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Exciting perspectives for Translational Myology in the Abstracts of the 2018Spring PaduaMuscleDays: Giovanni Salviati Memorial – Chapter IV - Abstracts of March 17, 2018

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Abstract

Myologists working in Padua (Italy) were able to continue a half-century tradition of studies of skeletal muscles, that started with a research on fever, specifically if and how skeletal muscle contribute to it by burning bacterial toxin. Beside main publications in high-impact-factor journals by Padua myologists, I hope to convince readers (and myself) of the relevance of the editing Basic and Applied Myology (BAM), retitled from 2010 European Journal of Translational Myology (EJTM), of the institution of the Interdepartmental Research Center of Myology of the University of Padova (CIR-Myo), and of a long series of International Conferences organized in Euganei Hills and Padova, that is, the PaduaMuscleDays. The 2018Spring PaduaMuscleDays (2018SpPMD), were held in Euganei Hills and Padua (Italy), in March 14-17, and were dedicated to Giovanni Salviati. The main event of the "Giovanni Salviati Memorial", was held in the Aula Guariento, Accademia Galileiana di Scienze, Lettere ed Arti of Padua to honor a beloved friend and excellent scientist 20 years after his premature passing. Using the words of Prof. Nicola Rizzuto, we all share his believe that Giovanni "*will be remembered not only for his talent and originality as a biochemist, but also for his unassuming and humanistic personality, a rare quality in highly successful people like Giovanni. The best way to remember such a person is to gather pupils and colleagues, who shared with him the same scientific interests and ask them to discuss recent advances in their own fields, just as Giovanni have liked to do*". Since Giovanni's friends sent many abstracts still influenced by their previous collaboration with him, all the Sessions of the 2018SpPMD reflect both to the research aims of Giovanni Salviati and the traditional topics of the PaduaMuscleDays, that is, basics and applications of physical, molecular and cellular strategies to maintain or recover functions of skeletal muscles. The translational researches summarized in the 2018SpPMD Abstracts are at the appropriate high level to attract endorsement of Ethical Committees, the interest of International Granting Agencies and approval for publication in top quality international journals. The abstracts of the presentations of the March 16, 2018 Padua Muscle Day and those of the remaining Posters are listed in this chapter IV. The Author Index of the 2018Spring PaduaMuscleDays follows at page 78.

Key Words: Giovanni Salviati, proof of concept, translational myology, PaduaMuscleDays

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Abstracts of the 2018Spring PaduaMuscleDay, March 17, 2018

Pain&Mobility, past, present, future: Topics to be discussed

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Key words: Pain&Mobility, past, present, future: Topics to be discussed

An incomplete list of topics follows that should be discussed between experts on still open scientific question,[157-163] such as:

A - 10 years post RISE

1. Training parameters on DDM
2. Improvement of exhausted muscle fibers in DDM by cell therapy
3. Stimulation parameter depending on time from SCI, age, magnetic stim, stim during rest, etc

B - New research for new solutions on old questions

1. Common new research projects as ageing, pain, ostheo-articular-muscular mobility impairments, etc.
2. Translational research of new topics
3. New research markers (only from blood, saliva, skin, hair samples?)

C - Working groups on:

1. nerve regeneration
2. training-parameters of seniors in different ages
3. muscle adaptation
4. connective tissue, cartilage

157. *Kern H, Carraro U, Adami N, et al. Home-based Functional Electrical Stimulation (h-b FES) recovers permanently denervated muscles in paraplegic patients with complete lower motor neuron lesion. Neurorehab Neur Rep 2010;24:709-21.*
158. *Carraro U, Boncompagni S, Gobbo V, et al. Persistent Muscle Fiber Regeneration in Long Term Denervation. Past, Present, Future. Eur J Transl Myol 2015 Mar 11;25:4832. doi: 10.4081/ejtm.2015.4832. eCollection 2015 Mar 11. Review.*
159. *Carraro U, Kern H. Severely Atrophic Human Muscle Fibers With Nuclear Misplacement Survive Many Years of Permanent Denervation. Eur J Transl Myol. 2016 Jun 13;26:5894. doi: 10.4081/ejtm.2016.5894. PMID: 27478559.*
160. *Zampieri S, Mosole S, Löfler S, et al. Physical Exercise in Aging: Nine Weeks of Leg Press or Electrical Stimulation Training in 70 Years Old Sedentary Elderly People. Eur J Transl Myol 2015; 25: 237–42. doi: 10.4081/ejtm. 2015.5374.*
161. *Robbins WR, Staats PS, Levine J, et al. Treatment of intractable pain with topical large-dose capsaicin: preliminary report. Anesthesia and analgesia. 1998 Mar;86:579-83. PubMed PMID: 9495419. Epub 1998/03/12. eng.*
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The physiopathologic dialogue between muscle and nerve

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Key Words: Aging, ALS, NMJ, PKC, muscle-nerve interplay, SOD1^{G93A}

A crucial system severely affected in several neuromuscular diseases is the loss of effective connection between muscle and nerve, leading to a pathological non-communication between the two

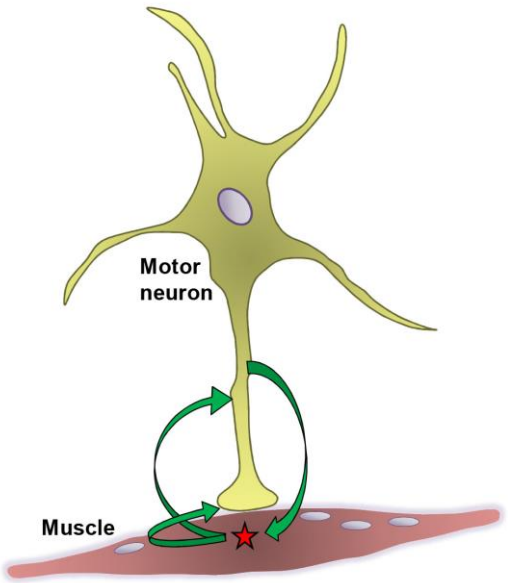

***Fig 26**. A schematic model depicting the physiopathologic interplay between nerve and muscle. Alterations in structural, physiological and metabolic parameters in motor neurons and muscle might act synergistically to exacerbate the disease. Moreover, morphological and functional alterations in muscle (red star) can negatively impact motor neuron in a sort of "dying-back" process.*

tissues. Aging-sarcopenia and several neuromuscular diseases, including Amyotrophic Lateral Sclerosis (ALS) and muscular dystrophies, are characterized by alteration in the functional connection between nerve and muscle, creating a sort of short-circuit that impinges the proper function between the two systems (Rudolf et al, 2014).[164] Neuromuscular junctions (NMJ) serve as the interface between the nervous and skeletal muscular systems, and thus they may receive pathophysiological input of both pre- and post-synaptic origin (Figure 26). However, controversy exists over whether NMJ dismantlement is a pathogenic event directly associated with the primary defects occurring in motor neurons or whether it occurs independently from motor neuron degeneration. To address this question, we made use of MLC/SOD1G93A transgenic mice (Dobrowolny et al, 2008),[165] which represent an ideal model to separate the ubiquitous toxic effects of mutant SOD1G93A with that of tissue-specific effects. We recently provided evidence that increased oxidative stress, induced by muscle-specific accumulation of mutant SOD1G93A, is causally linked to morphological alterations of the neuromuscular presynaptic terminals, high turnover rate of Acetylcholine Receptor (AChR), and NMJ dismantlement (Dobrowolny et al, 2017).[166] We then disclosed the molecular mechanisms by which oxidative stress, mediated by SOD1G93A gene expression, induces NMJ dismantlement. Interestingly, we found that muscle expression of toxic SOD1G93A gene induces the re-activation of PKCθ, a serine/threonine kinase implicated in the clustering and stability of AChRs during development (Lanuza et al, 2001).[167] We demonstrated that PKCθ selectively colocalizes with AChR in the muscle of MLC/SOD1G93A mice. To validate the hypothesis that the re-activation of PKCθ expression and activity was mechanistically associated with the dismantlement of NMJ, we pharmacologically interfered with PKCθ activity. We demonstrated that the inhibition of PKCθ activity was sufficient to reduce PKCθ - AChR co-localization, to restore mitochondrial functionality, to rescue the morphological complexity of NMJ, and to stabilize AChR turnover (Dobrowolny et al, 2017).[166] These results indicate that increased levels of oxidative stress and up-regulation of PKCθ are causally linked to NMJ dismantlement and suggest that primary muscle defects impact the functional connection between muscle and nerve at the level of NMJ. This might represent an early pathogenic signature of sarcopenia and neuromuscular diseases. In conclusion, our study discloses the molecular mechanism that triggers functional denervation associated with increased levels of oxidative stress within the muscle and suggests pharmacological intervention to attenuate muscle dysfunction, NMJ loss and eventually disease progression, typical of sarcopenia, ALS, and muscle diseases,[168,169] in a sort of "saving-back" process.

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Mobility impairment in low back pain patients and its improvement by multi-modal physical therapies

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Keywords: capsaicin, pain, sensation, inflammation.

Low back pain (LBP) is one of the most common health problem of the musculoskeletal system. The aim of our clinical study was to test differences in asymptomatic participants vs. LBP patients from different perspectives of movement function. Additionally, we were interested in testing the efficiency of different multi-modal regimens of multi-modal therapy, primarily based on physical therapy modalities. Altogether, 260 subjects were included (30 asymptomatic) and underwent the same testing protocol consisting of: (1) questionnaires on daily movement function and symptoms, (2) isometric strength tests – maximal voluntary force during trunk flexion, extension and lateral flexion, (3) flexibility and repositioning error of the trunk during trunk bending from a standing position, (4) chair rising and stand-up-

and-go test as functional locomotion tests. Patients underwent the same testing protocol before and after 10 consecutive physiotherapy visits (2 x/week = 5 weeks). The patients underwent 3 different multi-modal therapy groups that involved the following therapy modalities: massage, electrical stimulation, ultrasound, capsaicin-based patches, and movement exercise. The results showed statistically significant differences ($p < .05$) between asymptomatic and LBP subjects in the tested parameters. 2-way RM ANOVA revealed significant time effects and in majority of cases non-significant time x group interaction effects. The outcomes of this study add important knowledge to the evidence based practice of LBP treatment. Additionally, it sets a foundation for future studies of this project aiming at optimization of clinical practice.

