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European Project RISE: Partners, protocols, demography

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Abstract

Following the pioneering work of Helmut Kern and Winfried Mayr, the FES Vienna Group developed and implemented the EU RISE project. The persons involved in the clinical studies at the Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien were H Kern, C Hofer, S Löfler, M Vogelauer, M Mödlin, and C Forstner. At the Center for Biomedical Engineering and Physics, Medical University Vienna, Austria W Mayr, M Bijak, D Rafolt, S Sauermann, E Unger, and H Lanmüller made the stimulation devices and surface electrodes needed to stimulate long term denervated muscle of subjects sufferingt low thoracic-lumbar spinal cord injury (SCI). Here we report enrollment criteria, follow-up analyses, home based FES protocols and demography data. The scientific collaboration of the European partners of the clinical study, and of the 25 SCI participants that accepted to be muscle biopsied both pre and post h-b FES training are warmly acknowledged.
Key Words: RISE, human denervate muscle, SCI, partners, selection and enrollment criteria, follow-up analyses, home based FES protocols, demography of participants

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Source of Participants

The participants of the EU-RISE Project: Use of electrical stimulation to restore standing in paraplegics with long-term denervated degenerated muscles (Contract No: QLG5-CT-2001-02191) were enrolled among the patients hospitalized at:

Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Wien, (Austria);

Orthopädisches Spital Speising, Wien, (Austria);

Rehabilitationszentrum Weißer Hof, Klosterneuburg, (Austria);
Rehabzentrum Bad Häring, Bad Häring, Tirol, (Austria);

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University of Chieti, CESI, Centro scienze dell'invecchiamento, University G. D'Annunzio, Chieti, (Italy).

Table 1. Enrollment inclusion and exclusion criteria

Inclusion criteria
Complete lesion of *Conus Cauda* and/or of pelvis plexus with chronic denervation of the *quadriceps muscle*
Complete denervation time span between 9 months and 9 years
Absence of sensation in the thigh
Neurologically demonstrated flaccid paralysis with no spasticity, absence of segmental reflex activity
Intact skin
Exclusion criteria
Implants:
passive implants in hip, thigh and knee
pacemaker and ICD (Implantable Cardioverter Defibrillator)
active implants such as Brindley stimulator, pumps, pain simulator
In case of other implants, special considerations should be made
Diseases or hazardous infections: HIV, Hepatitis B, C
Pregnancy

Table 2. Demography of enrolled patients and intervals between SCI and muscle analyses of LMN denervated *vastus lateralis* before and after h-b FES training.

Patient	Age	Sex	Site of lesion		Time (years) between SCI and		Time of FES
			skeletal level	sensory level	enrollment	after FES	(years)
01	38	F	TH12	TH11	0·7	2·9	2·2
02	30	M	TH11,TH12	TH9	0·7	3·1	2·4
03	20	M	L1	TH11	0·8	3·3	2·5
04	25	M	TH11/12	TH12	0·8	2·9	2·1
05	29	M	TH11/12	TH10	0·8		
06	20	M	TH11	TH10	0·8	2·8	2·0
07	40	M	TH11/12	TH8	0·8	2·7	1·9
08	31	M	TH12,L4/5	TH10	1·1		
09	42	M	TH12,L1	L2	1·2	3·3	2·1
10	37	M	TH12	TH11	1·4		
11	26	M	L1	L1	1·7	4·0	2·3
12	37	M	TH11/12	TH10	1·7	3·8	2·1
13	44	M	TH12	TH10	2·9	5·3	2·4
14	49	F	TH12,L1	TH11	3·2	5·3	2·1
15	49	M	TH9/10	TH9	3·3	5·9	2·6
16	53	M	TH12,L1	TH6	3·5		
17	24	M	TH12,L1	TH10	4·1	6·7	2·6
18	47	M	TH11/12	TH9	5·4	7·5	2·1
19	37	M	TH12	L1	6·1	8·7	2·6
20	39	M	TH8/9,TH11	higher than TH6	6·1	8·2	2·1
21	44	M	TH12,L1	TH12	6·1		
22	55	M	TH12,L1	TH9	7·6	10·2	2·6
23	304	F	TH5	TH4	7·7	10·2	2·5
24	46	F	TH11/12	TH12	8·7	11·2	2·5
25	55	F	L1	TH11	8·7	10·9	2·2

