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Electrical stimulation alters muscle morphological properties in denervated upper limb muscles

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ABSTRACT

Background: Damage to lower motor neuron causes denervation and degeneration of the muscles affected. Experimental and clinical studies of muscle denervation in lower extremities demonstrated that direct electrical stimulation (ES) of muscle can prevent denervation atrophy and restore contractility. The aim of this study was to identify possible myogenic effect of ES on denervated forearm and hand muscles in persons with spinal cord injury (SCI) and tetraplegia.

Methods: This prospective interventional study with repeated measurement design included 22 patients aged 48·6 (± 15·7), 0·25 (0·1/46) years after spinal cord lesion, AIS A-D. In each patient, two electrophysiologically-confirmed denervated muscles in the hand and forearm were analyzed – one extrinsic (Extensor Carpi Ulnaris - ECU) and one intrinsic (1st Dorsal Interosseus - IOD1). Muscles were stimulated for 33 min, five times per week over a 12-weeks period. Using ultrasonography (USG), muscle thickness (MT) and pennation angle (PA) of these muscles were determined at start and end of the stimulation period.

Findings: MT of IOD1 increased from 6·3 mm (± 3·2 mm) to 9·2 mm (± 2·4 mm) (p = 0·004) and the PA from 5·5° (± 3·0°) to 11° (± 2·2°) (p = 0·001). The corresponding values for the ECU were 5·5 mm (± 2·5 mm) to 7·0 mm (± 2·2 mm) (p = 0·039) and 5·5° (± 3·4°) to 9·4° (± 3·8°) (p = 0·005), respectively. The correlation of MT between baseline and completion was r = 0·58 (p = 0·037) for the ECU and r = 0·63 (p = 0·008) for the IOD1.

Interpretation: 12 weeks of direct muscle stimulation increases the MT and PA of the denervated intrinsic and extrinsic hand muscles studied.

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1. Introduction

Denervation and atrophy of arm and hand muscles are regularly observed after cervical spinal cord injury and particularly in lesions affecting the lower segments of the cervical spine (C5-Th1). Depending on the degree of denervation, different treatment and rehabilitation protocols are applied [1]. For example, the decision-making process for nerve transfer surgery, and its success rate, appears to be highly dependent on the localization, magnitude, and distribution of the lower motoneuron (LMN) lesion [2]. Typically, denervated muscles occur in the coverage area of nerves originating at the level of injury where lower LMNs are frequently damaged [1]. Following a LMN lesion, structural and functional transformations of muscle properties occur different from those observed after an upper motor neuron (UMN) lesion. Denervation atrophy implies a decrease in muscle fiber diameters and a partial transformation into connective and adipose tissue in the muscle affected [3]. Interestingly, Helgason and coworkers found that electrical stimulation (ES) could restore contractile properties in structurally altered muscles of the lower extremities [4]. However, the impact of stimulation seems to be inversely correlated to the time elapsed since spinal cord injury (SCI) [5].

Due to the reduction of MT caused by degeneration, the orientation of the muscle fiber bundles changes [6]. Therefore, the angle between the direction of the muscle fibers and the axis of force generation in pennate muscles i.e., the PA, diminishes due to denervation/ muscle atrophy [7]. *In vivo*, USG has proven a feasible technique for assessing muscle architectural properties [8]. A strong positive correlation has been documented between MT and PA [6,9,10], i.e., an increase of PA is a sign of muscle restitution. Muscle architectural properties i.e., muscle fiber length, muscle physiological cross-sectional area (PCSA) and PA are determinants for muscle force generation. Muscles with large PCSA and short fiber length, generate high levels of force. In contrast, muscles with smaller PA and PCSA but long fibers are designed for excursion and higher contraction

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Research in context

Evidence before this study

Electrical stimulation is a common and established method in the rehabilitation of persons with spinal cord injury. Several studies investigated the effect of electrical stimulation to avoid disuse and denervation atrophy, improve muscle force, power output and endurance, change muscle fiber type, increase cross sectional area of muscles, increase muscle mass, enhance nerve sprouting and motor learning. Most of the studies investigated the function of the lower limbs. For the upper limbs fewer studies exist but none for denervated muscles. It is supposed that the effects of electrical stimulation are similar. In the upper limbs, in contrast to the lower limbs, the combination of spastic paralysis and flaccid paralysis is more frequent. This enhances the complexity in the application of electrical stimulation. The mentioned effects of electrical stimulation may be an important supplement in reconstructive tetraplegia hand surgery and mainly nerve transfers. In previously published studies by the authors (2018,2019), the topographic distribution of stimulation points (motor points) for the extensors and flexors of the forearm and the hand was mapped and described by means of electrical stimulation. The electrical stimulation according to this mapping led to remarkable findings in patients with tetraplegia. One key finding was that the presence of denervated muscles is considerably higher among the flexor than in extensor forearm muscles. That fact may explain the clinical observation of a better functional outcome after surgical nerve transfer to the extensors compared to the inconclusive result after nerve transfer to the flexors. A continuation was therefore to perform an interventional study with the clear goal to maintain the muscle properties in denervated muscles by direct muscle stimulation.

Added value of this study

This study reports that direct muscle stimulation appears to ameliorate the morphological condition of denervated forearm muscles, using the example of an intrinsic and extrinsic muscle. Neither time since injury nor ethiology is a limitation for the beneficial effect of the stimulation of denervated muscles. However, it is likely that early onset of direct electrical stimulation after lower motoneuron damage improves the preconditions for successful reinnervation after nerve transfers. In addition, the time between spinal cord injury and nerve transfer can be prolonged by the use of electrical stimulation without impairing the outcome. Furthermore, measuring muscle thickness by ultrasound can be used as a clinical, feasible assessment to predict the increase over a defined stimulation period. These findings enable new therapeutical approaches to optimize reinnervation and function in denervated muscles.