170. *Gore M, Sadosky A, Stacey BR, et al. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. Spine 2012;37:E668-E77.*
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172. *Sarabon N, Löfler S, Jan Cvecka J, Hübl W, Zampieri S. Acute effect of different concentrations of cayenne pepper cataplasm on sensory-motor functions and serum levels of inflammation-related biomarkers in healthy subjects. Eur J Transl Myol 2018;28,in press.*

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Histopathological analyses of skin in SCI. New results and hopes for h-bFES and beyond

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Key Words: skin thickness, biomarkers, denervated muscle, recovery by FES.

The skin is the body's heaviest sensory organ, accounting for approximately 16% of the body's weight. Other functions are protection from chemical, physical and biological insults and maintenance of the internal environment.[173] Several pathologies are associated to skin changes affecting skin cells and other structural proteins, thickness of the various epidermal layers, inflammatory cells, and amount of water.[174] Simultaneous qualitative and quantitative analysis of several components and properties of the skin are necessary to understand these disorders and to follow-up eventual managements. Our previous studies have shown that denervated Quadriceps muscles of patients suffering with complete *conus* and *cauda equina* lesions were rescued by two years of home-based functional electrical stimulation (h-bFES) using a new electrical stimulator and very large skin electrodes.[175-177] Muscle mass, force, and structure of the stimulated Quadriceps muscles were determined before and after 2 years of h-bFES, using Computed Tomography (CT), measurements of knee torque during stimulation, and muscle biopsies which were analyzed by light and electron microscopy. To harvest muscle biopsies the overlying skin was also collected and evaluated by histological morphometry of H&E and immuno-stained on paraffin-embedded section (Figure 27). Analysis of the structural characteristics of epidermis, i.e., thickness, morphology of the papillae and content of hairs together with some neural and inflammatory molecular markers were organized. Preliminary results[178] are interesting and stimulate additional analyses to better describe skin adaptation to this peculiar type of electrical stimulation by surface electrodes. Our approaches offer also new opportunities to study adaptation of the skin to other physical and pharmacological therapies based on application of

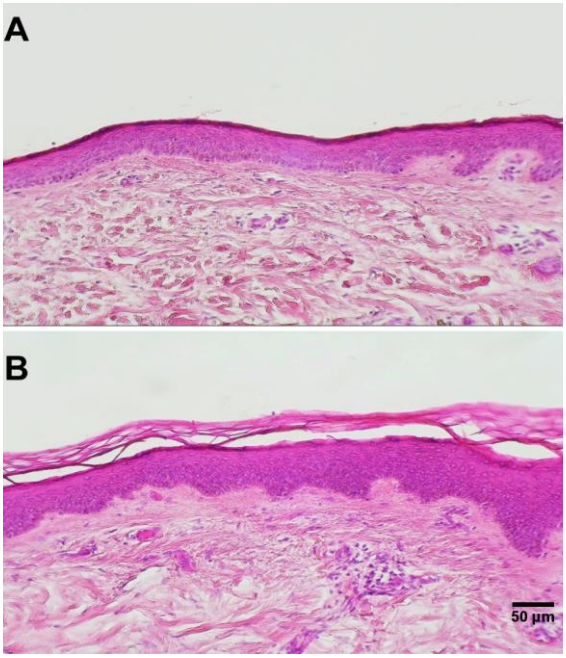

Fig 27. *A and B are H&E stains of skin harvested from the left thigh before and after h-bFES, respectively. Male, aged 48.4 years, 175 cm high and 100 kg weight. Th12/L1 SCI. Before-FES biopsy was harvested 3.3 years after SCI (A), and after 2.6 years of FES (B). Skin thickness increase: +35%, $p < 0.001$.*

rehabilitation managements through the skin, in particular for pain relief.

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Discovery of Calcium Entry Units: role in skeletal muscle function and disease

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Key Words: *muscle fatigue; sarcoplasmic reticulum (SR); skeletal muscle; store-operated Ca^{2+} entry (SOCE)*

Store-operated Ca2+ entry (SOCE) is a ubiquitous cellular Ca2+ influx mechanism, first described in non-excitable cells, that is triggered by depletion of intracellular Ca2+ stores (endoplasmic reticulum, ER).[179] A major breakthrough in the field was the identification of the two essential molecular players in SOCE: STIM1, the Ca2+ sensor in the ER,[180] and Orai1, a Ca2+ permeable channel in the plasma membrane (PM).[181] Question addressed by this study: SOCE is also well-documented in skeletal muscle where it limits muscle fatigue during repetitive fatiguing stimulation.[182,183] Also in muscle SOCE is mediated by interactions between STIM1 in the SR and Orai1 channels in the PM4. However, the precise subcellular location of STIM1-Orai1 SOCE complexes in skeletal muscle was not yet been unequivocally identified. We used a combination of ultrastructural and biochemical analyses (electron microscopy, immunohistochemistry and confocal microscopy, immunogold, and western blot) to compare and carefully quantify STIM1 and Orai1 subcellular localization in skeletal muscle of adult mice under resting conditions and after a single bout of treadmill running designed to promote STIM1 aggregation and coupling with Orai1. Using this experimental approach, we discovered: a) previously unidentified intracellular junctions between the sarcoplasmic reticulum (SR) and invaginations of the external membrane, i.e. the transverse tubules (TTs); b) that this remodeling allows for the assembly of the molecular machinery required to activate SOCE (i.e. STIM1 and Orai1); c) that muscles containing these new SR-TT junctions are more resistant to fatigue in presence of extracellular Ca2+; d) that TTs are more dynamic than SR and retracts from the new junctions in a few hours following the exercise protocol. We propose that these previously unidentified SR-TT junctions function as Ca2+ Entry Units (CEUs) (Figure), providing a preferential pathway for rapid reuptake of Ca2+ into the SR during repetitive muscle activity.[184] Significance of our study. Our work represents a pioneer study that identifies exercise-driven dynamic formation of new intracellular structures, an endogenous mechanism potentially quite important for the delay of muscle fatigue. In addition, as: a) altered SOCE activity contributes to muscle dysfunction in ageing and b) mutations in STIM1 and Orai1 are linked to Tubular Aggregate Myopathy (TAM), our findings may also have important implications for a deeper understanding of mechanisms involved in muscular dysfunction in pathophysiological conditions.

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Myofascial force transmission and muscle weakness

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Key Words: skeletal muscle, ageing, sarcopenia, muscle quality, extracellular matrix

Sarcopenia, the age-related loss of muscle mass, affects >50% of the population aged 75 years and over and is a main cause of impaired physical performance and reduced mobility. Amongst the several factors contributing to sarcopenia neuroendocrine changes are regarded as primary drivers of this condition.[185-187] They are responsible for α-motoneuron- and neuromuscular junction (NMJ) degeneration as well as muscle fiber denervation that, also fueled by mitochondrial dysfunction and oxidative damage,[188] leads to loss of motor units and muscle weakness. One of the major functional characteristics of sarcopenia is the disproportionate loss of muscle strength. At the age of 80 years, the loss of muscle strength is about 4-fold greater than that of muscle size.[189] This intrinsic muscle weakness, also known as a deterioration in 'muscle quality', has traditionally been attributed to changes in muscle fiber type composition, a decrease in fiber specific tension, reduced excitation-contraction coupling and reduced neural drive. However, new evidence suggests that this disproportionate loss of force also arises from changes in the extracellular matrix (ECM) and of associated proteins,[190,191] which in young muscle normally contributes to over 50% of muscle force output.[192] Indeed, as recently reported for rat muscle, lateral force transmission is reduced by up to 44% in old animals.[193] Direct measurements of lateral force transmission involve highly invasive surgical procedures (tenotomies and myotomies), precluding human studies. In this pilot *in-vivo* study, we tested the hypothesis that MR imaging-based indices derived from strain rate (SR) tensor maps reflect lateral force transmission (Figure 28).[194] SR tensors were derived from velocity encoded magnetic resonance phase-contrast images in nine young (28 years) and eight senior (78 years) women. The central finding of this study was that the angle enclosed by the SR along the fiber (indicative of the principal axis of muscle shortening) and the muscle fiber axis was significantly smaller in older women (proximal: -19.2%, central: -17.6%). Under the assumption that the SR-fiber angle would be 0° if force was solely transmitted along the fiber, this finding indicates lower lateral transmission of force in older compared to younger women. This observation seems consistent with the hypothesis, and with observations in animal muscle, that a reduction in lateral force transmission may contribute to the intrinsic muscle weakness of older human muscle.

