Eur J Trans Myol - Basic Appl Myol 2014; 24 (1): 41-54

History, mechanisms and clinical value of fibrillation analyses in muscle denervation and reinnervation by Single Fiber Electromyology and Dynamic Echomyography

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Abstract

This work reviews history, current clinical relevance and future of fibrillation, a functional marker of skeletal muscle denervated fibers. Fibrillations, i.e., spontaneous contraction, in denervated muscle were first described during the nineteenth century. It is known that alterations in membrane potential are responsible for the phenomenon and that they are related to changes in electrophysiological factors, cellular metabolism, cell turnover and gene expression. They are known to inhibit muscle atrophy to some degree and are used to diagnose neural injury and reinnervation that are occurring in patients. Electromyography (EMG) is useful in determining progress, prognosis and efficacy of therapeutic interventions and their eventual change. For patients with peripheral nerve injury, and thus without the option of volitional contractions, electrical muscle stimulation may be helpful in preserving the contractility and extensibility of denervated muscle tissue and in retarding/counteracting muscle atrophy. It is obvious from the paucity of recent literature that research in this area has declined over the years. This is likely a consequence of the decrease in funding available for research and the fact that the fibrillations do not appear to cause serious health issues. Nonetheless, further exploration of them as diagnostic tools in long-term denervation is merited, in particular if Single Fiber EMG (SFEMG) is combined with Dynamic Echomyography (DyEM), an Ultra Sound muscle approach we recently designed and developed to explore denervated and reinnervating muscles.
Key Words: skeletal muscle, denervation, atrophy, fibrillation, clinical electromyography, Single Fiber EMG (SFEMG), dynamic echomyography (DyEM)

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Skeletal muscle fibrillation refers to small, local muscular contractions that occur in response to spontaneous activation of single muscle fibers which then contract independently of surrounding fibers. This activity contrasts with muscle fasciculations which occur when muscle fibers of a motor unit are stimulated and contract in a synchronous manner.[1-3] Spontaneous fibrillations have been described in cases of certain muscle disorders such as muscular dystrophy,[4] polymyositis [5,6] and amyotrophic lateral sclerosis,[2,7] conditions in which muscle fiber regeneration and /or denervation are often or very often present.[8,9] However, they are considered to be a defining characteristic of motor nerve disruption to the skeletal muscle fiber in humans 10-15 and in animal models of spinal cord injury (SCI) and peripheral denervation.[1,3,16-18] These spontaneous fibrillations are often used clinically to determine the severity and magnitude of neural injury [19-21] and nerve regeneration status.[22] It also has been proposed that these fibrillations could be useful in assessing the muscle

atrophy occurring subsequent to denervation.[11,23] We would like to add the mechanism of pre-neural stages of muscle fiber development that are mandatory events of muscle fiber regeneration.[24]

History

The first recorded observations of muscle fibrillations after denervation have been attributed to Schiff[25] who reported, in his 1851 article entitled "Ubermotorische Lahmung der Zunge," visual observations of fibrillations in the tongue muscles of dogs after bilateral hypoglossal nerve section. In 1915, experiments by Langley and Kato[26,27] were the first to show explicitly that fibrillations followed denervation. Interestingly, three years later Langley and Hashimoto,[28] in continued experiments along the same vein, arguably may have come close to observing length dependency (that is, the currently accepted idea that the time of fibrillation potential appearance is dependent upon the distance of the muscle from the lesion, with potentials appearing earlier when the distance between the neural lesion and the muscle is smaller).[21,29,30] In multiple experiments, Langley and Kato found stronger fibrillations in proximal as compared to distal muscles. These early observations were not necessarily clinically applicable, as they had all been obtained by surgically exposing the denervated muscles and visually inspecting the surface. More reliable means of recording fibrillation potentials became available in the 1930s and, thus, more intense investigation of this phenomenon became possible.[21] In 1937, Brown[1] unilaterally sectioned an unspecified number of cat sciatic nerves. He then allowed 4 to 27 days of denervation to pass before exposing the gastrocnemius muscles and examining them both visually for fibrillations and electrically for fibrillation potentials with concentric needle electrodes. From this work, Brown concluded that: i) fibrillation potentials "*…are to be attributed to the activity of single muscle fibers*"; and ii) "*These observations are in agreement with those of Langley and Kato, who stated that fibrillation does not commence until the fifth day after nerve section.*" In 1944, Feinstein[18] reported a large number of human nerve injury cases related to the Second World War. They noted that"*… in no case of proved axonal interruption have we failed to find repetitive action potentials indicative of fibrillation*." In the same article, they reported that, in a series of 25 patients undergoing laminectomies for prolapsed disks, the earliest onset of electrically detectable sacrospinalis muscle fibrillation potentials occurred 12 days after surgery. Publications by Feinstein et al.(1945)[27] and Denny-Brown and Pennybacker (1938)[2] became the cited basis upon which the timing of onset of fibrillation potentials was determined. In essence, these 2 articles, particularly the former, defined the accepted wisdom as typified by Jasper and Ballem in 1949[31]: "*Suffice it to repeat here that fibrillation…was found most active two to four weeks following nerve lesion*." The often cited Eaton and Lambert paper[32] "*Electromyography and Electric Stimulation of Nerves in Diseases of Motor Unit*" from 1957 summarized fibrillation potential timing as: "*They are observed regularly in denervated muscle, beginning 2 to 3 weeks after interruption of the axon and persisting for a variable time up to many years thereafter*."