Implications of all the available evidence

Direct muscle stimulation is a safe and beneficial treatment option for denervated upper extremity muscles. It is well accepted in patients either in clinical setting or in domestic use. The daily application over a 12-week stimulation period showed a significant increase in muscle thickness and pennation angle. Both assessed parameters served as a surrogate for muscle morphology properties. The significant correlation of the increase in muscle thickness from baseline to completion of the stimulation period allows prediction of the gain in muscle thickness. This predictive information is critical for the time of preconditioning needed for a denervated muscle that will be target for nerve transfer. After nerve transfer, the stimulation maintains the muscle structure and provides better physiological condition for reinnervation.

velocity [7]. ES with long pulse duration and high amplitude prevents and reverses the degeneration process of skeletal muscle tissue [5,11]. Muscle activity is the key factor for regulation of muscle size and the biochemical and physiological properties of muscle fibers rather than neurotrophic substances [12]. Typically, the stimulation protocol for denervated muscles starts with single twitches followed by tetanic stimulation patterns [5,11].

Achieving a stimulation training that elicits a tetanic contraction, consisting of 20 Hz with 40 ms pulse duration, a pulse pause of 10 ms and bursts of 2 s/2 s pause applied over 30 min 5 times a week, could require some months in chronic stage after SCI [13]. However, previous studies of the effect of ES on muscle structure and characteristics in denervated muscles in SCI patients focused on the lower extremities [5].

To date, it remains unclear if ES of denervated muscles in the upper extremities in SCI is as effective as in the lower extremities. Animal studies have shown that the cross-sectional area of denervated muscle fibers may be increased by early ES. The shoulder girdle is, however, loosely anchored to the trunk i.e., flexibly connected to the rib cage, and the stimulation dose-response relationship may differ from that of the lower limb. In contrast, the pelvic girdle is inserted into the trunk enclosure and is almost immobile. Consequently, the upper extremity has a large workspace while the lower extremity, is supported by the vertebral column and serves primarily for locomotion. Furthermore, the intrinsic muscles of the hand, designed for precision and postural control, exhibit smaller motor units and different muscle fiber type composition (predominantly type 1). Hence, stimulation amplitudes and frequencies have to be differently adapted to achieve morphological and functional changes and without harming the stimulation area.

Optimal stimulation time and the most effective intensity needed to detect morphological change in the upper extremity musculature has yet to be proven. At the molecular level, the altered expression of myosin heavy chain isoforms following denervation could be reversed by electrical stimulation [14].

The interest in nerve transfers targeting denervated muscles to restore wrist extension and hand opening and closure in SCI has grown in recent years [2,15]. By a nerve transfer, an intact donor LMNs is sutured to a non-functioning recipient at or below the level of lesion. A recipient muscle affected by a LMN lesion normally degenerates and transform into fat- and connective tissue within two years [11]. This fact is essential for the eligibility of nerve transfers. A LMN lesion at the level of the recipient muscle would exclude a late nerve transfer more than 12 months post injury and affirms the necessity of early intervention [16]. If a nerve branch is transferred into a denervated or partially denervated muscle, it is reasonable to believe that preserved contractile structures would be favorable for outcome. Early ES onset of the crucial muscles is likely to preserve structure and gain time for eventual surgical intervention [17].

This prospective interventional study hypothesized that direct electrical muscle stimulation of two denervated forearm and hand muscles – one extrinsic and one intrinsic - can regain their structural integrity.

2. Subjects and methods

2.1. Recruitment and screening of patients

22 patients, 20 males and two females with a mean age of 48·6 (± 15·7 standard deviation (SD)) and a median time since lesion of 0·25 years and an interquartile range of (0·1/0·2/0·25/2·68/46) and a traumatic or non-traumatic spinal cord lesion, C3-C7, AIS Score A/B/C/D were included in the single arm-study (Table 1). Finally, data of 20 participants were analyzed. Two patients dropped out, one due to reinnervation in the stimulated muscle, the other due to diffuse leg pain that he subjectively attributed to the stimulation (Fig. 1). Ten

Table 1
Patents' characteristics.

ID	Age	Gender	Time since lesion years	Ethiology	Level of lesion	AIS
1	46	m	1	traumatic	C7	A
2	47	m	0.2	traumatic	C7	B
3	42	m	42	CMT	nt	nt
4	71	m	1.8	non traumatic	C7	D
5	26	m	0.25	traumatic	C6	A
6	34	m	0.25	traumatic	C6	A
7	36	m	2.25	traumatic	C7	B
8	72	f	0.25	traumatic	C5	D
9	64	f	0.1	non traumatic	C4	D
10	31	m	0.1	traumatic	C6	A
11	33	m	0.2	traumatic	C6	A
12	28	m	0.25	traumatic	C5	A
13	42	m	0.1	traumatic	C6	A
14	51	m	20	GBS	C5	D
15	60	m	46	non traumatic	C5	D
16	71	m	0.4	GBS	C4	C
17	55	m	0.2	traumatic	C5	A
18	28	m	0.25	traumatic	C6	B
19	48	m	4	GBS	C5	D
20	63	m	11	GBS	nt	nt
21	49	m	0.5	non traumatic	C5	A
22	74	m	0.2	non traumatic	C6	D

Abbreviations: *m* = male, *f* = female, AIS = American Spinal Injury Association Score, nt = not tested, GBS = Guillain Barré Syndrome, CMT = Charcot Marie Tooth disease, *n* = 22.

participants had a traumatic and 10 non-traumatic SCI. One of them had a Charcot - Marie -Tooth (CMT) disease that caused an incomplete tetraplegia and four patients had Guillain Barré Syndrome (GBS).