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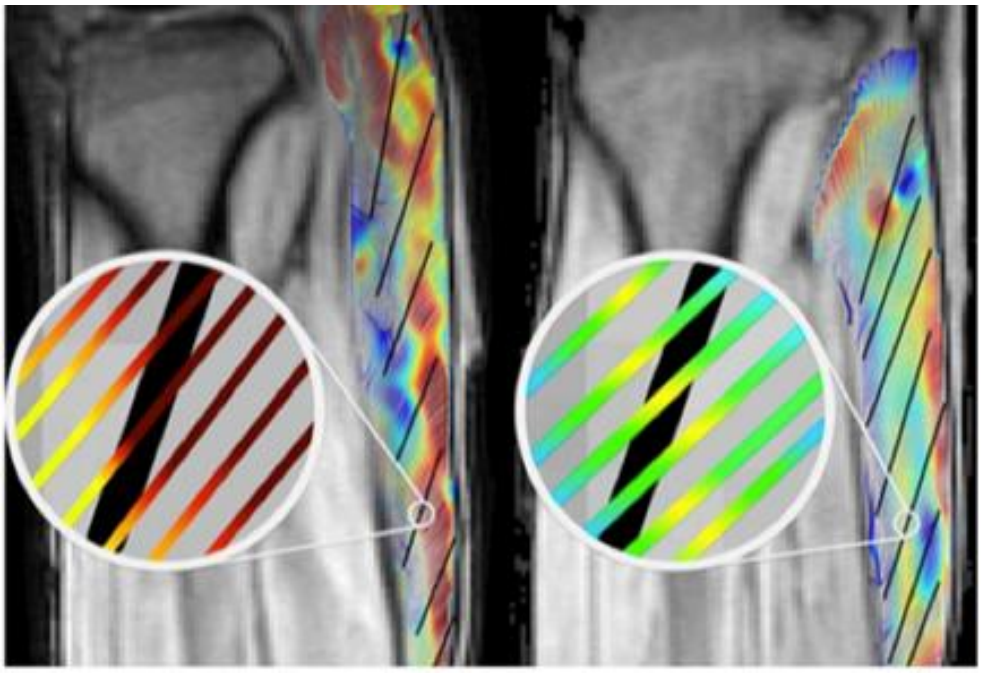

Fig 28. *Difference in angle enclosed by the fibre SR and the muscle fiber orientation*

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E-T Coupling of muscle trophism modulation

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Key words: Aging, muscle remodeling, electrical stimulation, Ca-handling-proteins, NFATc1

Physical activity plays an important role in preventing muscle atrophy in elderly. Calcium cycling and activation of specific molecular pathways are essential in contraction-induced muscle adaptation. Muscle sections and total homogenates were prepared from biopsies obtained before and after nine weeks of electrical stimulation (ES) on a group of volunteers. NFATc1 nuclear localization, kinase activation and expression of Sarcalumenin, Calsequestrin and sarco/endoplasmic reticulum Ca2+-ATPase (Serca) isoforms were determined by immunofluorescence and western blot. Results support the conclusion that ES is able to modulate expression of key Ca-handling proteins promoting positive fiber remodeling in sedentary seniors.[195-199]

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Physiotherapy for knee osteoarthritis: effects on lower limb strength, function and gait

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Key Words: Knee osteoarthritis, physiotherapy, gait

According clinical guideline recommendations, lower limb strengthening exercises are key components of knee osteoarthritis (OA) management.[200]. Exercise has shown to have beneficial effects on decreasing symptoms of pain and improving physical function in knee OA patients. However, its effects on knee biomechanics are still unclear. Although changes in knee biomechanics during gait have been recently reported following a physiotherapy treatment,[201] other studies were not conclusive.[202] Interpreting gait data is challenging due to intersubject variability observed in the gait pattern of both normal and pathological populations. In this study, we investigated the impact of using principal component analysis for clustering knee osteoarthritis (OA) patients' gait data when studying the effect of a physiotherapy treatment focusing on lower limb strengthening exercises. 3D knee kinematic and kinetic data were recorded during the gait of 29 participants diagnosed with knee OA before and after they received 12 weeks of physiotherapy treatment. Pain, stiffness, function and knee muscle strength were also measured. The physiotherapy program was standardized and it was mainly oriented towards muscle strengthening and stretching exercises, proprioceptive exercises and aerobic training. In most gait studies, dynamic joint angles and moment data as a function of the gait cycle are presented in the form of curves.[203] Specific kinematic or kinetic gait parameters, such as the mean of peak values, are extracted at particular periods of the gait cycle and used for group comparison. However, limitations can be encountered using this technique. Although human gait is a cyclic and repeatable activity, every person has a fairly unique gait pattern, leading to intersubject variability in curve profiles. Averaging can collapse information to the point of removing important intersubject variability within a given group, whether before or after physiotherapy. In this study, we used principal component analysis to extract clusters of knee flexion/extension, adduction/abduction and internal/external rotation angle and moment data. The treatment's effect was assessed using paired t-tests performed before and after clustering the knee kinematic data into subgroups. At the end of the physiotherapy program, patients had less knee pain, less stiffness and felt less disabled; isometric quadriceps and hamstring strength was also improved ($p<0.05$). Except for the knee flexion/extension angle, two different clusters (C1 and C2) were extracted from the angle and moment data. When pre- and post-treatment analyses were performed on the clustered data, participants exhibiting a C2 knee moment pattern demonstrated a greater first peak flexion moment (Figure 29), lower adduction moment impulse and smaller rotation angle range post-treatment ($p<0.05$). Pre- and post-treatment comparisons performed on unclustered data showed no treatment effect. The results of the present study suggest that the proposed physiotherapy exercices program is effective to improve the clinical status of patients with knee OA. Morevover, the results demonstrate that the effects of exercices on knee biomechanics may be masked or underestimated if the gait data are not clustered into more homogeneous subgroups when performing pre- and post-treatment comparisons.

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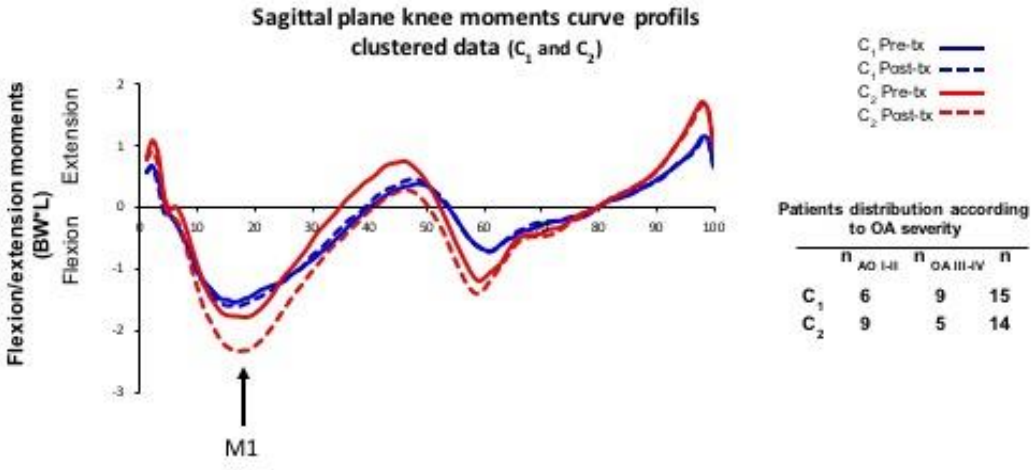

Fig 29. *Sagittal plane knee moments curve profils, clustered data (C1 and C2) as a function of normalize gait cycle. When pre- and post-treatment analyses were performed using the clustered data, a significantly greater first peak flexion moment (M1) was observed for patients exhibiting C2 patterns ($p<0.05$).*

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FES-cycling at the 1st CYBATHLON competition

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Key Words: adaptive control of movements, gait and cycling assistance, Functional Electrical Stimulation, FES leisure cycling for SCI, CYBATHLON

Functional Electrical Stimulation (FES) can elicit muscular contraction and restore motor function in paralyzed limbs. FES can assist cycling for training purposes mainly in clinical environments using cycle ergometers. Different events have promoted FES-cycling as a recreational practice. In 2016, twelve teams participated in the first Cybathlon competition in the FES-cycling discipline for persons with motor-complete spinal cord injury. Different approaches have been considered by the different participating teams.[204] The objective of the race was to travel 742 m in less than 8 minutes. 7 pilots were able to cover the total distance in 5.5mn on average.[205] This communication intends to comment the results and performances during this competition and discuss the scientific challenges to improve FES-assisted cycling applications. Taking the example of the experience of Freewheels team we will also discuss the experience of participating in such an event.[206] Online modulation of functional electrical stimulation (FES) parameters is necessary to adapt timings and levels of muscle activations. This requires information on the system state evolution and involves the design of robust closed-loop control approaches. Open-loop control should be limited to non functional situations and to motion control not requiring reproducibility or adaptability (e.g facing fatigue occurrence or external changes). Voluntary actions of the user through his/her healthy limbs or residual control of his/her deficient limbs should also be considered and integrated in the global functional task control as well and not only under the form of external disturbances. Our team has been working for several years on the observation of voluntary movements through artificial sensors in order to extract useful information about the on-going postural task.[207-209] Indeed, depending on the considered function to be restored (posture, gait, grasping, cycling), different information can be extracted from embedded sensors, going from discrete events to continuous measurements of variables (joint angles, gait evolution…). As illustrated in Figure 30, this method involves combining a natural controller (i.e. the subject with his/her disability and his/her partial voluntary control) with an artificial FES-based controller fed with information from one or multiple sensors located on the subject. We will discuss possible improvements of FES-cycling assistance, using at a deeper level sensors embedded either on the tricycle or on the user, paving the way to adaptive assistive solutions

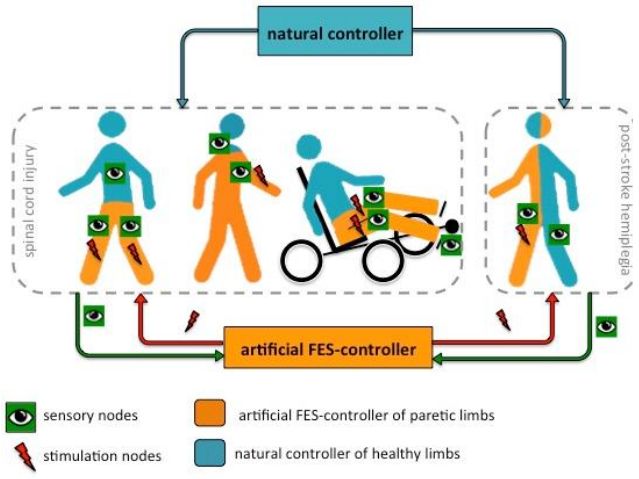

Fig 30. *Illustrative examples of a closed-loop approach of FES-assisted postural tasks restoration.*

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Functional electrical stimulation for ageing laryngeal muscles

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Key words: Evaluation, surface FES, voice rejuvenation

The percentage of elderly people is increasing steadily during the last decades in most Western societies and large parts of Asia which is accompanied by a steady increase of age related diseases. These changes do not spare the larynx. Weakening of the voice has long been neglected, but gained consideration more recently, as vocal endurance is required in many professions up to higher ages. A large retrospective study identified vocal fold (VF) atrophy as the most prevalent finding. The noticeable glottal gap and VF bowing are the most prominent video-laryngoscopic findings in these patients, and are related to the atrophy of the thyroarytenoid muscle (TAM). In a recent trial we could demonstrate that functional electrical stimulation (FES) of the afferent nerves with specific parameters led to a muscle fiber hypertrophy.[210] In subsequent trials we explored not only different stimulation patterns, but also different anatomical locations and outcome parameters such as 3D-volumetry.[211] Aged sheep were used as animal model. FES was delivered in predesigned training patterns unilaterally at the common trunk of the recurrent laryngeal nerve, as well as at more distal ends of the same nerve (mere adduction fibers). After a maximum time of eleven weeks permanent stimulation we observed significant increases of mean fiber diameters and volumes of the TAM using different stimulation patterns. Noticeably, we did not elicit a fiber type change with any regime, as proved with RT-qPCR.
FES is possible new treatment option to counteract laryngeal sarcopenia. Further work will comprise identification of optimized patterns in terms of training- and current-efficacy.