To determine the length dependent nature of the onset of fibrillation potentials, Luco and Eyzaguirre[29] experimentally sectioned the sciatic nerve of two groups of animals: 1) the sciatic nerves of one group were sectioned high in the pelvis, while 2) the sciatic nerves of the animals in the second group were sectioned at the nerve twig to the tenuissimus muscle; the result was that there was a difference in residual nerve length of 25–35 mm between the two groups. In the former group, fibrillation potentials were first detectable at 140 h, whereas in the latter, fibrillation potentials were present in all by 120 h. From this work, the investigators concluded: "*…if the cut portion of a nerve is short the above phenomena [fibrillation potentials] appear earlier than when a greater length of nerve is left to degenerate*." Salafsky et al.[30] published similar findings in 1968 based on experiments designed to determine if there was a difference between the onset of fibrillation potentials in fast versus slow twitch muscle and to investigate length dependency of onset. As with Luco and Eyzaguirre,[29] they had demonstrated length dependency, however, only in muscles presumed to be slow twitch. The significance of this result is unclear, as neither the sampling interval nor the number of data points was detailed in the article. With the onset timing of fibrillation potentials issue thought to be relatively settled, scientific study in the late 1940s and early 1950s shifted more toward study of the underlying mechanism of fibrillation potentials and their origin.

Origins and consequences of muscle fibrillation

Obviously, when a muscle is severed from its nerve, the muscle loses both the electrical stimulus the nerve provided as well as any chemical "neurotrophic" factors the nerve would have supplied. During the 1940's and 50's, Jarcho et al.33 expanded upon the "neurotrophic" hypothesis as put forward by Tower et al.34 in 1941, to experimentally localize the origin of fibrillation potentials to the end-plate. In 1957, Li et al.35 experimentally determined "*…membrane resting potentials of denervated muscle fibers tended to oscillate.*" This was ultimately an important finding, as membrane oscillation is one of the key concepts in the etiology of fibrillation potentials. Over the years, researchers have investigated losses of both electrophysiological input as well as potential neurotrophic factors as possible origins of fibrillation potentials and have provided numerous reports which ultimately demonstrate that it is mainly the loss of

electrophysiological stimulation that is responsible for the changes that occur in the sarcolemmal membrane and propagate the spontaneous fibrillations. In turn, the fibrillations themselves may affect cellular metabolism and muscle fiber size.

