i)** 3.3 yr denervation, **(j)** to **(l)** 19 yr denervation. Inset in **(b)** is of normal human muscle. Cryosections are stained with hematoxylin-eosin (H-E). **(a)**, **(d)**, **(g)**, and **(j)** scale bar: 1 mm; **(b)**, **(e)**, **(h)**, and **(k)** scale bar: 100 µm. Heavy fat infiltration and increased content of fibrous connective tissue were present in biopsies taken more than 2 years after denervation. **(c)**, **(f)**, **(i)**, and **(l)**: fiber size spectra show that in all samples, 50% of myofibers had diameters smaller than 20 µm. (Den = denervated myofibers; numbers after "Den" identify biopsy.)

Table 2.
Time intervals between spinal cord injury (SCI) and muscle biopsy, percentile cryosection area covered by myofibers or connective tissues, and mean minimum diameter of denervated myofibers (Den) or of functional electrical stimulation (FES)-trained denervated myofibers (FESdm) in long-term spinal motoneuron denervation of human *vastus lateralis*.

Biopsy	Time (yr)		Percentile Area Covered by			Diameter† (Mean ± SD)
	Den*	FESdm	Fibers†	Adipocyte‡	Connective Tissue†	
Den1	0.8	—	70.0	1.2	28.8	15.6 ± 6.8
Den2	0.8	—	86.0	2.4	11.6	19.2 ± 7.8
Den3	1.3	—	72.4	3.1	24.5	16.2 ± 10.0
Den4	2.9	—	26.4	12.6	61.0	15.9 ± 10.3
Den5	2.9	—	11.6	3.1	85.3	22.3 ± 18.0
Den6	3.3	—	5.4	44.8	49.8	8.12 ± 4.5
Den7	19.0	—	28.4	2.6	69.0	15.3 ± 15.3
Den8	19.0	—	22.5	25.5	52.0	21.6 ± 25.3
Mean ± SD	6.3 ± 7.9	—	40.3 ± 30.9	11.9 ± 15.6	47.8 ± 24.7	16.8 ± 4.5§
FESdm1	3.6	2.4	97.5	1.3	1.2	30.7 ± 23.7
FESdm2	3.6	2.4	73.4	1.3	25.3	26.7 ± 22.9
FESdm3	6.3	4.3	97.5	0.2	2.3	45.7 ± 18.3
FESdm4	6.3	4.3	98.0	0.5	1.5	46.5 ± 14.3
FESdm5	10.6	9.4	97.7	0.5	1.8	48.1 ± 12.2
FESdm6	10.6	9.4	97.4	0.5	2.1	39.2 ± 18.0
FESdm7	13.5	4.8	96.3	1.2	2.5	58.2 ± 24.1
Mean ± SD	7.8 ± 3.8	—	94.0 ± 9.1	0.8 ± 0.5	5.2 ± 8.9	42.2 ± 10.8¶

*Den vs. FESdm, student's *t*-test (independent groups), $p = 0.324$
†$p < 0.001$
‡$p = 0.042$
§Cumulative values of 8 Den biopsies
¶Cumulative values of 7 FESdm biopsies
SD = standard deviation

HISTOLOGY OF LONG-TERM DDM WITH FES TRAINING OF DENERVATED MUSCLES

Figures 3 and **4** show four examples of the effects of FES training on human long-term spinal-motoneuron-denervated *vastus lateralis* muscles. In **Figure 3**, more than 75 percent of the cryosection area is covered by myofiber profiles, and in **Figure 4**, the biopsies show round myofibers of heterogeneous size, but with hypertrophic fibers clearly prevailing.