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Selective surface stimulation of denervated muscles in the larynx

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Keywords: unilateral vocal fold paralysis, electrotherapy, electrical stimulation, hoarseness

The laryngeal innervation bears the risk of unilateral and bilateral vocal fold paralysis due to trauma, injury during surgery, and inflammation. Vocal fold paralysis is a pathological motion impairment of the vocal fold, mostly caused by damage of the N. *vagus* or the N. *laryngeus recurrens*. Bilateral vocal fold paralysis usually causes dyspnea with inspiratory stridor and requires immediate

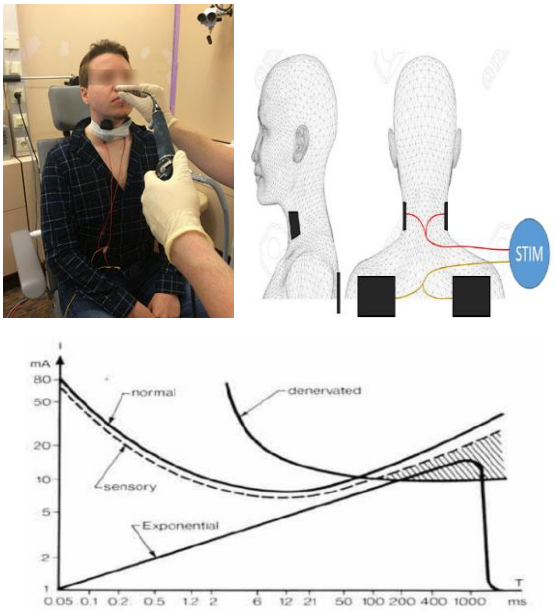

Fig 31. ***Upper left panel:*** *Uni-polar electrode configuration.*
Upper right panel*: Two electrodes (same as bipolar set-up) are places above the larynx and two indifferent electrodes on the shoulder blades (large self-adhesive electrodes with 13 x 8 cm).*
Lower panel: *The principle of selective stimulation of denervated muscle utilizing a long exponentially progressive current form. The strength duration curves for normally innervated muscle, denervated muscle, and intact sensory nerves are illustrated. Selective stimulation of the denervated muscle without recruitment of either normally innervated muscle or the sensory axons is possible (shaded area).*

an intervention to stabilize the airway. The unilateral vocal fold paralysis (ULVP) causes dysphonia, thus patients are in need for therapy to improve the voice quality. In temporary ULVP, direct superficial electrical muscle stimulation (EMS) can be used for activation of intrinsic laryngeal muscles as an alternative to conservative voice therapy.[212-217] The EMS should promote the regeneration without negatively interfering with normal regeneration processes. Little is known about the effect of electric current on the larynx. In this study, 30 patients with ULVP (20 male/10 female, average age 57,4 y) underwent superficial electrical muscle stimulation (EMS) with flexible endoscopic laryngoscopy (Figure 31). Criteria for a selective laryngeal activation are an ab- or adduction of the immobile vocal fold. In 10/30 patients a selective laryngeal activity could be achieved using conventional stimulation parameters. The other patients did not present any selective vocal fold movement or the test had to be ended prematurely due to nonselective reaction (activity of the strap muscles, unintentional swallowing, pain) before a selective laryngeal activity could be achieved. Nevertheless, the superficial electrical muscle stimulation can be regarded as a therapy alternative to only "wait and see"-strategy and a sole voice therapy in early treatment of ULVP. In future studies, position and size of electrodes and the stimulation parameters need to be improved in order to reach a reliable and effective selective laryngeal stimulation.

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Reinnervation is not prevented by surface electrical stimulation (ES): clinical experience of 17 patients with facial nerve paralysis

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Keywords: Electrostimulation; Reinnervation; Facial Paralysis; Facial Pacing; Surface electrodes; triangular pulses

Facial nerve paralysis as a complete peripheral nerve injury results in neuromuscular atrophy or in a combination of muscle atrophy and aberrant reinnervation of facial muscles. The symptoms include significant aesthetic, functional and often life-altering consequences. Facial pacing systems show controversial but promising results to treat facial paralysis.[218] Electrical stimulation (ES) can increase the mass and force generation of denervated lower extremity muscles.[219] Nevertheless, there is an ongoing discussion, whether the potential benefit of ES to maintain the muscle might be compromised by reducing the chance of reinnervation. The few studies published show no clear benefit for ES in the face.[220] We present data after a new regime of home-based stimulation. Before analysing the potential positive effects of optimal ES, negative effects have to be excluded. From April 2016 till November 2017 17 patients (11 women) with an age between 18 and 78 years, with a complete denervation of one facial nerve (caused by benign tumor: 8, malign tumor: 4, idiopathic: 1) confirmed by needle electromyography, have received home-based ES devices for exponential impulses training to induce contractions of the denervated muscles of the mid and lower face (Figure 32). These 17 patients have performed ES-training for on average 12months (min. 4months, max. 21months). The average amplitude was 14mA (min. 6mA, max. 25mA), the average phase-duration of the biphasic triangular impulses was 100ms (min. 30ms, max. 500ms), the frequency 0.9Hz. Out of this heterogeneous group 7 patients got a Hypoglossal-Facial-Jump-Anastomosis (HFJA), a surgical procedure to allow axons from the hypoglossal nerve to reinnervate the facial muscles using a nerve-bypass. While waiting

Fig 32. *Placement of two 6x4cm surface electrodes on the cheek of a patient suffering with postoperative complete denervation of the left facial muscles.*

for reinnervation, these patients performed ES-training for on average 12 months (min. 9 months, max. 18 months). The average amplitude was 14mA (min. 10mA, max. 25mA), the average phase-duration of the biphasic triangular impulses was 100ms (min. 70ms, max. 500ms), the frequency 0.9Hz. Most patients performed the training twice a day for 10 minutes and on 5 days a week according to their diary. Reinnervation was confirmed by volitional movements and by needle EMG of the formerly paralysed muscles.
None of our patients had to stop the ES training due to negative side-effects. In 6 patients there were first signs of reinnervation before beginning the ES-training. With ES, 5 of them showed a progressive reinnervation (more muscles innervated and stronger EMG activity per muscle). Only one had no improvement of the EMG. Reinnervation was observed in 7 patients that had no sign of innervation at the start of the training. In 1 patient, even 12 month after denervation, no signs of reinnervation were detected and in 3 patients, the follow up time without reinnervation is less than 12 month. Therefore these patients are excluded for our retrospective study. So, reinnervation or improvement of reinnervation was observed in 85% (12 from 14 patients with sufficient follow up) despite electrostimulation. On average, it took 8 months after the initial denervating infection or surgery until first signs of reinnervation could be detected by visual inspection and needle-EMG. In the sub-group of patients that received a HFJA, reinnervation was observed in 4 of the 7 patients. In one patient there are no signs of reinnervation yet due to the short period since the HFJA (surgery November 2017). Therefore this patient was excluded for our retrospective study of the 6 patients reinnervated within 6 months. In another patient it took 4 years for stable reinnervation with sufficient volitional movements. The defect in the cerebellum after extirpation of an astrocytoma could explain this unusual long reinnervation time. Two patients had first signs of reinnervation before they began the ES-training. In the follow up an improvement of reinnervation (more muscles innervated and stronger EMG activity per muscle) was shown. So reinnervation and improvement of reinnervation was observed in 100% of the patients with sufficient follow up, despite electrostimulation. In conclusions, surface stimulation of facial muscles with long triangular biphasic pulses did not prevent reinnervation in this series. Most of our patients reported a comfortable home based ES training recruiting visible muscle response, an improvement in facial symmetry and tone, and later on also in muscle movement. In the future, these subjective reports have to be quantified and examined in detail to differentiate the benefit of ES and reinnervation. Seeing no hint of severe side effects, facial pacing should be further investigated to prove therapeutic effects like reduction of muscle atrophy.[221]

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Opposite effects of tumor-derived cytokines and mechanical stimulation on muscle stem cell activity and muscle homeostasis

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Key Words: skeletal muscle differentiation; myotube atrophy; mechanical stimulation; myokines; tumor-derived factors

Cancer cachexia is a muscle wasting syndrome, characterized by muscle fiber atrophy and hampered satellite cell (SC) myogenic potential, ultimately leading to morbidity, lowered quality of life, and death. Exercise training improves quality of life and survival of cancer patients and its beneficial effects can be mimicked by wheel running in mice.[222,223] In an animal model of cancer cachexia we demonstrated that wheel running counteracts cachexia by releasing the autophagic flux and by lowering Pax7 expression, which blocks SC myogenic progression.[224,225]. Exercise pleiotropic effects include the alteration of circulating factors in favor of an anti-inflammatory environment and the activation of mechanotransduction pathways in muscle cells. Serum response factor (SRF) is a transcription factor of pivotal importance for muscle homeostasis, which is activated with its co-factor MRTF by mechanotransduction in a way dependent on actin polymerization.[226] Our goal was to assess whether mechanotransduction per se is sufficient to elicit exercise effects in the presence of pro-cachectic factors of tumor origin and to characterize the mechano-transduction signals involved. We used C26 tumor-bearing mice, in the absence or presence of wheel running, and mixed cultures of C2C12 myotubes and