The ECU and IOD1 were tested bilaterally with surface ES to determine whether an UMN or LMN lesion was present. The muscle was defined as denervated when no muscle contraction could be elicited with short pulses. For testing, the EMPI 300 PV (ID B36711008L00, AXIOBIONICS, Minnesota, US) portable neuromuscular stimulator was used. The stimulation was performed with a pulse duration of 300 μs, a frequency of 35 Hz and an amplitude of 20 to 50 mA. One self-adhesive 3 cm round electrode (Axelgaard Pals, Lystrup, Denmark) was placed on the median and ulnar nerve in the area of the medial epicondyle. A corresponding pen electrode with a diameter of 5.0 mm was placed over the motor point of each tested muscle [17].

If at least one of the four tested muscles (IOD1 right/left, ECU right/left) was denervated, the patient was eligible for the study. Consequently, exclusion criteria were innervation or partial innervation of the two abovementioned muscles. A muscle yielded as partially innervated or innervated if any detectable muscle contraction could be evoked by ES testing. The Medical Research Council (MRC) for muscle testing was used to define the detectability of innervation, partial denervation or denervation. As described in a previous paper [1], a muscle is considered innervated when it achieves full range of motion (MRC $\geq$ 3) under stimulation, partially denervated at MRC $<$ 3 and denervated in the absence of any visible and palpable contraction. In case of an inconclusive determination of partial denervation, stimulation was performed under ultrasound imaging to verify the contraction.

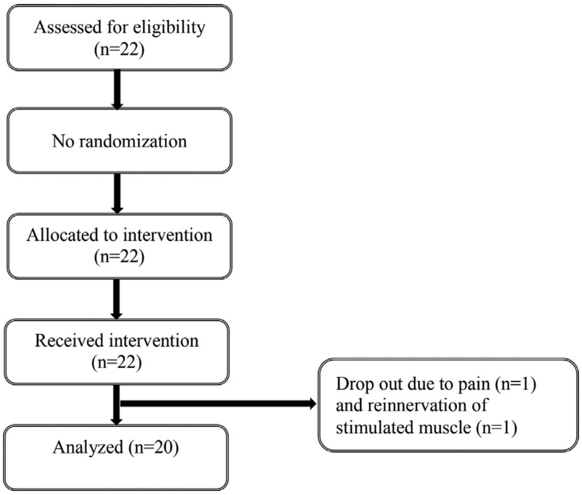


Fig. 1. Flow chart of the single arm study (*n* = 20).

The study was approved by the Swiss National Ethics Committee (Swiss Ethics, 2018 –01,238) and registered on ClinicalTrials.gov (NCT03698136). After signing the informed consent, the patient was enrolled in the study. The study protocol consisted of an examination at the beginning of the study including USG measurement of the PA and MT of the selected muscles, followed by a 12-weeks stimulation period. At the end of the stimulation period, the identical PA and MT measurements were performed. In addition, a questionnaire of the patient perceived treatment's effectiveness was completed.

2.2. *Ultrasonographic measurements*

An ultrasound Hitachi Arietta Fields Prologue (Steinhausen, Switzerland) with a peak frequency of 13 MHz, maximal gain, 70%, image depth 3 cm and focal point depth of 1 cm was used for analyses. The linear probe size was 4·2 cm. Imaging and analyses were performed by two research physiotherapists. For each participant, the imaging, measurements and directly ensuing calculations of MT and PA were conducted by the same examiner. The second examiner repeated the analysis of the USG image for reliability verification. MT (mm) and PA (°) were measured by ultrasound as a surrogate for structure of muscle contractile components (muscle bundles). The MT was measured at the midpoint of its longitudinal axis. The intra-class correlation coefficient (ICC) for USG measurements of MT in our lab is 0.95.and in line with ICC for ultrasound imaging of MT and PA presented by others [6]. Fig. 2 is a representative image for measurement of PA.

For measurement, the participants' forearm was placed in a relaxed position (semi-flexed elbow and pronated forearm) on a table. A thick layer of contact gel was applied between probe and skin. The probe was applied with minimal pressure to avoid

Fig. 2. Representative ultrasound image used for measurement of Pennation Angle (ECU).

compression of the muscle. Three consecutive images were collected per muscle, with the ultrasound transducer removed and repositioned between each image acquisition. The mean of the three measurements of MT and PA was taken for statistical analysis.

For each examined muscle, individual landmarks in relation to the forearm length (lateral epicondyle to ulnar head prominence) were defined and marked. The probe was centred over the mark and longitudinally aligned along the muscle. The probe was then tilted to receive the sharpest possible muscle border fascia.

The PA of the IOD1 was defined by the angle of insertion of the superficial fibers to the superficial aponeurosis of the muscle. It was measured in a relaxed, supported position of the hand with the fingers slightly flexed and the thumb radially abducted (Fig. 3A). Therefore, the wrist was positioned in neutral (0°) dorsiflexion and no radial-ulnar deviation (3rd metacarpal aligned with radius longitudinal axis). The index' finger flexion ranged from 0° to 20° in the metacarpo-phalangeal (MCP) joint and 0° to 10° in the proximal (PIP) and distal interphalangeal (DIP) joints. The radial abduction of the thumb was 20° to 40°, ensuring that the 1st web space remained relaxed.