Electrophysiological factors

Numerous studies report that changes in resting membrane potential (rmp) occur within a few days of denervation, concomitant with the onset of fibrillation potentials.[36-40] Indeed, a 10-15 mV depolarization of denervated muscle membrane has been rather consistently reported,[21,41] and the onset of this change in rmp begins sooner when the denervation is closer to the muscle.[42,43] Purves and Sakman[44] reported in 1974 that spontaneous fibrillations arose from both rhythmically and irregularly discharging fibers. Their studies showed that the rhythmic fibrillations were associated with membrane potential oscillations while random discrete depolarizations were responsible for the fibrillation of the irregularly discharging fibers. Both types of event were inhibited when they removed sodium from the extracellular solution of the rat diaphragm preparations and by treatment with the sodium channel blocker tetrodotoxin (TTX). The researchers concluded, therefore, that both fibrillation types arose from localized changes in sodium conductance. This was supported by subsequent studies of Thesleff and Ward[45] whose work on denervated extensor digitorum longus (EDL) muscle in rats also revealed that fibrillation potentials result from spontaneous biphasic membrane potential oscillations of increasing amplitude. They suggested that the hyperpolarizing phase of the oscillation relieves sodium channel inactivation so that more of these channels may open during the depolarizing phase of the oscillation, allowing the depolarizing phase to exceed threshold and produce an action potential. They pointed out that this mechanism, important for the initiation and continuation of fibrillation potentials, is similar to anode break excitation and suggested that the physiological calcium levels in denervated muscle may not be sufficient to stabilize muscle fiber membranes. Further, Smith and Thesleff[46] used denervated mouse diaphragm muscle to demonstrate that spontaneous fibrillations were induced by changes in sodium conductance and could be abolished by disruption of the t-tubule system. Gage and co-workers described a persistent TTX-sensitive (Na^+) current of similar amplitude in both innervated and denervated fibers and suggested that this could bring about fibrillation potentials in the denervated muscles (which have an increased input resistance) by producing greater depolarization in the denervated fibers.[44=47] Pappone characterized sodium currents in innervated and denervated rat skeletal muscle.[48] She noted that activation and inactivation of sodium currents were shifted to more negative potentials by 10 mV and suggested that, because the resting membrane potential is depolarized relative to normal muscle, that the shift in activation could explain the fibrillations. These shifts in voltage dependence of both activation and inactivation, however, were not detected in rabbit muscle preparations.[49] Instead, changes in channel inactivation were detected that would be expected to accelerate recovery from fast inactivation during the after potentials and, thereby, increase membrane excitability. Another proffered explanation for increases in sodium conductance in denervated muscle comes from [^{3}H]-ouabain binding studies which suggest that there is an increase in the number of Na^+/K^+ ATPase molecules on the cell membrane of denervated cat muscle.[50] Pappone also reported the discovery of a TTX-resistant sodium current.[45=48] Expression of the TTX-resistant "cardiac-type" sodium channel has indeed been reported in denervated muscle[51,52] and Sekiguchi and colleagues have recently reported a TTX-resistant channel that is expressed in denervated muscle with the onset of fibrillation potentials.[53] This latter group used northern blot analysis to demonstrate that the TTX-resistant "cardiac-type" voltage-gated Nav1.5 sodium channel mRNA is up-regulated by day 2 post denervation in rat EDL and that its expression increases on day 3 with obvious decreased expression by day 6. Immunohistochemistry using confocal microscopy revealed that Nav1.5 protein abundance continued to increase past day 3, with day 6 having the greatest signal. Indeed this time frame corresponds well with their measured onset of spontaneous fibrillations. Further, treatment of the animals with lidocaine (which has a greater affinity for TTX-resistant sodium channels than for the TTX-sensitive ones) decreased fibrillation activity without affecting compound muscle action potentials. This suggests that: 1) the lidocaine worked as a selective Nav1.5 blocker; and 2) Nav1.5 channels contribute to the production of TTX-resistant spontaneous fibrillations. It is not possible that these TTX-resistant channels are solely responsible for spontaneous fibrillation activity, however, because the membrane potential oscillations and random discrete depolarizations reported to be responsible (at least in part) for fibrillation [37=40,41=44,51=54] are described as TTX-sensitive.[43=46,52=55] Indeed, Prabhu and Oesterhave reported that the spontaneous fibrillation potentials of denervated rabbit muscle are TTX-sensitive.[56]

Changes in potassium currents and channel expression have also been reported to occur in skeletal muscle membrane soon after denervation. For example, decreases in potassium permeability have been reported to occur in denervated muscle[57,58] and it is suggested that this could alter the sodium to potassium permeability ratio and contribute to the decline in membrane potential.[59] Using patch clamp of membrane vesicles from 8-16 day denervated mouse EDL,

Escobar and co-workers[60] reported the disappearance of an 81 pS voltage-dependent K^+ channel from the denervated muscle and suggested this may contribute to membrane depolarization and reported decreases in K^+ permeability.[61] However, they also reported the appearance of a 16 pS channel in denervated muscle similar to SK channels. Indeed, also using patch clamp, Neelands and colleagues described a small conductance calcium-activated K^+ (SK) current in denervated mouse skeletal muscle which was absent from innervated controls.[62] Citing studies which show that a specific inhibitor of SK channels suppresses both hyper-excitability in denervated rat muscle[63] and the myotonic runs in patients with myotonic muscular dystrophy,[64] Neelands and collaborators suggested that this SK channel contributes to the hyper-excitability of denervated skeletal muscle.[62] Because SK channels are found in T-tubules, these investigators further suggested that potassium ions may pass into and accumulate inside the tubules and produce a localized depolarization in an area on the membrane and, thus, contribute to the propagation of fibrillation action potentials.

Some studies also suggest that changes in cellular calcium levels/handling may be involved in modulation of fibrillation potentials. Indeed, it has been shown that post denervation, intracellular calcium levels increase in skeletal muscle[65] and that the rate of calcium uptake into the sarcoplasmic reticulum (SR) declines;[66] this could contribute to membrane depolarization. Accordingly, increases in extracellular calcium have been demonstrated to decrease the spontaneous activity in denervated muscle

careful studies, Lomo and co-workers demonstrated that chronic electrical stimulation of denervated rat muscles prevented the development of sensitivity to ACh and further caused ACh-sensitivity to disappear from denervated muscles already ACh supersensitive.[80,81] These studies strongly demonstrated that the muscle sensitivity to ACh is dependent on electrical input to the muscles and not on trophic factors contributed by the nerves.[82]