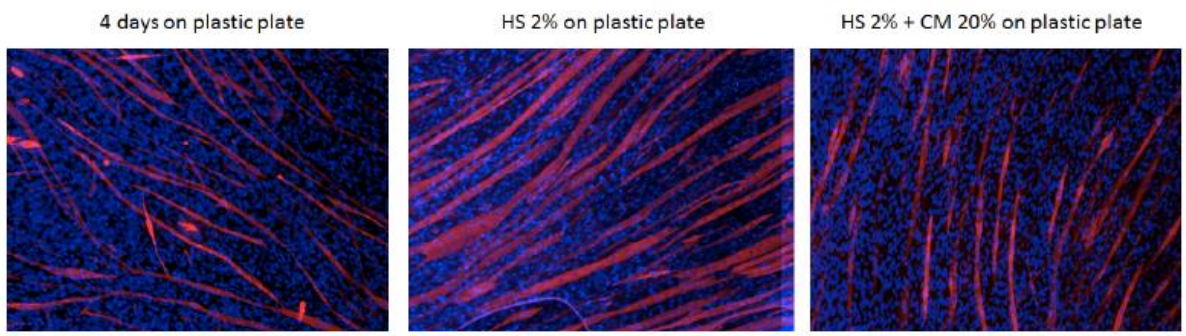

Fig 33. *Tumor cell conditioned medium (CM) induces myotube atrophy and hampers myogenesis. Immunofluorescence analysis for MHC (red) on mixed cultures consisting of nascent myotubes and myoblasts (nuclei are counterstained in blue), following 4 d of culture (left panel). After additional 2 d of culture in differentiation medium (2% HS, Horse Serum) myotube size increases (central panel), while in the presence of C26 tumor cell conditioned medium (2% HS + 20% CM) the myotubes appear atrophic and no additional nuclei are present in the myotubes in respect to 4 d of culture. This experimental model to test the effect of tumor-derived factors on muscle cells, can be combined with mechanical stimulation when the cells are cultured in the FlexCell apparatus.*

myoblasts treated with C26 conditioned medium (CM) in the absence or presence of cyclic stretch to mimic the mechanical stimulation occurring upon exercise (Figure 33). In vivo both SRF expression and activity were differentially modulated by the C26 tumor, i.e. by humoral factors, and by exercise. In vitro we showed that CM had a negative effect on muscle cell cultures, both in terms of myotube atrophy and of myoblast recruitment and fusion, and that these effects were counteracted by cyclic stretch. We showed that CM repressed SRF-MRTF transcriptional activity, while mechanical stretch rescued their transcriptional activity; in addition, loss of function experiments demonstrated that SRF was necessary to mediate the beneficial effects of mechanical stimulation on muscle cells. At least part of the observed effects was mediated by the balance of pro- and anti-myogenic factor, such as IL-4 and members of the TGFbeta superfamily. in conclusion, we propose that the positive effects of exercise on cancer patients and mice may be specifically due to a mechanical response of muscle fibers affecting the secretion of myokines.

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Seeking economy of charge injection in activation of motor nerves

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Key Words: neural activation, nerve, pulse shaping, biphasic pulse, depolarisation,

How does one choose a pattern of electrical stimulation for therapeutic effect? Often there is a useful guide from normal physiology, and many therapeutic strategies try to mimic or replace a natural activation pattern.[227] Another strategy is to try to generate a numerical model of the excitable tissue to be stimulated so that trials can be achieved *in silico*.[228] Many optimised activation strategies are based on the results of such simulations. They have been used to identify activating pulses that best answer the requirement of charge balance to minimize damage to electrode and tissue, and the requirement to activate nerve tissue with minimal use of energy. This latter requirement is critical to improve the lifetime of implantable neuromodulation devices. We have tested some of the conclusions of studies that have investigated the charge efficiency of activation both in numerical models and in nerve-muscle preparations.[229-234] We have used the simple experimental model of a single motor nerve trunk activated by two electrodes

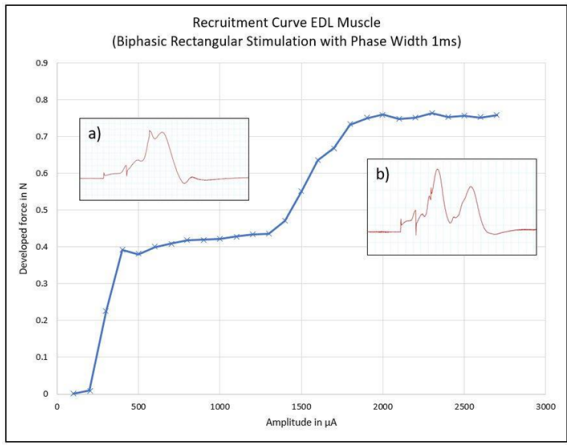

Fig 34. *Recruitment Curve EDL Muscle. Force developed by the extensor digitorum longus (EDL) muscle following a biphasic rectangular stimulation (phase width 1ms) of the Common Peroneal Nerve. The Common Peroneal Nerve was isolated from the central nervous system. Insert a) EMG signal recorded on the EDL muscle at full muscle recruitment (first force plateau 400-1300µA). Insert b) EMG signal recorded on the EDL muscle at double muscle activation (second force plateau 2000-2700µA)*

placed near to the nerve (common peroneal in rats). The degree of activation has been monitored indirectly by measuring the isometric force of the extensor digitorum longus muscle because it has discrete proximal and distal tendons and can thus be mechanically isolated between a proximal clamp and a distal load sensor. Our latest results show that the relative benefit of Gaussian or sinusoidal shaping of biphasic pulses over rectangular shaping reduces with phase width (Figure 34). We will also present new data on the complex recruitment curves that are generated when the range of phase width and amplitude is extended, adding electromyographic recordings from the activated muscle to try to understand the effect of the primary, secondary and tertiary transitions in electrode voltage in a biphasic pulse. Such fine differences are important when designing low energy implanted stimulators such as may be used in retinal stimulation or brain stimulation or activation of fine autonomic nerves.

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Enhanced muscle coordination by transcutaneous spinal cord stimulation in brain injured people

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Key Words: Transcutaneous spinal cord stimulation, treatment of spasticity, brain damage, EMG

Transcutaneous spinal cord stimulation (tSCS) is used in our clinic as a therapy option as it has been shown to abbreviate spasticity in lower limbs in people with incomplete spinal cord injury (SCI) people.[235,236] This is thought to be inhibiting effects of action potentials entering the neural network of the spine where the motor neuron for the muscle in question exits.[237,238] By stimulating the posterior roots of sensory fibres this can be reached without stimulating the motor neuron of the same muscle. The sensory fibres have bigger diameter and therefore lower stimulation threshold compared to the motor neurons. The question arises how the tSCS influences the spinal cord circuitry. In order to investigate on that we looked at EMG in pendulum test and in Achilles tendon test by brain injured people. In this work four hemiplegic subjects with severe spasm are stimulated in the lumbar area, at the height of Th11-Th12. One man (67) with brain damage after falling, a woman (48) after stroke, a woman (72) with brain tumour and a woman (68) with a bleeding aneurism. An assessment of the spasm was made in the morning followed by a 30 minutes posterior root sensory fibres stimulating treatment with 50 Hz with no EMG responses

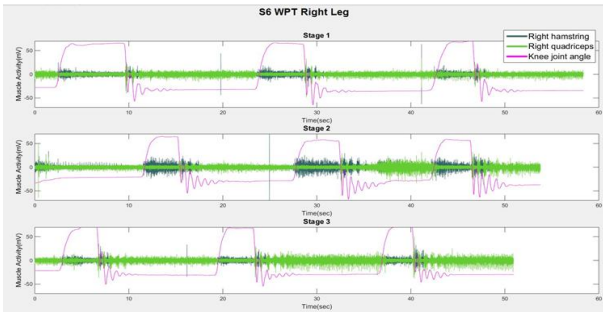

Fig 35. *Pendulum test EMG recordings from a female stroke subject. First line before tSCS treatment, second line immediately after tSCS treatment and third line four to five hours after treatment. Second line shows lighter knee angel oscillations and the EMG coordinated responses of quadriceps and hamstring muscle after tSCS therapy. The effect lasts less than five hours.*

of the corresponding muscles (sub motor threshold). The assessment consist of EMG measurements and evaluation of passive and active movements.[236] Stimulating electrodes where placed on the surface on the skin at the back and two big different electrodes where placed on the abdomen just beside the umbilicus. Immediately after the treatment a second assessment was made and a third one four to six hours later. Changes in the reflex responses of the muscles are recorded. EMG activity during Wattenberg pendulum test and during Achilles tendon stretch test is changed towards a clear division between the antagonistic muscles. Plantar and dorsiflexion of the foot is done easier. EMG shows less co-contractions and therefore les effort for the movement. Three out of four subject report easier movements. The EMG recordings show clear changes towards coordination in the responses of the thigh muscles after tSCS treatment for 30 minutes showing. This is detected both by the Wartenberg pendulum test and by the Achilles tendon test. This is in line with the results obtained by SCI people. In the SCI group the reduction of spasticity was though obvious whereas in the brain injured group the effects is not as clear. The effects of tSCS on the spinal neural network has to be further investigated.