To warrant reproducibility of measurement, two landmarks were defined. Two skin lines were drawn, one longitudinal over the second metacarpal and the second perpendicular to the first at the level of the MCP joint of the thumb. The distance between the first and second MCP joints was measured with a ruler and the probe was placed at the midpoint (Fig. 3A). The thumb was kept in a resting position throughout examination. The probe angle was tilted to give the sharpest IOD1 border.

The hand positioning at baseline and completion measurement of each participant was identical.

For ECU measurement, the probe was placed with its centre over the motor point of the ECU and longitudinally to the trajectory of the muscle (Fig. 3B). The motor point localization of the ECU was calculated relative to the forearm length and based on a previously developed standardized motor point mapping system [17]. The probe angle was tilted to pose the sharpest border of the muscle. The

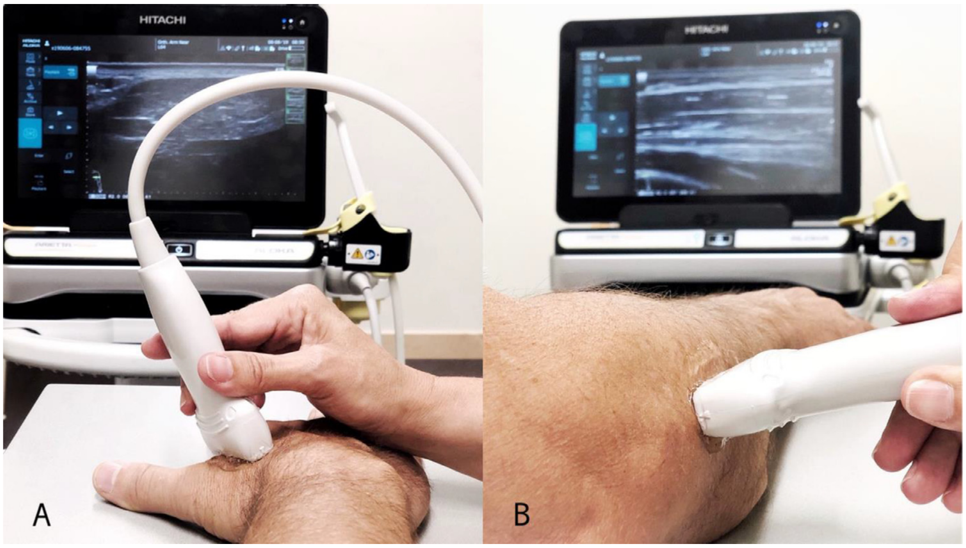


Fig. 3. Position of the probe for ultrasound imaging of IOD1 (A) and ECU (B).

forearm positioning (80° of elbow flexion and full pronation) was identical at baseline and completion measurement for each participant.

2.3. *Questionnaire*

A questionnaire of the perception and effectiveness of treatment was carried out to understand whether stimulation is accepted by patients and how they personally assess the benefit in relation of the time expenditure for the treatment. The questionnaire included one question each about the time of the stimulation, the individual acceptance of a long-term utilization for more than two years and the users' convenience of the device. Questions asked were based on patient interviews and comments reflecting the most cited reasons for not performing stimulation.

2.4. *Stimulation of the denervated muscles*

Stimulation of the denervated muscles was applied with the Stimulette Den2x, (Schuhfried GmbH, Mödling, Austria). Muscles were stimulated for 33 min, 5 times per week over a stimulation period of 12 weeks. The stimulation consisted of a 3 min warm up phase and a 30 min training phase. Stimulation parameters for the warm-up phase were: 200 ms pulse duration, 500 ms pause and a frequency of 1.42 Hz in bursts of 11 s on and 11 s off with an amplitude of 20–30 mA. The treatment phase included 35 ms pulse duration, 10 ms pause and a frequency of 22 Hz in bursts of 2 s on and 2 s off and an amplitude of 30–45 mA. In the treatment phase, the amplitude was considered to be efficient when a visible muscle contraction and/or movement occurred of the thumb and index for the stimulation of the IOD1, and ulnar deviation of the wrist for the ECU. The stimulation was applied over water humidified sponges with rubber electrodes, sized 5 × 5 cm fixed onto the muscle belly with a bandage (Fig. 4).

2.5. *Statistical methods*

20 patients were recruited for the study. Since this investigation represents a pilot study, the sample size chosen was attributable to feasibility.

Shapiro-Wilk and Kolmogorov-Smirnov test of Gaussian distribution was applied for age, time after injury, MT, and PA of both muscles at the two measurement time points.

Data for normally distributed values (age, MT and PA) are presented as means with standard deviation. Interval scale data for time since injury is presented as median with interquartile range. The group comparison between pre- and post-intervention PA and MT were analysed by paired *t*-test and are expressed as means with standard deviation. The level of significance was set at $\alpha = 0{\cdot}05$. A Pearson correlation was conducted to analyse the relation between MT and PA at baseline and completion for the IOD1 and ECU as well as the correlation between MT at baseline and completion of the treatment. The correlation tests were used to examine if the two muscle architectural parameters chosen (MT and PA) responded physiologically to ES in the same (or different) way to growth as stated in literature [10].