Enrollment of patients in the project and follow-up

All subjects enrolled in the project - suffering from *Conus Cauda* lesion (up to 9·0 years of complete peripheral denervation) - were volunteers that received detailed information and signed an informed consent. Patients were included in the study from December 2003 until August 2004. Clinical and functional assessments, as well as follow-up and muscle biopsies, were performed at the Wilhelminenspital, Vienna, Austria. *Quadriceps muscle* complete denervation was assessed by electrophysiological testing, i.e. by test electrical stimulation, needle electromyography (EMG), brain motor control assessment (BMCA), transcranial and lumbosacral magnetic stimulation (TMS, LMS) as described in [6]. Every 12 months, subjects underwent a full clinical assessment.

Enrollment criteria are reported in Table 1, demographic details of the enrolled subjects are described in Table 2.

114 patients were examined for eligibility, and 38 were confirmed to be eligible. 25 patients (aged 20 to 55 years; 5 female and 20 male) were included in the study since they accepted to perform muscle biopsies both pre and post h-b FES training. Five patients dropped out because they decline to visit the Wilhelminenspital, in Vienna for follow-up analyses and end-point follow-up biopsy. The average follow-up time of the 20 compliant paraplegics was 2.2 years (minimum 1.9, maximum 2.6).

Home-based FES training strategy

The training strategy [3-8] is based on four different stimulation programs described in details in Table 3. In all subjects biphasic stimulation impulses of very long

Table 3. Home-based TES training programs for complete LMN denervated muscles of paraplegic patients.

Training Program	Timeline (months) #	Stimulation parameters	Training parameters
1	0-4	120-150ms ID / 400ms IP; 4sec SD / 2sec SP	3-4 x 3min with 1-2min pause; 5d/week
2	2-6	70-100ms ID / 400ms IP; 5sec SD / 2sec SP	4-5 x 3min with 1-2min pause; 5d/week
3	4-12	40-50ms ID / 10ms IP; 2sec SD / 2sec SP	4-6 x 20-40 rep 1-2min pause; 5d/week
4	8-24	40ms ID / 10ms IP; continuous stimulation	standing, stepping-in-place and walking; 4-6 x 20-40 rep (1-5min) 1min pause; 5d/week

duration (120-150 ms, 60-75 ms per phase) and high intensity (up to ± 80V and up to ± 250 mA) were applied at the beginning of the treatment (Training Program 1). Enrolled patients were provided with stimulators and electrodes to be used at home. The large (180 cm^2) electrodes (Schuhfried GmbH, Mödling, Austria) made of conductive polyurethane were placed at the skin surface using a wet sponge cloth (early training) and fixed via elastic textile cuffs. Later on, when the skin was accustomed to the necessary high current density we used gel under the polyurethane electrode to achieve minimal transition impedance. The electrodes were flexible enough to maintain evenly distributed contact pressure to the in general uneven and moving skin surface, thus providing homogeneous current distribution throughout the entire contact area. The subjects underwent clinical assessment and stimulated knee torque measurement every 12 weeks by physiatrists, who progressively modified the stimulation parameters and training protocol according to the patient's improvements. The daily training may consist of combined twitch and tetanic stimulation patterns (Training Programs 2 and 3 in Table 3) in sessions lasting up to 30 min for each group of muscles (gluteus, thigh and lower leg muscles on both sides).

Electrophysiological testing for the assessment of complete LMN denervation

Test stimulation of the quadriceps muscle was performed by bidirectional rectangular impulses, which in normal innervated muscles last 0.3 – 0.7 ms. Needle EMG of the quadriceps muscle was performed according to [11] The needle was inserted into the rectus femoris, vastus lateralis and medialis muscles, which were examined at different depths and in four directions. We tested for insertion and spontaneous activities during muscle relaxation (fibrillation potentials, positive sharp waves and fasciculations) and for volitional activity. Transcranial stimulation was carried out by placing a double cone coil (MAGSTIM 200, Magstim Company Ltd, Wales, UK) over the medial caput. Lumbosacral stimulation was applied at vertebral levels T12, L2, and L4 by using a circular coil. Magnetic stimulation amplitude was increased from 0.4 to 4 Tesla in steps of 10%.