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Effect of ageing on the myosin heavy chain composition of the human sternocleidomastoid muscle

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Key words: *Human, Ageing, Sternocleidomastoid muscle, Immunohistochemistry, Myosin heavy chain isoforms, Muscle fiber types*

The underlying cause of sarcopenia, or the age-related loss of muscle mass, in limb muscles appears to be slowly progressive denervation due to age related motor neuron loss in the lumbar and cervical spinal cord with incomplete reinnervation of muscle fibres.[239,240] In ageing limb muscles morphological studies demonstrated a slower phenotype,[241] in accordance with the preferential loss of fast motor neurons.[242] Concomitant muscle fiber atrophy, mainly of type 2,[243] is thought to be related to disuse. Thus disuse seems to act in the opposite direction to partial denervation, that either determines a shift towards the faster phenotype or no change in the phenotype.[243] The Sternocleidomastoid (SCM) muscle is an interesting skeletal muscle to investigate since SCM activity is essential for everyday tasks and its activity during life is relatively unchanged, avoiding to some extent inactivity-related atrophy to which the limb muscles are prone. By analyzing immunohistochemically myosin heavy chain (MyHC) composition we have demonstrated that a similar fiber type shift, i.e. towards a slower phenotype, as in ageing limb muscles occurs also in an ageing SCM (Figure 36),[244] despite a significant inactivity atrophy is avoided. In the ageing, SCM the percentage of slow-twitch fibres was nearly equal to the share of fast-twitch fibres (44.6 vs. 45.8% for numerical proportion and 57.2 vs. 50.6% for area proportion); in comparison, the SCM of young males was a fast-twitch muscle, of which approximately one-third was slow-twitch fibres and two-thirds were fast-twitch fibres (31.5 vs. 60.8% for numerical proportion and 38.4 vs. 63.5 % for areal proportion). Regarding the subtypes of fast fibres, the numerical proportion of hybrid 2a-2x was statistically significantly diminished in ageing SCM (14.1 vs. 26.8 %), due to decreased expression of MyHC-2x (19 vs. 34.5 % for area proportion). The remaining 10% of muscle fibres

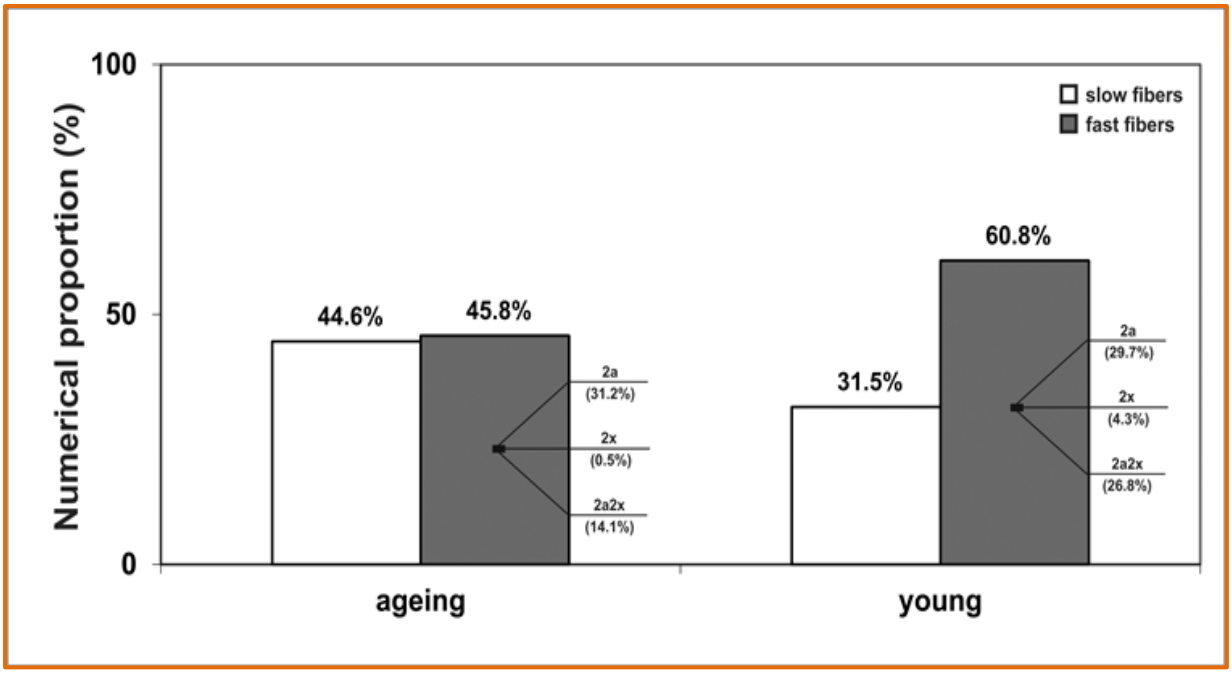

Fig 36. *Numerical proportion (%) of slow- and fast-twitch fibres and their subtypes based on the expression of myosin heavy chains.*

were either 1/2a fibres, fibres co-expressing MyHC-neo with adult myosin heavy chain isoforms or untraceable/unclassified fibres and were present in similar percentages in the ageing and young male SCM. A trend towards a smaller fiber diameter was noticed in all fiber types in the ageing SCM, but the differences were not statistically significant. A slower muscle phenotype with the preferential loss of the fibres co-expressing the fastest myosin isoform MyHC-2x in the ageing SCM 6 provide a circumstantial evidence for the selective loss of fast-twitch motor neurons in the cervical spinal cord and the reinnervation process in the ageing SCM. The ageing SCM appears to be an excellent subject for studying the effects of ageing on MyHC composition without significant inactivity atrophy.

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Fiber typing and isomyosin analyses of rat *sternomastoideus* muscle: Constrains to muscle sampling from its peculiar distribution of muscle fiber types

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Key words: neck muscles, rat, histochemistry, isomyosins, sternomastoideus

The sternomastoid muscle (SM) in rodents is known to have a peculiar distribution of fiber types with a steep gradient from surface to deep region.[245] We better characterized this peculiar regional distribution by quantitative histochemical analyses (Figure 37). Transverse sections of the SM muscle stained by SDH reaction clearly show two distinct regions, toward the deep surface of the muscle a 40% area that contains packed SDH-positive myofibers, while the other 60% area of the SM toward the external surface presents with a more checker-board appearance. In the deep region of SM the type 1 (slow type) muscle fibers identified by positive acidic ATPase pH 4.35 are only the 24.5% of the fiber in the deep area of SM muscle and they are

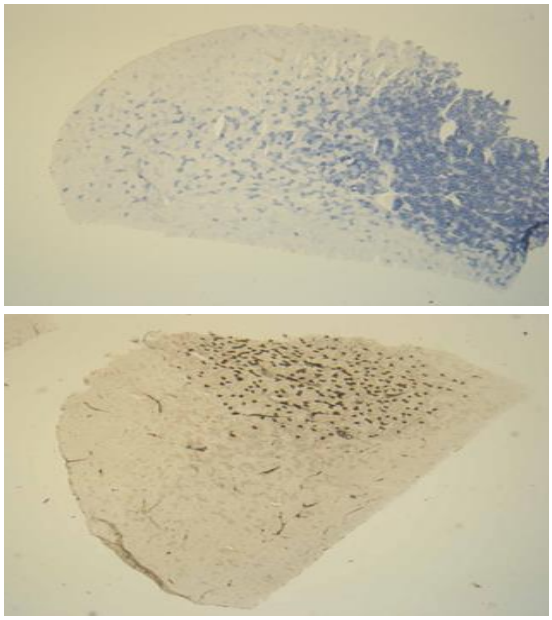

Fig 37. *Transverse cross-section of normal rat Sternomastoid muscle*
Upper panel: SDH reaction
Lower panel: ATPase pH 4.35.

restricted in the deepest region. The 75.5% of the myofibers in the deep region are of the fast contracting types (48.4% 2A, SDH –positive fibers and 27.1% 2B, SDH-negative fibers, respectively). Based on present and previous observations changes in absolute and relative number of fiber types, in any experimental model,[246] will ask for morphometry of the whole muscle cross-section, while after muscle sampling only the size of the different types of muscle fibers could be computed.[247]

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Fighting muscle weakness in premature and advanced aging by take-home strategies: Full-body In-Bed Gym

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Key Words: *Fighting muscle weakness, premature and advanced aging, take-home strategies, Full-body In-Bed Gym*

All progressive muscle contractile impairments need permanent managements, including aging-related muscle-strength decline. Beside the eventual pharmacology therapy, a home-based physical exercise approach is helpful and education of hospitalized patients to take-home physical exercise managements is an effective low cost alternative. Frail elderly persons due to advanced age or associated diseases are often hospitalized for long periods of time. There, their already modest amount of daily physical activity is reduced, contributing to limit their independence up to force them to bed. Immobility is associated with neuromuscular weakness, functional limitations, thromboembolism and high costs.[248-250] Inspired by the proven capability to recover skeletal muscle strength by home-based Functional Electrical Stimulation even in the worse cases of neuromuscular traumatic injuries,[251] we suggest a brief

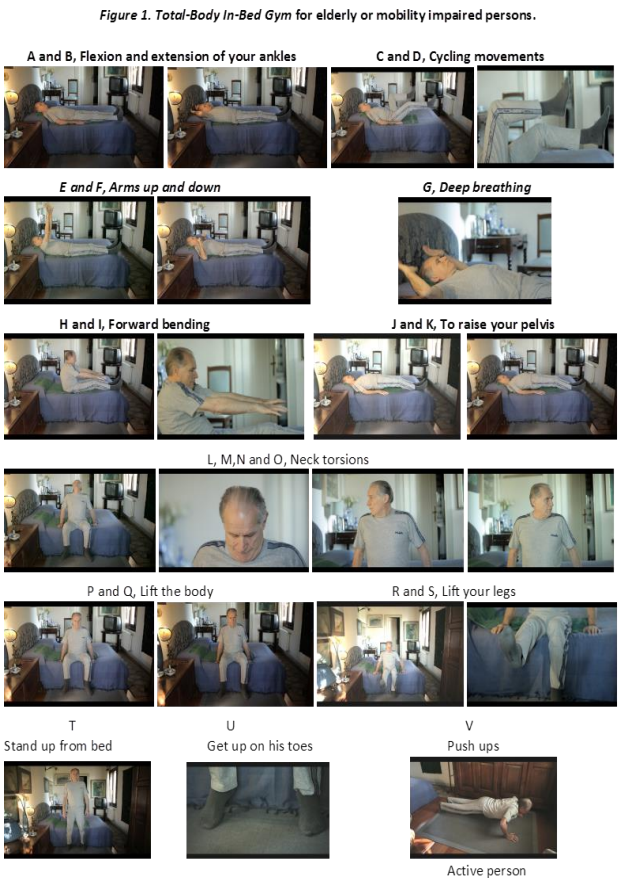

Fig 38. *Active persons, able to make 20 consecutive push-up in 3 minutes (Figure 1, V), do not need In-Bed-Gym, but sedentary people may gradually start with 5 repetition, and add weekly up to 20.*