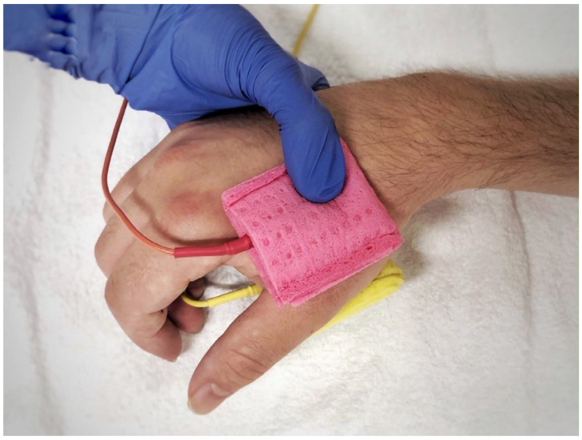


Fig. 4. Position of the 5 × 5 cm electrodes to cover the IOD1.

2.6. *Role of the funding source*

The funding source (Swiss Paraplegic Centre Nottwil) had no role in study design; in the collection, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication. The corresponding author confirms that she had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

Altogether, MT increased in 35 out of 48 (73%) and PA in 43 out 48 (90%) muscles studied. Five ECU and four IOD1 muscles showed a decreased MT. Two IOD1 remained unchanged. The mean MT with (± SD) of the IOD1 increased significantly from 6·3 mm (± 3·2 mm) to 9·2 mm (± 2·4) ($p = 0{\cdot}004$) (paired *t*-test). PA increased from 5·5° (± 3°) to 11° (± 2·2°) ($p = 0{\cdot}001$) (paired *t*-test) (Fig. 5).

In addition, the statistically significant change in the mean MT (± SD) of the ECU was from 5·5 mm (± 2·5) to 7 mm (± 2·2 mm) ($p = 0{\cdot}039$) and from 5·5° (± 3·4°) to 9·4° (± 3·8°) ($p = 0{\cdot}005$) in the PA (paired *t*-test) (Fig. 6). Overall, the ECU was stimulated in 22 arms and the IOD1 in 26 hands.

The correlation (Pearson correlation) of the MT and PA of the IOD1 at baseline was moderate ($r = 0{\cdot}579$) and proved to be significant ($p = 0{\cdot}002$), whereas the correlation for the same muscle at completion was poor ($r = 0{\cdot}209$) and not significant ($p = 0{\cdot}305$). In contrast, the correlation of MT and PA for the ECU was significant at both baseline ($p = 0{\cdot}000$) and completion ($p = 0.000$) and was considered strong ($r = 0{\cdot}788$) and moderate ($r = 0{\cdot}685$), respectively.

There was a statistically significant correlation (Pearson correlation) of MT for both ECU and IOD1 from the baseline measurement to the completion. The correlation coefficient, both classified as

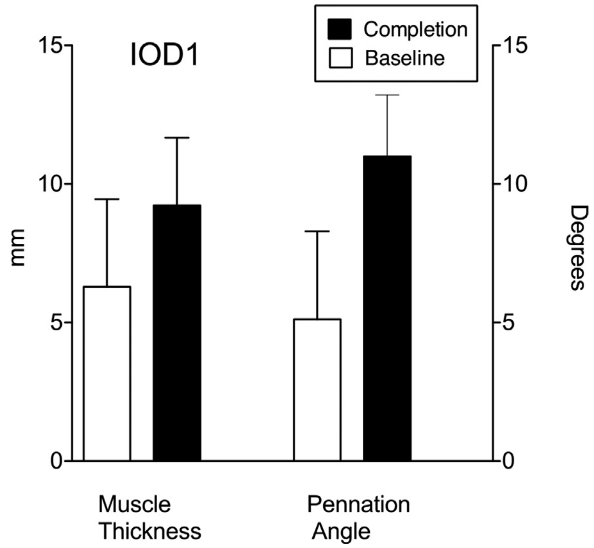


Fig. 5. Effect of electrical stimulation on Muscle Thickness ($p = 0{\cdot}004$) and Pennation Angle ($p = 0{\cdot}001$) of the IOD1 muscle from start (Baseline) to end (Completion). The mean difference for Muscle Thickness was 2·94 mm (± 2·79) and for Pennation Angle 5·87° (± 3·31) (paired *t*-test). $n = 20$. Values are expressed as mean + SD.

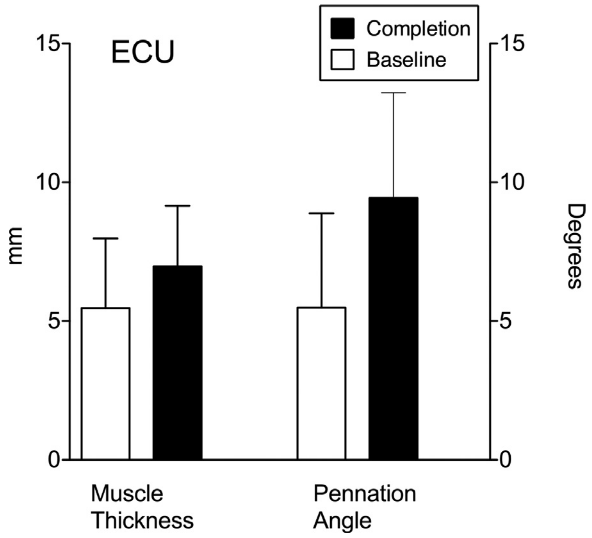


Fig. 6. Effect of electrical stimulation on Muscle Thickness ($p = 0{\cdot}039$) and Pennation Angle ($p = 0{\cdot}005$) of the ECU muscle from start (Baseline) to end (Completion). The mean difference for Muscle Thickness was 1·50 mm (± 2·28) and for the Pennation Angle 3·95° (± 3·56) (paired *t*-test). $n = 20$. Values are expressed as mean + SD.

moderate, was $r = 0{\cdot}58$, ($p = 0{\cdot}037$) for the ECU and $r = 0{\cdot}63$ ($p = 0{\cdot}008$) for the IOD1 (Fig. 7).