Metabolic factors

In 1945, Feinstein and associates demonstrated with animal models that the time to onset of fibrillation potentials is decreased by thyroidectomy while treatment with desiccated thyroid increases it.[27] Further, they showed that small changes in temperature have a large direct effect on spontaneous fibrillation in denervated skeletal muscle. These data suggest that the level of spontaneous fibrillation activity is related to muscle metabolic rate; however, (as has been noted by numerous authors) it is often difficult to determine which changes contribute to development of fibrillation and which are a consequence of it. Indeed, denervated muscle exhibits decreased levels of glycogen[83-85] and changes in activity levels of numerous enzymes (e.g., decreased succinic dehydrogenase[86] and cytochrome oxidase activities[87] with increased hexokinase activity[88]) and other factors involved in carbohydrate metabolism.[83,84,86,88] In 1950, Humoller and co-workers compared metabolic parameters in denervated and tenotomized muscle and found that glycogen levels dropped in both sets of samples and that in the tenotomized muscles this decline started before the commencement of the fibrillation they detected, suggesting that decreased glycogen levels may contribute to production of the spontaneous activity.[83] This group also carefully assayed for inorganic phosphates, ATP and creatine phosphate to address inconsistencies in reports of these parameters in the (even) older literature. They reported increased phosphocreatine and drops in organic phosphates within 24 hrs; however, after 3 days, while the organic phosphate was still low, the phosphocreatine returned to near normal. ATP levels remained nearly constant for the first 5 days post denervation, but then began to slowly drop afterward. In 1954, Ware and co-workers suggested that the decreased rmp of denervated skeletal muscle could be brought about by the metabolic changes which deprived the tissue of adequate energy supplies and could disrupt the sodium pump which contributes to maintenance of the rmp.[40] Subsequently, Corsi and colleagues severed the sciatic nerve of the hind limbs of dogs and measured both lactate oxidation and fibrillation potentials.[89] They report that lactate oxidation levels and fibrillations were low and absent, respectively, for the first four days post denervation and were both most obvious at 10-20 days post procedure. The lactate oxidation decreased when fibrillation activity was no longer continuous. Interestingly, when they suppressed the fibrillation activity with either dihydroquinidine or procainamide, the lactate oxidation did not increase in the denervated muscles. The researchers suggest that the increase in lactate oxidation that occurs with denervation is caused by fibrillation. Izumi and associates measured fibrillation potentials in denervated rat muscle under both ischemic (blood flow interrupted) and hypoxic conditions.[90] They reported that the fibrillations disappeared within minutes under the ischemic conditions and reappeared with blood flow, suggesting that blood may provide a factor important to fibrillation potential initiation. Although fibrillation potential amplitudes were not affected significantly by the low oxygen conditions, the fibrillation firing rate was positively correlated with both PaO_2 and temperature. The group suggested that the rate of fibrillation potential initiation is proportional to the rate of aerobic metabolism.

Cell Factors.

One of the last result of myology research is the discovery in denervated muscle fiber of the surprising extent of satellite cells activation, proliferation (they indeed increase in number), fusion to myotubes and grow to regenerated muscle fibers that reach a third of those innervated even in aneural muscles, much more than that usually happens in muscle cultures (see for review in this issue Bruce M. Carlson,[3]). As it is well known from *in vitro* myogenesis, the new fibers soon or later start to contract and thus it is conceivably that they may contribute to fibrillation of denervated muscles, in particular of long term denervated muscles (see above and below for re-expression of embryonic genes, in particular of the myosin heavy chains genes).[24,94-103]

Fibrillation Potentials and Atrophy

In the early nineteenth century, it was suggested that the atrophy which occurs subsequent to denervation was a consequence of the excessive energy use incurred by the spontaneous fibrillations and so denervation-induced atrophy was referred to as "*fatigue atrophy*".[85,104-109] However, in contrast to this idea, Fischer and collaborators (1939) showed that stimulation of denervated muscle slowed the loss of muscle (dry weight) and excitability, suggesting that the fibrillation activity might have a similar effect and, therefore, not cause but (in fact) inhibit muscle loss.[105] The "fatigue atrophy" theory was indeed dealt a big blow in 1940 when researchers demonstrated that inhibiting fibrillation in denervated muscle with either quinidine or quinine did not stop atrophy.[15,106] More recently, Herbison and co-workers (1983) demonstrated that electrical stimulation could actually reduce the degree of atrophy and the number of

fibrillations in denervated muscle.[107] Accordingly, Lewis and colleagues (1988-89) showed that the rate of atrophy in the denervated muscle of rats and guinea pigs varies inversely with the percentage of fibers which exhibit fibrillation,[108,109] suggesting that the fibrillations actually inhibit muscle atrophy to some degree. Studies also show that fiber type transitions known to occur in denervated rat muscle may stem from the differences in the fibrillatory properties of their muscles.[110,111] Thus, it is commonly accepted that the stimulation from fibrillation potentials actually counteracts muscle loss to some extent.