(15-20 minutes) daily routine of easy-to-perform

physical exercises that may be performed in bed: Full-body In-Bed Gym.[252,253] Full-body In-bed Gym is an extension to all body muscles of well-established physiotherapy approaches of in-bed cardio-circulation-ventilation workouts (Figure 38). If sedentary borderline persons challenge them-self, in hospital Full-body In-Bed Gym may increase muscle strength and fatigue resistance. In surgical units this will grant standing of patients soon after operation, a mandatory measure to prevent risk of thromboembolism. Full-body In-Bed Gym helps also to mitigate the bad mood that accompanies mobility limitations, strengthening patients' confidence in recovering partial or total independence. Continued regularly, Full-body In-Bed Gym may help to maintain independence of older people reducing the risks of the possible serious consequences of accidental falls.

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Altered differentiation of primary myocytes in an ALS mouse model

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Key Words: Amyotrophic lateral sclerosis, differentiation, primary myocytes, ALS mouse model

Amyotrophic lateral sclerosis (ALS) is a neuro-degenerative disease characterized by motor neuron (MN) degeneration, muscle atrophy, paralysis and, ultimately, death. While the great majority of ALS cases are sporadic (sALS), 10% are inherited (fALS), and approximately 20% of fALS are caused by mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene. Accumulating evidence suggests that non-cell-autonomous processes involving the interaction with neighbouring cells contribute to MN pathology in ALS, possibly through "dying-back" mechanisms. In addition, numerous studies indicate that skeletal muscle is one of the primary targets for mutant SOD1 (mSOD1) toxicity both in human sALS and fALS,[254,255] and in transgenic (Tg) mice.[256,257] Little is known about muscle fibre regeneration capacity in ALS. Muscle skeletal regeneration is an important homeostatic process that guarantees maintenance of muscle integrity and

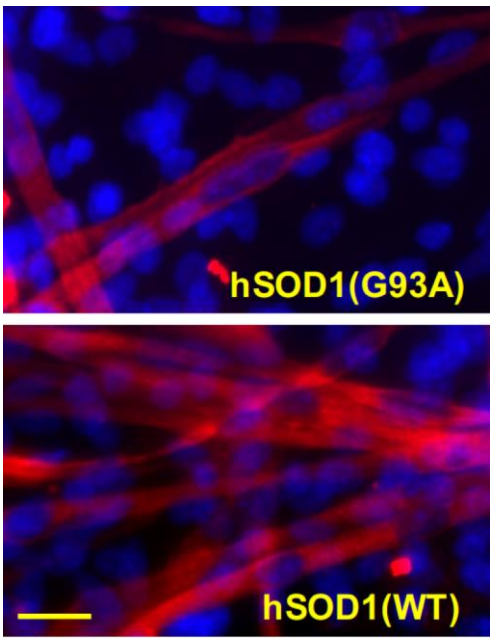

Fig 39. *After 4 days of differentiation in vitro, primary myocytes from neonatal hSOD1(G93A) (upper panel) and hSOD1(WT) (lower panel) Tg mice, were fixed, permeabilised, immuno-stained with an antibody to embryonic myosin heavy chain (eMyHC) (red signal), and then counter-stained with the nuclear fluoro-probe Hoechst-33342 (blue signal). It is evident that cultures from hSOD1(G93A)-expressing mice contain less eMyHC-positive myotubes compared to the hSOD1(WT) control. Scale bar = 20 μm.*

plasticity. In normal muscles, satellite cells became activated, proliferate and differentiate in response to tissue damage to regenerate muscle fibers. A satellite cell impairment, with consequent less efficient regeneration of skeletal muscles, however, has been recently demonstrated in an ALS mouse model expressing the SOD1(G93A) mutant.[258] Such a reduced regenerative potential could limit the efficacy of compensatory processes, thereby contributing to the progression and/or severity of muscle atrophy and weakness. To determine if mSOD1 perturbs the myogenic program, we carried out an in vitro study using primary cultured myocytes from neonatal Tg mice expressing human (h) SOD1(G93A) or the wildtype (WT) hSOD1 counterpart. We found that, 4 days after switching to a differentiative culture medium, hSOD1(G93A) cells show less differentiated myotubes, and reduced expression of embryonal myosin heavy chain, compared to hSOD1(WT) cultures (Figure 39). The finding that expression of both Pax7 and MyoD is decreased in 2 day-old cultured mSOD1 myocytes, and preliminary results from proliferation assays suggest that SOD1(G93A) myoblasts suffer from defective proliferation in the early stages of the myogenic program. As a future perspective, we envisage to get insight into the molecular mechanism that may perturb adult myogenesis in SOD1(G93A) mice by analysing miRNAs (and targets thereof) that are known both to play a leading role in the myogenic program, and to be altered in ALS (i.e., miR1, miR206, miR133a).[259]

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MicroRNA expression in genetic and sporadic ALS

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Key Words: MicroRNA expression, genetic and sporadic ALS

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of upper and lower motor neurons and the progressive loss of synaptic connection between nerve and muscle. The disease leads to a decline in strength, severe muscle atrophy, paralysis and death within 3-5 years after its diagnosis. While the majority of ALS cases are sporadic (SALS), about 10% of ALS cases have a familial inheritance (FALS). Among the familial cases the most frequent genetic cause is associated with an abnormal repeat in the 3' untranslated region of C9orf72 gene. The role of this expansion in ALS disease is still unclear: it is possible that the expansion causes a reduction of C9orf72 protein that in turn contributes to the disease; another more convincing hypothesis is that the abnormal repeats causes the accumulation of intranuclear RNA-foci sequestering RNA-binding proteins as a consequence; a third possible mechanism proposes that the expansion causes the accumulation of toxic dipeptide repeat (DPR) protein.[260,261] Another frequent genetic cause leading to ALS is due to mutation in the gene SOD1, coding for a superoxide dismutase enzyme with an important antioxidant function. Although the exact mechanism that leads to motor neurons degeneration is unknown, the general consensus is that the toxicity arises from the accumulation of the mutant SOD1 protein leading to motor neurons degeneration.[262] MicroRNAs are small non-coding RNAs that regulate the expression of specific genes by binding to the 3' untranslated region of the target mRNA (Figure 40). The binding leads to the cleavage of the target mRNA or to translation impairment, therefore an increase of a specific microRNA leads to the decrease of the corresponding mRNA and to the down-regulation of the corresponding protein. Some microRNAs are expressed in a tissue-specific manner. In muscle a group of microRNAs called myomiRNA regulate specific myogenic processes such as skeletal muscle proliferation, myogenic differentiation and atrophy.[263]

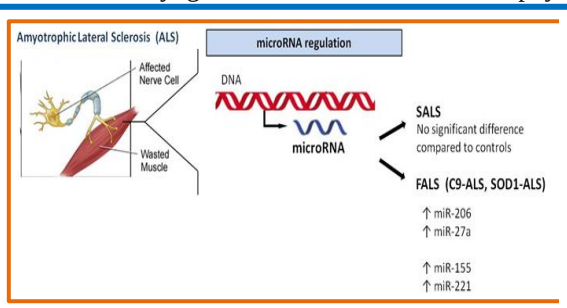

Fig 40. *MicroRNA in ALS muscle are diversely expressed in SALS and FALS patients, with a significant increase in muscle-specific miRNAs, miR-206 and miR-27a, and inflammatory miRNAs, miR-155 and miR-221 in FALS samples.*

Other microRNAs, such as miR-155, miR-146a and miR-221, are involved in the regulation of the immune system and in the inflammatory response during muscle damage or disease promoting the regeneration process.[264,265] In this study we analyzed the expression of five muscle-specific miRNA (miR-1, miR-206, miR133a, miR-133b, miR-27a) and of three inflammatory miRNA (miR-155, miR-146a and miR-221) in muscle biopsies of 18 ALS patients with different genetic form of ALS disease: four carried the C9orf72 hexanucleotide repeat expansion (C9-ALS), four carried the SOD1 mutation (SOD1-ALS) and other 10 were considered SALS. Our results show different expression of miR-206 and miR-27a in genetic (C9-ALS and SOD1-ALS) against SALS. Inflammatory miRNAs were also up-regulated in FALS cases, in particular there was a significant increase in miR-155 and miR-221 in SOD1-ALS against SALS and miR-221 in C9-ALS. Our data suggest that a diverse regulatory mechanism may exist between different forms of ALS.