All 20 participants completed the questionnaire. The questionnaire showed that both the device itself and the time spent on it was well tolerated. Seventeen participants could imagine continuing with the stimulation for more than two years (Fig. 8).

The study intervention was carried out in all participants without adverse events.

4. Discussion

The present study shows that direct muscle stimulation of a denervated upper limb muscle increases MT and PA over a stimulation period of 12 weeks. IOD1 and ECU muscles respond with a significant increase of both MT and PA. Furthermore, the moderate correlation of MT from the beginning to the end of the stimulation period indicates that this measurement might be a predictor for the extent of MT increase over a defined stimulation period in clinical practice. In contrast to this finding, the increase of MT does not correlate with the increase of PA at the time of stimulation completion in both muscles, possibly due to the heterogeneity of the patient group studied. Indeed, the study population included persons with acute/subacute (days/weeks), traumatic and non-traumatic lesions as well as GBS patients. Assuming that in the acute and subacute phase after a LMN lesion, the PA has only changed to a small extent, the difference to reach its "physiological" level again is small. On the other hand, if the PA is 0° due to long-term degeneration, the difference to reach its physiological level is greater. Similarly, the increase of MT in a degenerated muscle does not progress linearly relative to stimulation time. In the acute/subacute phase after the LMN lesion, contractile muscle fibers are still present and the effect of hypertrophy through direct muscle stimulation is immediately possible. However, the denervation process includes a conversion of slow- to fast-twitch muscle fibers accompanied with a denervation induced muscle atrophy that leads to an adaptation of myosin heavy chain (MHC) expression in which MHC content decreases and the relative proportions of MHC isoforms change. As a result, hybrid fibers expressing multiple MHC isoforms increases in denervated muscle [14]. Electrostimulation elicits muscle contraction with the intent of minimizing muscle atrophy and replacing neural input during denervation [14]. The rationale for using electrical stimulation of denervated muscles is to maintain contractility as the most important factor in regulating the properties of muscle fibers [14]. By eliciting muscle contractions, the muscle is activated and “strengthened” and can at least regain MT to a limited extent. A possible test to prove the effect of stimulation is therefore the measurement of force under stimulation. This might be useful for muscles that involve a high range of motion, come under load and are therefore to be used functionally. Hence, the present study investigated if direct muscle stimulation is able to regain structural integrity of denervated, non-voluntary active muscles, independent of their force generation capacity.

Nevertheless, muscles that have been already degenerated for many years and altered by fat and connective tissue transformation, initially convert to contractile muscle tissue before measurable hypertrophy occurs and evidently this process takes longer time [18]. In the lower extremity muscles examined (vastus lateralis), a stimulation period of approximately one year was indicated [19]. A literature search revealed that there were no study data available for the upper extremities in humans, but animal studies demonstrated that the effect achieved by direct muscle stimulation was not muscle specific regardless of their localization, function and fiber type composition [3]. That observation may imply the usefulness of ES also to any denervated human muscle. Not to be ignored is the fact of the relatively small number of study participants in the current study. That might be another possible explanation why the correlation between MT and PA was not statistically significant over the study period.

Although the interpretation of the differential response across the individual participants to stimulation itself and both muscles is not clear, we acknowledge the importance of both different stimulation-related and muscle architecture and anatomical factors. For example, the sponge electrodes entirely cover the relatively small muscle IOD1 throughout ES. Therefore, the muscle receives a maximal training to increase thickness and to maintain contractibility while performing