Diagnostic and prognostic values of fibrillations in peripheral nervous system injuries and disorders

Diagnostic value of fibrillations in peripheral nervous system injuries and disorders

Clinically, the presence or absence of fibrillation potentials is used to help determine whether a nerve injury is either axonal or demyelinating in nature. Fibrillation potentials are easily detected by electromyography (EMG) and, in general, are first detected within days to 1-4 weeks post denervation.[17,23] The variable time of onset is attributed to nerve degeneration being length-dependent and, therefore, fibrillation potentials are expected to occur earlier in muscles closer to the site of nerve injury than in muscles further away.[112,113] It is common in medical practice due to the intrinsic slowness of nerve recovery, however, to wait 3 to 4 weeks after an injury to do needle EMG studies to ensure that, should the injury be axonal, the electrical evidence for it is present.[114] This time frame was not derived from

Usefulness of fibrillations for monitoring therapeutic interventions: human case reports and anecdotal experiences

Wallerian degeneration is the process whereby nerves degenerate after axonal injury. It is characterized histologically by axonal blebbing and fragmentation into ovoids.[125,126] The clinical correlation of Wallerian degeneration is generally taken to be failure of electrical conduction *by the nerve distal to the site of injury* and the onset of denervation potentials.[112-114,124,125] In turn, this information is used to prognosticate the time course and extent of recovery in response to therapeutic interventions.

An example is the case of a 47 year old man with a complete right median nerve lesion at the wrist, following an injury by cutting of the forearm. The complex trauma also caused a lesion of the radial artery, a total cross-section of the deep flexor tendon of the first toe and an injury to the flexor carpi radialis tendon. Three hours after the trauma, the patient underwent a surgical suture of artery, tendons and neurorrhaphy of the median nerve. After 10 days of healing, the patient was submitted to a neuromuscular electrical stimulation protocol 5 times a week, each session lasting 30 minutes, using these parameters: pulse duration 0.3 msec, current intensity < 25 mAmp (according to pain sensation), stimulation frequency 50 Hz for 1 second and pause of 4 seconds. After 1 month of this training protocol, the patient reported an improvement of subjective sensitivity of the second and third fingers of the hand. At that time, we performed a dynamic echomyographic scan of the of the tenar muscles (TM) that showed initial muscular atrophy (innervated left hand 14.5 mm vs. denervated right hand (13.3mm) and an increased echogenicity of the muscle, both accepted direct signs of denervation. To avoid muscular wasting, we decided to combine the neuromuscular electrical stimulation protocol with direct stimulation of the denervated tenar muscles, using the following parameters with the Demitalia SM1, Stimulator for Denervated Muscle of the Medical Technology,Turin (Italy): rectangular biphasic waves, pulse duration 150 msec and pause of 2 seconds, applied every day for 30 minutes at 5 mAmp. Two months after the trauma, the needle EMG exam of the tenar muscle showed low level of spontaneous activity (fibrillations) and no voluntary recruitment of motor units. The nerve conduction studies showed no sensitive response of the median nerve with orthodromic stimulation of the first and third fingers.

After an additional 4 months, the EMG showed minor modifications of the spontaneous activity, further increase of subjective sensitivity of the second and third fingers of the hand, but absence of volitional activity of the tenar muscles. On the other hand, under dynamic echomyography, all the tenar muscles responded with clear contractions when electrically stimulated at 10 mAmps, a threshold far from pain sensation. It is worth mentioning that electrical stimulation achieved the goal of maintaining the left denervated tenar muscles (14.0 mm) almost at the thickness of the contralateral innervated left hand (14.4 mm) and without any worsening during the period from 2 to 6 post-denervation months. Taken together, these results are clear evidence that combining fibrillation analyses with dynamic echomyography to monitor the denervation phase and to follow the rate of atrophy along with the effectiveness of the electrostimulation can help to develop a therapy that is finely tuned to specific patient's needs.[120,130]

Possible role of Single Fiber EMG in the follow up of peripheral nerve injuries

Single Fiber EMG (SFEMG) can be used to follow the course of reinnervation. The parameters of fiber density, mean jitter and percent blocking must each be followed and related to the type of injury to determine the stage of reinnervation.[131] The amplitude of the action potential recorded with an SFEMG electrode from an average muscle fiber decreases to 200 microvolts (μV) when the electrode is approximately 300 μm from the muscle fiber. Thus, we can infer that action potentials greater than 200 μV arise from muscle fibers within 300 μm of the recording surface. By measuringmany sites within a muscle, the mean number of time-locked action potentials (APs) that have an amplitude greater than 200 μV and rise time of less than 300 microseconds (μs) can be used to calculate the fiber density, which quantitates the local concentration of muscle fibers within the motor unit. This provides information that is analogous to type grouping in muscle biopsies. Also Bertorini et al. found that increased muscle-fiber density, delineated by SFEMG, was most prominent in diseases of ordinary denervation, namely motor neuron disorders and peripheral neuropathies, and it correlated with histochemical fiber type grouping.[134] SFEMG was also used to evaluate chronic demyelinating neuropathies, where fiber density was significantly increased in patients with fibrillation as measured with the conventional needle EMG. The study showed that the SFEMG is mildly abnormal in many patients with demyelinating neuropathy and that this test is useful in detecting and quantitating axonal degeneration in demyelinating neuropathy.[133]