A residual population of small myofibers is differentially present in the electrically stimulated muscles, as exemplified by fiber size spectra in **Figure 4(b)**, **(d)**, **(f)**, and **(h)**. The mean diameter is 26.7 ± 22.9 μm, 45.7 ± 18.3 μm, 39.2 ± 18.0 μm, and 58.2 ± 24.1 μm, respectively. The cumulative value for seven *vastus lateralis* biopsies is 42.2 ± 10.8 μm, which is significantly higher than the value of 16.8 ± 4.5 μm for the eight long-term denervated human muscles ($p < 0.001$ by student's *t*-test for independent groups). Right and left legs in all subjects have concordant mean fiber diameter and similar profiles of their fiber diameter spectra, but the two groups are small (five subjects in the DDM group and four subjects in the FES-trained denervated muscle group) and heterogeneous to have statistical significance. In conclusion, after several years of electrically induced exercise, FES-trained myofibers overcame the myofiber size of normal sedentary adults (37.9 ± 9.5 μm, inset of **Figure 4(a)**).

DISCUSSION

Our previous clinical assessments have shown that muscle atrophy/degeneration due to long-term spinal-motoneuron denervation can be reversed with the use of the FES protocol developed in Vienna during the last few

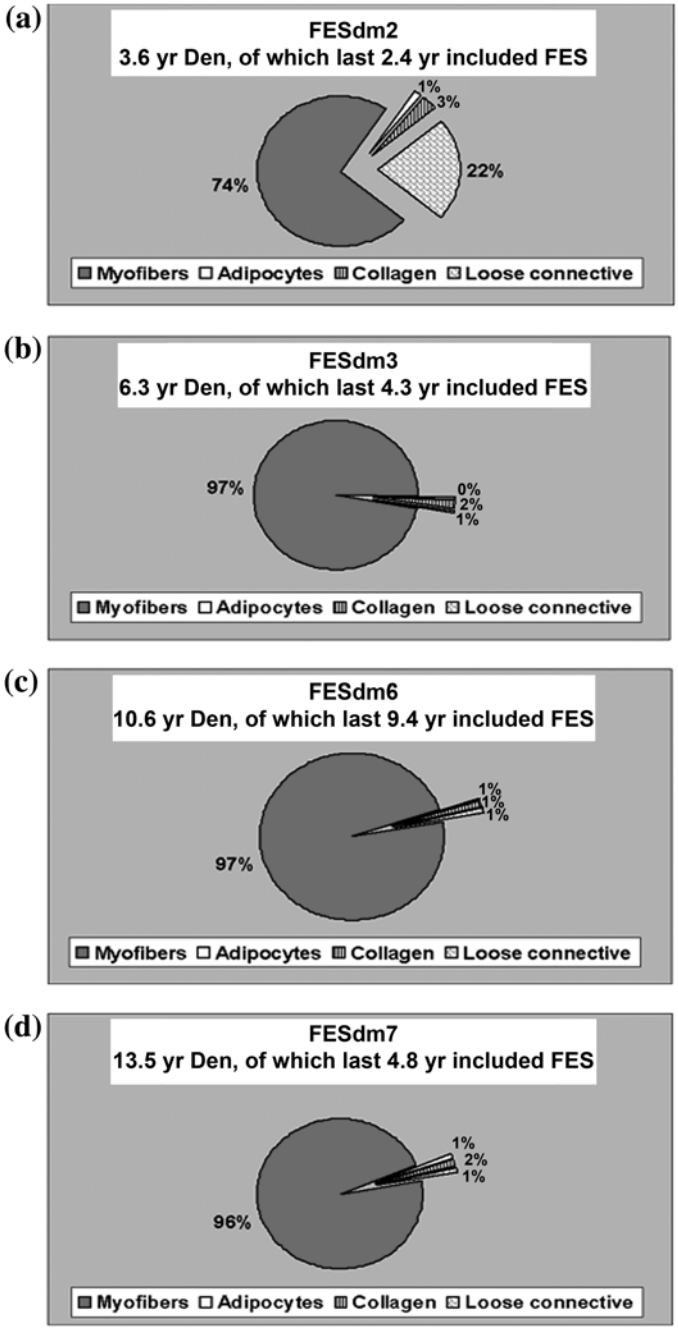

Figure 3.
Human long-term denervated *vastus lateralis* muscle. Percentile cryosection area covered by myofibers or connective tissue of functional electrical stimulation (FES)-trained long-term spinal-motoneuron denervated human *vastus lateralis* muscle (FESdm) after: **(a)** 3.6 yr of denervation including 2.4 yr of FES training, **(b)** 6.3 yr of denervation including 4.3 yr of FES training, **(c)** 10.6 yr of denervation including 9.4 yr of FES training, and **(d)** 13.5 yr of denervation including 4.8 yr of FES training. Only one relatively short-term stimulated muscle (2.4 yr FESdm) had more connective tissue than normal muscle. Den = denervated myofibers.