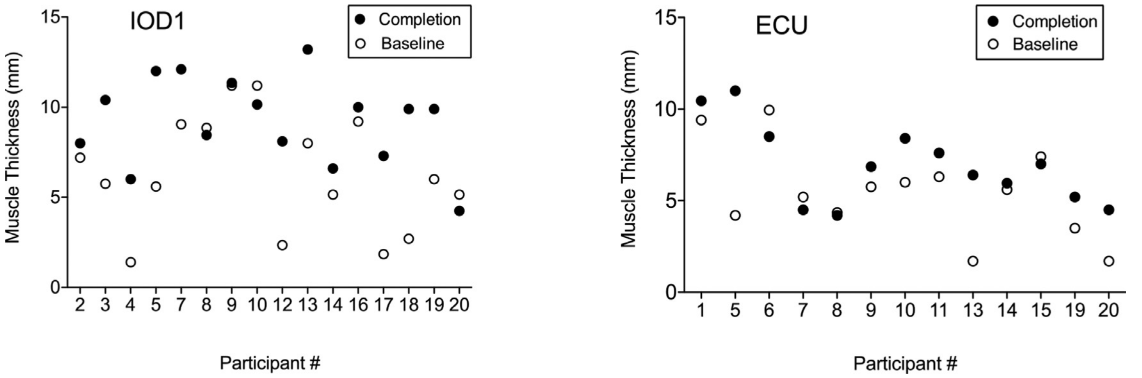
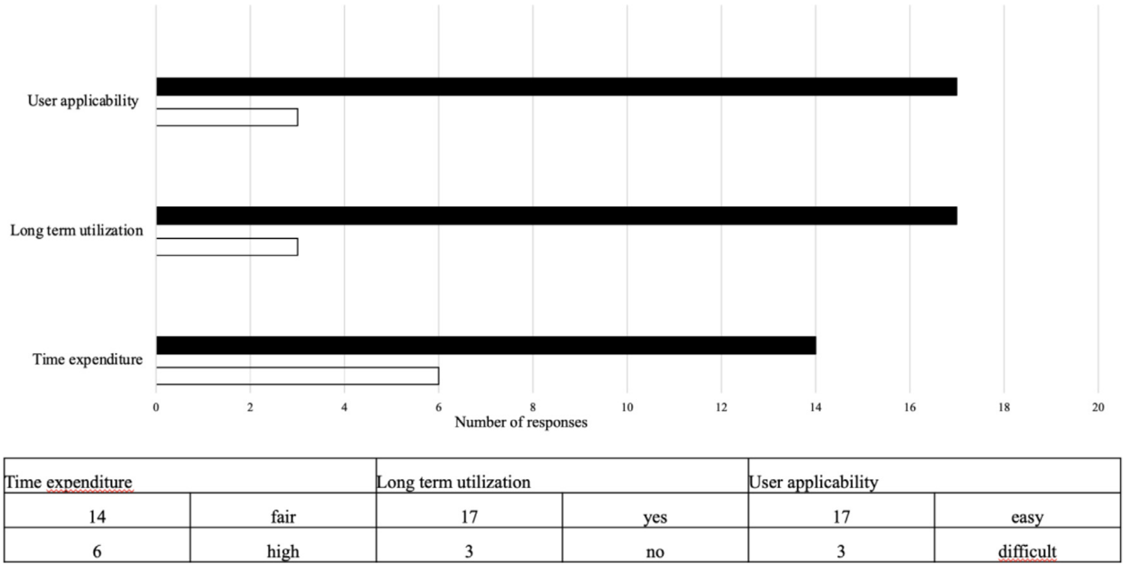


Fig. 7. Effect of 12 weeks electrical stimulation on muscle thickness for each participant.
Correlation coefficient for IOD1: $r = 0{\cdot}63$ ($p = 0{\cdot}008$) and ECU: $r = 0{\cdot}58$ ($p = 0{\cdot}037$) (Pearson correlation), $n = 20$.



Time expenditure		Long term utilization		User applicability	
14	fair	17	yes	17	easy
6	high	3	no	3	difficult

Fig. 8. Questionnaire
Question 1: How do you rate the stimulation device, including electrodes in the application?
Question 2: Could you imagine doing the stimulation for two years?
Question 3: How do you rate the time expenditure to perform the stimulation?, $n = 20$.

active motion under stimulation. In addition, there are other critical stimulation-specific factors such as the optimal stimulation amplitude and pulse shape necessary to induce growth or the number of stimulation sessions to be performed that have to be taken into account in order to explain differences between the muscles studied. In other words, the stimulated muscle should be circumferentially covered by the electrodes along the length of its muscle belly, the pulse shape should cause the greatest possible selectivity, and the amplitude should be high enough to ensure efficient contraction.

Our assumption about the stimulation parameters (shape form and amplitude) is consistent with the findings of Arnold and colleagues [20], who studied ES on denervated facial muscles. When stimulating facial muscles, selectivity is of utmost importance to avoid synkinesia, which would have a counterproductive effect on facial expressions. In addition, especially in the face, a very sensitive region of the body, the current tolerance and the stimulation comfort are of decisive importance. This can be compared to stimulation on a tetraplegic hand, that has hyperesthetic and paresthetic areas. Referring to the stimulation parameter composition, a rectangular pulse shape allows more selective stimulation at low amplitudes. The rise of triangular pulses is flatter, and the energy applied per pulse is half that of rectangular pulses. This may be one reason why patients experience stimulation in triangular pulses as more comfortable than that of rectangular pulses [20]. When setting up the stimulation parameters, we opted for the greatest possible selectivity with a stimulation amplitude calculated as a function of size. In our study, the target amplitude was reached by all participants after a few minutes. Furthermore, the importance of the number of stimulation sessions over the entire stimulation period became noticeable [19]. This might explain why, in the case of the ECU, three out of four patients did not respond to ES.

In order to interpret the different stimulation effects, some muscle design parameters need to be addressed. IOD1 is a bipennate muscle with short muscle fibers and large PAs [21] and thus designed primarily to generate force [7]. This muscle is easy to examine by ultrasound due to its location, size and design. This is in contrast to the ECU, the fourth strongest muscle in the forearm that based on architectural studies and measurements of PCSA [22], may require adjusted stimulation parameters in order to regenerate. Alternatively, muscle hypertrophy may result in an uneven increase of the cross-sectional area over the whole muscle length [23]. In our study the MT and the PA of the ECU were measured at a single scaled position defined relative to the forearm length. A variability of the stimulation effect among these points cannot be ruled out.

While direct ES of denervated lower limb muscles maintains muscle structure and induces hypertrophy [11] , this is the first study that investigated and identified an effect of direct muscle ES in denervated upper limb muscles. The use of electrostimulation for denervated muscles requires a high level of compliance in terms of time, application, and implementation on the part of the person and potential caregivers. The questionnaire of treatment effectiveness reflects the difference of inpatient and outpatient stimulation experience. Out of the 20 participants, seven performed the stimulation at home. Three of them rated the user applicability of the device as difficult and the time expenditure as high. In all cases, the fixation of the electrodes was the most challenging task and not the operation of the device itself. Not surprisingly, those three participants denied a long-term utilization of the treatment. Only three of the inpatients rated the time expenditure as high because they stimulated more than two muscles and thus committed themselves to a 75 min. session including preparation time. Stimulation accuracy can only be secured when the electrodes are prevented from displacement which requires a stable position of the extremity. The time expenditure is always critical because of the well-structured time schedule of a person with tetraplegia in the domestic setting. Additional therapies need to be noticeably beneficial in order to justifying inclusion into daily living. This is especially true for those modalities where there is no immediate effect on function. However, the results indicate that the ES of denervated muscles of the upper limbs could preserve the contractile muscle structures for possible reinnervation or further treatment options as reconstructive hand surgery.