In 1974 Hakelius evaluated the pattern of reinnervation in 26 free autogenous muscle transplants, performed in 21 patients with facial palsy using EMG and SFEMG recordings. These researchers reported that SFEMG revealed that increased jitter and blockings occurred in the first months. The researchers further reported that action potential complexes became progressively more stable, but blockings remained detectable even after 12 months.[135] Gray and colleagues used microstimulatory single-fiber electromyography to assess reinnervation

after motor nerve transplantation in rabbits.[136] Considering these data, we suggest that muscle fiber density data along with the quantitative analysis of fibrillation activity using the SFEMG technique could be the best approach for assessment of the denervation-reinnervation process in peripheral nerve injury.

Equally important are the results of Hofer et al. (2005) in the analyses of the electrophsiological properties of single muscle fibers in the permanently denervated muscle fibers of RISE subjects before and during h-b FES.[137] Dynamic Echomyography will, indeed, have even higer impact if combibed with stimulation needle electromyography (SNMEG) to test the electrophysiological properties of single muscle fibers. Hofer et al. measured the muscle fiber conduction velocity (MFCV) and the shortest interstimulus interval (ISI) still eliciting a response to the second stimulus delivered to the fiber. MFCV recorded in the denervated patients before and after h-b FES therapy showed a significant increase in conduction velocity (fastest and mean CV) and reduced refractory periods (shortest ISI). This suggests that electrical stimulation training is effective to improve the electrical properties of the muscle fibre and SNEMG could serve as an additional measurement technique to specify the status of the denervated muscle.

Fibrillation analyses: EMG and DyEM in the follow-up of denervation atrophy and h-b FES of denervated/reinnervating muscle

As a result of information gathered from the Rise2-Italy project, we have proposed and implemented a new protocol of quantitative ultrasonography (dubbed Dynamic Echomyography to stress the functional components of the analyses) for patients suffering with permanent or transitory Lower Motor Neuron denervation.[130,131,138] The protocol have been used to evaluate changes in Tibialis Anterior, Deltoid, and Quadriceps muscles undergoing h-bFES by either neuromuscular stimulation or direct surface stimulation of the denervated/reinnervating muscles.

A case of a subject with a lesion of the right sciatic nerve was followed for 12 months, 6 months for a subject suffering left brachial palsy and 8 months for a Spinal Cord Injury ASIA A subject suffering incomplete Conus Cauda Syndrome, we analyzed the denervated/reinnervating muscles before and during h-bFES training.r. When possible, we compared the affected muscles to the contralateral normal muscles using ultrasound to record: 1. Gross morphology and sonographic structure; 2. Tissue thickness; 3. Dynamic properties of voluntary and electrical stimulation-induced contraction-relaxation cycles; and 4. Short-term and long-term modifications of arterial perfusion in response to volitional and electrical stimulation-induced contractions. Morphology and ultrasonographic structure of the denervated muscles changed during the period of h-bFES training from a pattern typical of muscle atrophy to a pattern which might be considered "normal" when detected in an older patient. Thickness improved significantly more in the middle than in the proximal and distal thirds of the denervated muscles, reaching approximately the same thickness of the contra lateral innervated muscle in a year. In all measurements, arterial perfusion of the denervated muscles showed a low resistance pattern with Doppler ultrasonography at rest, and a pulsed pattern after several months of h-b FES, more similar to the triphasic high-resistance pattern of the innervated muscles. Contraction-relaxation kinetics, measured by recording movements during electrical stimulation, showed an abnormal behavior in the denervated muscles., In agreement with many experimental animal models and clinical human observations, contraction-relaxation kinetics of the denervated muscle were significantly longer than in the normal muscle, particularly during relaxation was,. The very high current energy needed to activate the denervated muscles, according to the Vienna h-b FES strategy, is strong evidence that the explored muscles were still denervated. If they recovered volitional function, the energy of muscle activation went down to the values of motor point stimulation of innervated/reinnervated muscles.

All together, our observations confirm that h-b FES, EMG and DyEM and are useful for diagnosis and adjustment of treatments of denervated and reinnervating muscles. In the latter case, Dynamic Echomyography provides information on the muscles and/or muscle parts contracting under voluntary or electrical activation. We are confident that combining sound invasive "time zero" and "end-point" analyses (e.g., tissue biopsy, 2D or 3D Color CT macro-morphometry with noninvasive, bed-side repeatable SMEMG (including fibrillation recording) and DyEM will allow researcher to address some of the following open issues: 1. Reliable evaluation of the extent of muscle denervation and reinnervation; 2. Reliable quantification of the progression of atrophy to degeneration in longstanding muscle denervation; 3. Influence (positive or negative) of electrical stimulation on muscle reinnervation (in well-defined sub-groups of patients). In this manner, these techniques will contribute to the much-needed evidence-based approaches in Physical Medicine and Rehabilitation.