years [14–18]. After several years of a new FES training strategy, electrical stimulation effectively elicited sustained muscle contractions, and measurements taken by CT scans revealed increases of the thigh muscle cross-sectional area and tissue density [15–16,18–19]. The present work shows that both percentage area of cryosection covered by myofibers and mean fiber diameter of FES-treated muscles are larger than those of untreated DDM subjects. Electron microscopy also has shown that the massive structural alteration of both the contractile and excitation-contraction coupling apparatus of human DDM is absent in myofibers that underwent several years of FES-induced tetanic contractions [26]. All together, these morphological findings provide the structural basis for the recovered contraction strength in the lower-limb muscles of the FES-treated subjects. Muscle power reached values that allow supported standing up and standing during FES training sessions. After 1 year of training, subjects are able to stand up by FES-induced contractions of the quadriceps muscles and partial support of their arms on parallel bars. While the subject is standing, the FES-stimulated quadriceps muscles maintain knee extension. The arms are used only for maintaining balance.

The preliminary results reported here compare independent groups of DDM subjects—one biopsied pre-FES training and another biopsied post-FES training; the latter group had not been biopsied before because ethical committees in the past were reluctant to grant approval to perform muscle biopsies before training in subjects suffering from permanent *conus cauda* lesions. Indeed, in these cases, 1 year after the lesion, a poor chance existed for reinnervation, and standard clinical stimulators offered no hope for electrical-stimulation-induced muscle contractions. Our analysis is the first and only series that compares spinal-motoneuron-denervated FES-trained subjects, who demonstrated clear clinical signs of quadriceps muscle mass and force improvements, with a different group of denervated subjects. The latter are subjects who recently enrolled in the EU RISE Trial, for whom pre- and post-FES-training biopsies were approved by ethical committees and accepted by subjects.

The stimulation protocol tested is potentially harmful for the patient's skin. The risk of skin lesions is higher in the very early phases of stimulation, and one must prevent it by properly instructing the subjects. In the next few years, by means of animal experiments and clinical research, the EU RISE project will aim to devise safer stimulation protocols, which are needed to induce earlier clinically relevant effects, while hopefully reducing the stimulation burden for