Classification of the electrical stimulation-induced functional behavior of the complete LMN denervated human SCI subjects

Grading of the electrical stimulation-induced functional behavior of the complete LMN denervated human muscles needs a special classification since the complete LMN denervated subjects are and ought to remain without sensory function in the thigh throughout the study to tolerate the high currents of the FES training for complete LMN denervated human muscles. In Table 4 the enrolled subjects were categorized in four Functional Classes on the basis of their electrical stimulation-induced muscle contraction performance.

Training strategy

The contractile response of denervated muscle to electrical stimulation depends on the stage of post-denervation muscle atrophy/degeneration, which in turn depends on the time period elapsed between the denervation event and the onset of stimulation [1,2,4-6,9]. With the exception of the subjects who had been injured since about one year, at the beginning of the treatment all others were treated with biphasic stimulation impulses of very long duration and high intensity [4-6,8,9]. The stimulation parameters were subsequently adjusted according to the increasing

Table 4. Functional classification of paraplegic patients with complete LMN denervation of the quadriceps muscle.

Subject	Denervation (years)	Functional Class at enrollment				
		0	1	2	3	4
1	0·7			●		
2	0·7				●	
3	0·8				●	
4	0·8			●		
5	0·8			●		
6	0·8		●			
7	0·8			●		
8	1·1			●		
9	1·2			●		
10	1·4			●		
11	1·7				●	
12	1·7		●			
13	2·9			●		
14	3·2		●			
15	3·3		●			
16	3·5		●			
17	4·1		●			
18	5·4			●		
19	6·1			●		
20	6·1		●			
21	6·1		●			
22	7·6		●			
23	7·7		●			
24	8·7		●			
25	8·7	●				

Functional Classes: 0 No torque measureable, no contraction/twitch visible; 1 No torque measureable, but contraction/twitch visible; 2 Torque measured between 0.1 – 2.9 Nm; 3 Torque measured more than 3.0 Nm, but not able to stand; 4 Able to stand in parallel bars/standing frame

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excitability induced by h-b FES training. Details of the four-phase FES Training are provided in Supplemental Table 2 and discussed in the Results section.

Computer Tomography (CT) scan

Complete cross sectional area of quadriceps muscle and hamstrings were determined as described by Modlin et al. [9]. The cross sectional area was measured manually by marking the muscles on the scan and calculating the area of the selected zone.

Electron microscopy and size distribution spectrum of total myofibers

The samples obtained by biopsies were fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 2h on ice followed by a buffer rinse and 1 hour fixation in 1% osmium tetroxide. The specimens were dehydrated in a graded series of ethanol solutions and embedded in epoxy resin. Semi thin sections (1µm), stained using conventional techniques with toluidine blue, were used to plot fiber size distribution [1]. The minimum transverse diameter of each myofiber was measured against a reference ruler. The myofibers were grouped and relative percentage plotted in 10µm steps. Ultrathin sections (approx. 40 nm) were cut in Leica Ultracut R (Leica Microsystem, Austria) using a Diatome diamond knife (DiatomeLtd. CH-2501 Biel, Switzerlad) and stained in 4% uranyl acetate and lead citrate. Sections were examined with a Philips M 301 or a FP 505 Morgagni Series 268D electron microscope (Philips), equipped with Megaview III digital camera and Soft Imaging System (Germany).

Analyses of human muscle biopsy

Needle muscle biopsies from the right and left muscles of each patient before and after two-years of h-b daily FES training were taken as described by Kern et al. [5].

Cryosections (10 µm thick) of muscle biopsies were stained with H&E, using conventional techniques. Images were acquired using a Zeiss microscope connected to a Leica DC 300F camera at low magnification, under the same conditions that were used to photograph a reference ruler. Tissue type distribution (relative content of interstitial tissue and cumulative muscle fiber areas) was determined using the Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA). The minimum transverse diameter of each muscle fiber was measured against the reference ruler. Muscle fibers were grouped and the relative percentile was plotted in 5 µm steps, as described [10]. Morphometric analysis was performed using Scion Image for Windows version Beta 4.0.2 (2000 Scion Corporation), a free software downloaded from the web site: www.scioncorp.com.

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