Summary

Fibrillation potentials of skeletal muscle are small spontaneous action potentials which are considered to be a hallmark response of skeletal muscle to denervation. They have been shown to arise from both oscillations in membrane potential and discrete membrane depolarizations resulting from changes in both sodium and potassium permeabilities. The

initial atrophy in denervated muscle and are sometimes used to assess the degree of neural injury and reinnervation that have occurred. It is suggested that these may also be useful to assess the degree of atrophy that exists. For patients with peripheral nerve injury, therapeutic interventions such as electrical muscle stimulation may be helpful in preserving the contractility and extensibility of denervated muscle tissue and in retarding muscle atrophy. There is no doubt that the literature highlights controversy over the benefits of electrical stimulation for peripheral neuropathies, but the overall goal of all is to keep muscle tissue healthier and viable until reinnervation is established. Monitoring fibrillations may be useful to verify that electrical stimulation is not detrimental to terminal nerve growth (i.e., the stimulus parameters are wisely selected and the amplitude is applied at a level that does not fatigue the regenerating nerve or stress the healing tissue). It is obvious from the paucity of recent literature that research in this area has declined over the years. This is likely a consequence of the decrease in funding available for research and the fact that the fibrillations do not appear to cause serious health issues. Nonetheless, further exploration of fibrillations as diagnostic tools is merited, in particular if combined with Dynamic Echomyography, an Ultra Sound muscle approach we designed and developed to explore denervated and reinnervating muscles.

Acknowledgements

The authors wish to thank the editor and editorial board of The European Journal of Translational Myology for the opportunity to write this review paper. It is an honor.
In memory of Herwig Thoma, without his creativity and his Vienna FES Meetings, neither the Vienna-Padua collaborations nor the Padua Muscle Days would have existed, nothing to say, the European Journal of Translational Myology.

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List of Abbreviations

Ach, acetylcholine, AChR, acetylcholine receptor, AP, action potential, DyEM, Dynamic EchoMyography; EDL, extensor digitorum longus, EMG, electromyography, h-b FES, home-based functional electrical stimulation, ISI, inter-stimulus interval, MFCV, muscle fiber conduction velocity, NMES, neuromuscular electrical stimulation, rmp, resting membrane potential, SCI, spinal cord injury, SFEMG, Single Fiber electromyography, SNMEG, Stimulation needle electromyography, SK, small conductance calcium-activated potassium channel (or its current), SR, sarcoplasmic reticulum, TTX, tetrodotoxin

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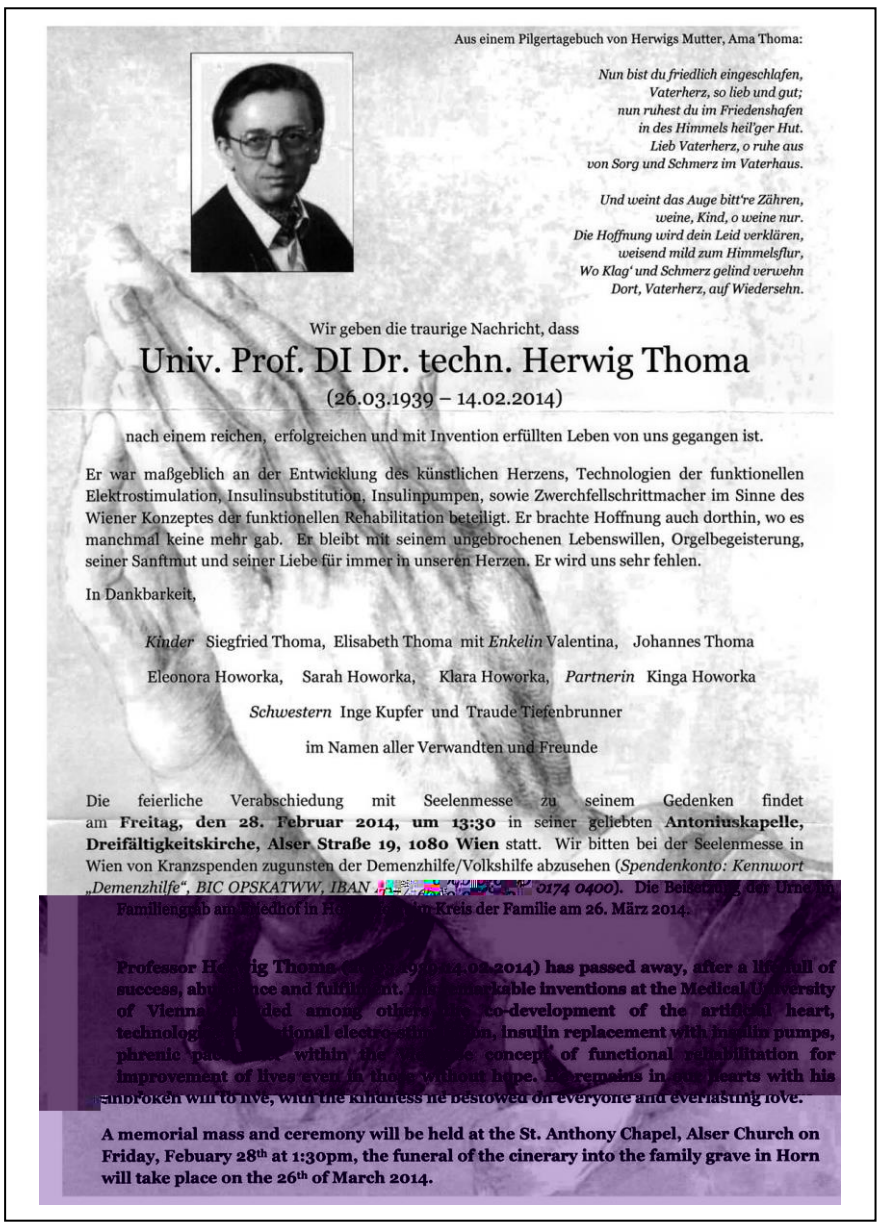