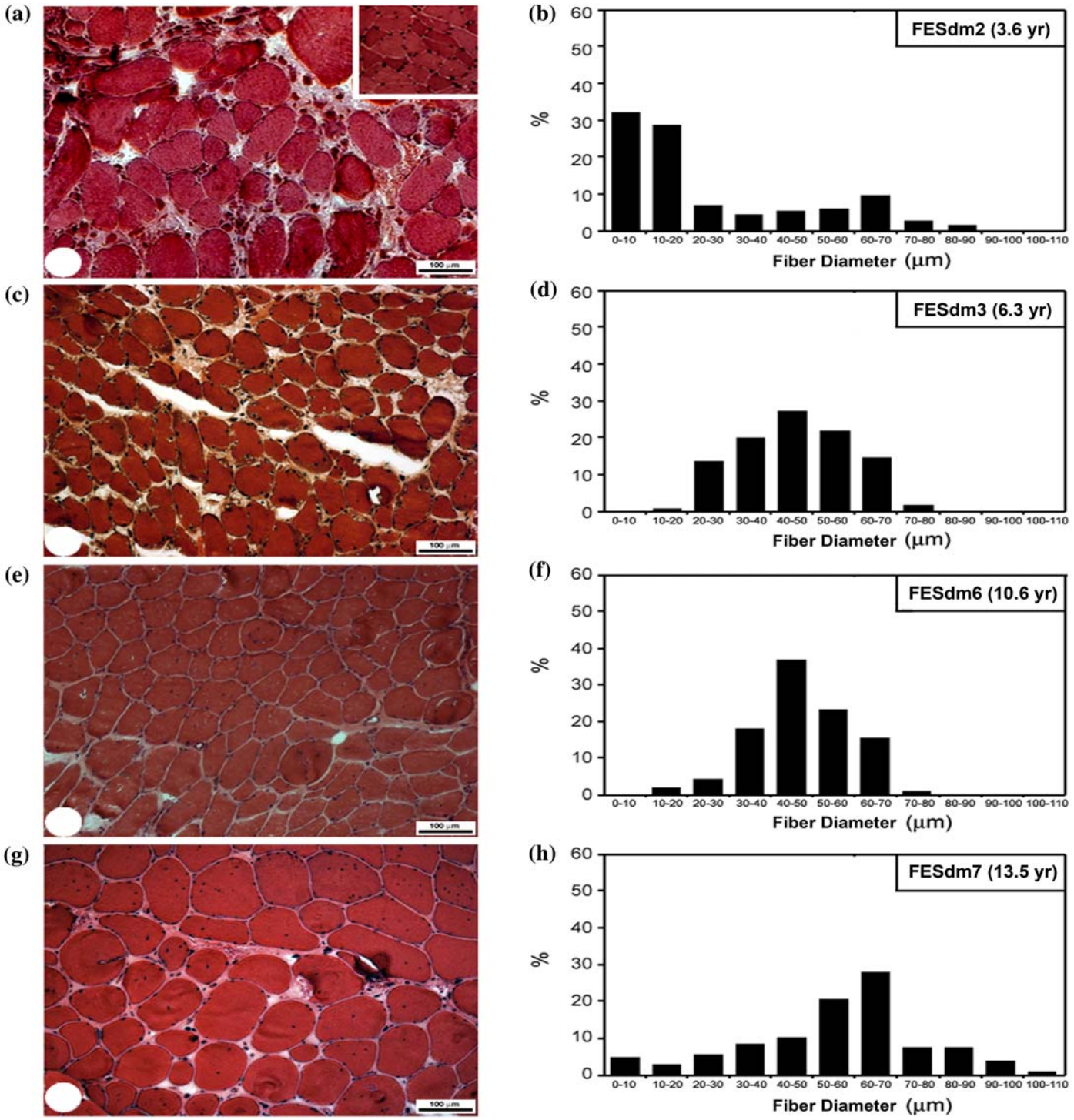

Figure 4.
Effects of functional electrical stimulation (FES) training on histology of long-term human denervated and degenerated muscle: **(a)** and **(b)** 3.6 yr of denervation of which 2.4 yr included FES, **(c)** and **(d)** 6.3 yr of denervation of which 4.3 yr included FES, **(e)** and **(f)** 10.6 yr of denervation of which 9.4 yr included FES, and **(g)** and **(h)** 13.5 yr of denervation of which 4.8 yr included FES. Inset in **(a)** is of normal human muscle. Cryosections are stained with hematoxylin-eosin (H-E). Scale bar: 100 μm. Round myofibers pave biopsy specimens. Residual population of small myofibers is differentially present in four electrical stimulated muscles, as also shown by fiber size spectra in **(b)**, **(d)**, **(f)**, and **(h)**. Severely atrophic myofibers are frequent in only relatively short-term stimulation cases **(a)** and **(b)**. FESdm = FES-trained denervated myofibers.

the subjects. A major improvement in subjects' quality of life would be attained if the number of sessions in the life-long stimulation program were successfully reduced to one session per day. Any successive change in stimulation parameters/modalities reducing the amount of current delivered will warrant a second-generation protocol.

An important by-product of our biopsy study is the unexpectedly healthy appearance of the spinal-motoneuron-denervated human muscle 1 year after SCI. If confirmed by independent studies, this result could open new perspectives in the management of subjects with spinal-motoneuron-denervated muscle who are awaiting reinnervation and/or FES training.

CONCLUSION

We believe that the effectiveness of FES training for the recovery of irreversibly spinal-motoneuron-denervated human muscles is sufficiently supported by our structural muscle analyses. These results justify further endeavors in this field on our part and encourage other researchers to independently verify our hypotheses and conclusions.

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