Therefore, a key factor that motivates the effort of daily stimulation for months is the implication for nerve transfer surgery. In persons with tetraplegia who benefit from nerve transfers, ES could help extending time to surgery without risking deteriorating the outcome [17]. If a nerve branch is transferred into a denervated or partially denervated muscle it is reasonable to assume that contractile structure and motor endplate integrity should be optimized to enable successful function restoration. Consequently, it is possible to hypothesize that these morphological conditions might be decisive in denervated flexor muscles and therefore should be investigated as a preconditioning treatment prior to a nerve transfer to reanimate thumb and finger flexion. Thus far, these surgeries have not convincingly shown a high degree of success [15]. Recently, Bersch and coworkers reported a high degree of lower motoneuron lesions in the target muscles flexor pollicis longus and flexor digitorum profundus [24]. ES as described in this study might potentially provide a promising intervention prior to surgery to improve the outcomes in nerve transfers also targeting forearm flexor muscles.

Stimulation can be considered also after nerve transfer. Apart from the structural benefit of ES in denervated muscles, the potential positive effect on motor cortex representation of the hand should be noted. Paralyzed muscles lose their representation on the motor cortex within two months [25]. Neuromuscular electrical stimulation in combination with motor training can, however, increases the excitability of motor cortical areas [26] although it is still unknown whether direct muscle stimulation has a similar effect.

The present study population also included patients with chronic muscle degeneration ($>$ 2 years). Our results indicate that ES has an effect on the mechanism of regeneration. Muscle degeneration after denervation does not occur immediately. In short term, the progressing atrophy of the muscle fibers is accompanied by the capability of the denervated muscle to produce new muscle fibers [11]. Structural changes include a loss of myofibrillar alignment and the loss of myosin and actin filaments, whereas muscle spindle integrity persists after denervation [27]. The capability of a chronically (more than two years) denervated muscle to maintain a satellite cell activity results in the formation of new muscle fibers. Furthermore, the fact that satellite cell activity decreases with age [28] and with time of denervation [27] also needs to be considered. After all, 30% of the study participants were 60 years or older. Consequently, a correlation between time after injury and the extent of structural recovery after stimulation of a muscle might be expected. To address this question a larger number of cases and more homogeneity of the investigated group is required.

In the present study population, there appeared two subgroups, a single individual with a CMT and four persons with GBS syndromes. We did not expect to see a remarkable increase of MT and PA in chronic patients after a 12-weeks stimulation period.

For this reason, the unexpected functional improvements in gross and fine motor skills in both hands in addition to doubling of MT and PA was remarkable. All chronic patients showed a stable neurologic status and reported no functional improvements in the last two or more years after disease onset. We assume that in small muscles with a direct access and superficially located with little fat tissue above, ES acts directly on the target with less overlapping effect on synergistic and/or neighboring muscles. The increase of recovery and the decrease of the atrophic fibers in patients treated with ES indicate a restoration of a muscle and, potentially, its function [18,29]. However, the reason for this functional improvement after years of denervation remains unclear.

There are only a few studies that investigated the effect of ES in GBS. A pilot study applied neuromuscular as well as direct muscle stimulation in the early stage of GBS [30] with the aim to avoid disuse and denervation atrophy of the quadriceps muscle during the neurological recovery [30]. In that study, the GBS disability score improved significantly but not muscle strength. We believe that the stimulated muscles are predominantly denervated and cannot functionally benefit from neuromuscular electrical stimulation (NMES). In contrast, direct muscle stimulation as it was applied in the current study acts as motor relearning vehicle and allows voluntary access to functions.

A logical consequence for future studies is the effect of direct muscle stimulation and its mode of action after nerve transfers on reinnervation and motor learning of restored hand function.

There are limitations as the relatively small sample size in this study that reduces the power of the shown effect. Ultrasound to measure muscle properties as the MT and PA is controversial and discussed. The reproducibility of the probe vis-à-vis the surface as well as the pressure applied to the skin with the probe is critical. This bias has been reduced given that only two trained study staff members performed the ultrasound examinations, in which the ultrasound on the patients' arm/hand and the measurement on the image were executed by the same person.

In conclusion, the early onset of electrical stimulation of denervated muscles in the upper limbs preserves the contractile muscle structure for possible reinnervation or further treatment options. Patients with tetraplegia who could benefit from nerve transfers, may improve target muscle quality as well as extend the time span for undergoing surgery with successful outcomes.

Data sharing statement

The study protocol and the datasets generated during and/or analyzed during the study, including deidentified participant data will be available with publication from the corresponding author on reasonable request.

Contributors

IB conceived the idea and designed the study. JF and IB interpreted the data and wrote and revised the manuscript. Both authors contributed to the literature search. Both, IB and JF have verified the underlying data. The final version of the manuscript was carefully read and approved by the two authors IB and JF.

Declaration of Competing Interest

The authors declare no competing interests in terms of financial and personal relationships.

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Supplementary materials

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