Aus einem Pilgertagebuch von Herwigs Mutter, Ama Thoma:

Nun bist du friedlich eingeschlafen,
Vaterherz, so lieb und gut;
nun ruhest du im Friedenshafen
in des Himmels heil'ger Hut.
Lieb Vaterherz, o ruhe aus
von Sorg und Schmerz im Vaterhaus.

Und weint das Auge bitt're Zähren,
weine, Kind, o weine nur.
Die Hoffnung wird dein Leid verklären,
weisend mild zum Himmelsflur,
Wo Klag' und Schmerz gelind verwehn
Dort, Vaterherz, auf Wiedersehn.

Wir geben die traurige Nachricht, dass

Univ. Prof. DI Dr. techn. Herwig Thoma

(26.03.1939 – 14.02.2014)

nach einem reichen, erfolgreichen und mit Invention erfüllten Leben von uns gegangen ist.

Er war maßgeblich an der Entwicklung des künstlichen Herzens, Technologien der funktionellen Elektrostimulation, Insulinsubstitution, Insulinpumpen, sowie Zwerchfellschrittmacher im Sinne des Wiener Konzeptes der funktionellen Rehabilitation beteiligt. Er brachte Hoffnung auch dorthin, wo es manchmal keine mehr gab. Er bleibt mit seinem ungebrochenen Lebenswillen, Orgelbegeisterung, seiner Sanftmut und seiner Liebe für immer in unseren Herzen. Er wird uns sehr fehlen.

In Dankbarkeit,

Kinder Siegfried Thoma, Elisabeth Thoma mit *Enkelin* Valentina, Johannes Thoma
Eleonora Howorka, Sarah Howorka, Klara Howorka, *Partnerin* Kinga Howorka
Schwestern Inge Kupfer und Traude Tiefenbrunner
im Namen aller Verwandten und Freunde

Die feierliche Verabschiedung mit Seelenmesse zu seinem Gedenken findet am **Freitag, den 28. Februar 2014, um 13:30** in seiner geliebten **Antoniuskapelle, Dreifältigkeitskirche, Alser Straße 19, 1080 Wien** statt. Wir bitten bei der Seelenmesse in Wien von Kranzspenden zugunsten der Demenzhilfe/Volkshilfe abzusehen (*Spendenkonto: Kennwort „Demenzhilfe", BIC OPSKATWW, IBAN [illegible] 0174 0400*). Die Beisetzung der Urne im Familiengrab am Friedhof in Ho[illegible] im Kreis der Familie am 26. März 2014.

Professor He[illegible]ig Thoma [illegible]4.02.2014) has passed away, after a life full of success, abu[illegible]nce and fulfilment. [illegible]kable inventions at the Medical University of Vienna [illegible]ded among others the co-development of the artificial heart, technolog[illegible]tional electro-stim[illegible]n, insulin replacement with insulin pumps, phrenic 'pac[illegible]' within the [illegible] concept of functional rehabilitation for improvement of lives even in those without hope. He remains in our hearts with his unbroken will to live, with the kindness he bestowed on everyone and everlasting love.

A memorial mass and ceremony will be held at the St. Anthony Chapel, Alser Church on Friday, Febuary 28th at 1:30pm, the funeral of the cinerary into the family grave in Horn will take place on the 26th of March 2014.