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Distinct pattern of microRNA expression in Becker dystrophy and LGMD

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Key Words: MicroRNA expression, Becker dystrophy, LGMD

Becker muscular dystrophy (BMD) is an X-linked recessive inherited disorder, due to a mutation in dystrophin gene that encodes for the sarcolemmal protein dystrophin. This mutation leads to translation of a truncated dystrophin, which is expressed at lower and more variable levels than full-length dystrophin. Individuals with this disorder typically present progressive weakness and wasting of the skeletal and cardiac muscles.[266,267] However, some BMD cases have no overt muscle weakness and wasting and their independent activity is maintained until late adulthood.[268] The limb-girdle muscular dystrophies (LGMDs) are an heterogeneous group of rare progressive genetic muscular disorders that are characterized by a progressive weakness with onset in the proximal limb girdle muscles.[269] Two subgroups of limb-girdle muscular dystrophies (LGMDs) are calpainopathy caused by mutations in the CAPN3 gene which encodes the skeletal muscle–specific member of the calpain family,[270] and sarcoglycanopathies in which the primary defect is in one sarcoglycan (SG) transmembrane glycoprotein (alpha, beta, gamma, delta) and results in a deficiency of the whole sarcoglycan complex. We studied sarcoglycanopathies due to mutations in genes encoding two of these glycoproteins, more precisely the beta-sarcoglycan protein and gamma-sarcoglycan protein. MicroRNAs (miRNAs) are small non-coding RNA molecules approximately 22 nucleotides in length, which are involved in gene expression at the post-transcriptional level and regulate many cellular functions. A group of miRNAs are highly expressed in skeletal and cardiac muscle and they are called myomiRs. The myomiR family includes miR-1, miR-133a, miR-133b, miR-206 which are used as non-invasive biomarkers in neuromuscular diseases.[271] We analysed miRNA expression level by Real Time PCR in the serum of 4 BMD patients (Table 3), 6 LGMD patients (4 female, 2 male) and 10 control subjects. The most highly dysregulated serum miRNA in BMD was miR-206, a skeletal muscle–specific miRNA. One patient affected by BMD, who presented a major clinical and MRI alterations with calf hypertrophy, showed a marked increase of miR-206 and miR-133b and a slight up-regulation of the other miRNA, compared to the control group. In LGMD cases it was possible to observe an increase of miR-133a and miR-206 in two patients affected respectively by beta-sarcoglycanopathy and gamma-sarcoglycanopathy while miR-1 and miR-133b levels exhibited no significant dysregulation. These data highlight the potential use of miRNA as biomarkers of BMD and LGMD.

Table 3. *Becker muscular dystrophy patients: clinical, radiological and miRNAs data*

	Age	MRI changes	Treatment	miRNAs changes
Patient 1	41	+++	steroids	↑ miR-1, miR-206, miR-133a, miR-133b
Patient 2	39	++	steroids	↑ miR-206
Patient 3	32	no changes	—	no significant dysregulation
Patient 4	41	+	—	↑ miR-206

↑ = *Up-regulation*

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Rehabilitation in DM1 patients: observation in circulating myomiRNAs and myostatin

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Key Words: Rehabilitation, DM1 patients,: circulating myomiRNAs, myostatin

Myotonic Dystrophy type 1 (DM1), a multi-systemic autosomic dominant disorder caused by a trinucleotide (CTG) expansion, is the most common inherited muscular dystrophy in adulthood. DM1 is characterized mainly by progressive muscular weakness, myotonia, respiratory failure, cardiac defects, cataracts and cerebral involvement. MicroRNAs (miRNAs) are small non-coding RNAs that regulate post-transcriptional mRNA typically by binding to the 3'-untranslated region of the complementary mRNA sequence, resulting in translational repression and gene silencing. Therefore, an increase in a specific miRNA results in a decreased expression of the corresponding protein product. MiRNAs have recently gained attention for their potential as minimally invasive and cost-effective disease biomarkers. MiRNAs are known to be secreted by various cell types and, unlike most mRNAs, they are markedly stable in circulating body fluids due to proteic protection from ribonucleases. Because of their stability in plasma and serum, they can be reliably detected even at low concentration and used not only as markers of disease but also to measure the effectiveness of novel drug therapies and rehabilitation. MiR-1, miR-206, miR-133a and miR-133b are called "myomiRNAs" and are involved in myogenesis, manteinement and recovery of muscles. In DM1 patients miRNA have been extensively studied in both muscle and serum/plasma samples demonstrating that their expression level correlate with loss of muscle strength and with the different disease stage.[272-274] Perfetti and collaborators found an up-regulation of myomiRNA in DM1 patients. Myostatin, also known growth factor 8 (GDF8), belongs to the TGF-β (transforming growth factor-β) superfamily and it acts as negative regulator of muscle mass and it could be used as an important indicator of muscle atrophy. Recently it was reported that myostatin is involved in regulation of pathways of skeletal muscle mass induced by exercise training.[275] MyomiRNAs and myostatin are considered possible biomarkers of muscle atrophy.[276] Rehabilitative intervention is useful to optimize muscle trophism and to

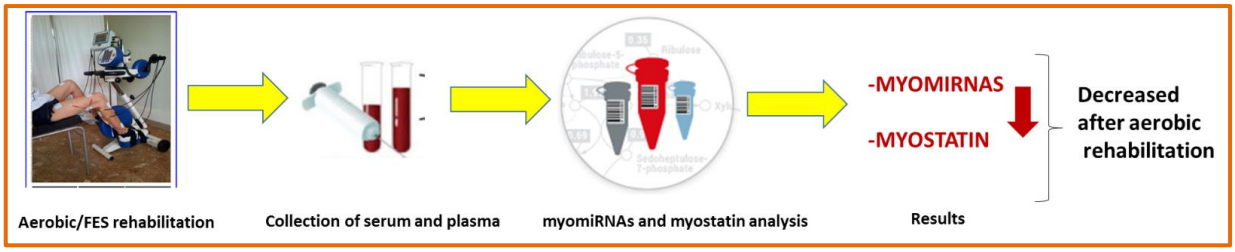

Fig 41. *Experimental flow-chart of the rehabilitation protocol: the DM1 patients make aerobic/FES rehabilitation: before and after training blood sample were collected to analyze myomiRNA and myostatin. Both myomiRNAs (miR-1, miR-206, miR-133a and miR-133b) and myostatin significantly decreased after aerobic/FES rehabilitation*

prevent additional disuse muscle atrophy. Aerobic and resistance training as well as Functional electrical stimulation (FES) combined with cycling improved muscle function and strength in patients with DM1.[277] In this study we collected serum and plasma of 10 DM1 patients (9 male, 1 female) before (T0) and after (T1) a period of 3-6 weeks of physical FES/aerobic rehabilitation. We measured circulating muscle-specific microRNAs by qRT-PCR and myostatin by ELISA. To evaluate endurance and gait speed we used the 6-minute walking test and the time 10-m walk test respectable. Both myomiRNAs (miR-1, miR-206, miR-133a and miR-133b) and myostatin significantly decreased after aerobic/FES rehabilitation and the DM1 patients showed an improvement of muscle strength and fitness performance Our study suggest that myomiR and myostatin levels could support clinical data. MicroRNAs should be considered a good serum biomarkers of functional response to rehabilitation.

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Role of nutrition in neurodegenerative diseases

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Neurodegenerative diseases are characterized by the progressive loss of neuronal function in the brain causing cognitive impairment. The most common form is Alzheimer disease but is also included amyotrophic lateral sclerosis (ALS) and Parkinson disease (PD). Despite inflammation and hormonal deficiencies play an essential role in the pathogenesis of neurodegeneration, nutrition is one of the modifiable factors that has been included in the physiopathology of these diseases. A systematic literature search was performed using PubMed Medline and Cochrane Central Register of Controlled Trials. A combination of the following keywords was used: "nutrition" with "Alzheimer's' disease," "nutrition" with Amyotrophic lateral sclerosis," and "nutrition" with "neurodegeneration." The search included the filter "clinical trials" and "humans." Uncompleted studies that did not evaluate the mental impairment have been excluded. We found 124 articles, of these 27 have been selected including 9058 patients with a mean age of 71,5±7,7. A significant difference between the duration of the studies, (varying from 3 weeks until 51 months) and the number of patients (from 24 until 2911) has been observed. Furthermore, in various studies, information about the type of diet, macronutrients, and calories ingested by the patients are lacking. The most common date emerged is that malnutrition and low body mass index correlated with the higher development of dementia and mortality, showing that nutrition is involved in the neurodegenerative process and SLA.[278] The administration of

polyunsaturated fatty acids, either alone or in combination, had no significant effects on cognitive decline. High-dose B vitamin supplementation seemed beneficial in patients with cognitive dysfunction. Also, the administration of ginkgo biloba did not support any improving cognitive function A high-glycemic diet was associated with the higher cerebral amyloid burden and AD development. Dietary supplementation with protein showed a positive effect on cognitive function. The pathogenesis of motoneuron degeneration is not yet entirely understood, but nutrition represents a critical co-factor regulating the development of the disease. High protein diet and ketogenic diet seem to be the most effective increasing the IGF-1 plasma level and stimulating the IGF-1 receptor expression in the brain.[279] Circulating IGF-1 level has a protective effect on the brain and mediates the formation of new neurons in the adult hippocampus.[280] Insulin signaling is a specific independent inhibitor of neurons regeneration in aging,[281] while the insulin/IGF-1 signaling has a neuroprotective effect.[282] So that favoring lower insulin and higher IGF-1 plasma level has a protective effect on the neuron, and a low carbohydrates-high protein diet seems to support this hormonal attitude while a high-fat diet predisposes to neuroinflammation in central and peripheral nervous systems. Nutrition plays a fundamental role in maintaining brain health and reduce neurodegenerative diseases development. However, an insufficient number of the clinical trial has investigated the interaction of macronutrients, hormonal impact and the decline of brain function. The drawback is represented by the small group of patients evaluated, too short time of observation and limited protocols with insufficient information about macronutrients ingested hormonal evaluation and cognitive evaluation.

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Regional regulation of mechano-sensitive proteins is related to human skeletal muscle remodelling in response to concentric vs. eccentric loading

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Concentric (CON) vs. eccentric (ECC) resistance training (RT) can lead to similar hypertrophy but with distinct changes in muscle architecture (i.e., fascicle length and pennation angle).[283] To gain insights into the molecular mechanisms underlying these remodelling patterns, we collected biopsies at mid-belly and 4 am above distal myotendinous junction (MTJ) of the vastus lateralis (VL) muscle at 0, 4 and 8 weeks of either CON or ECC RT.[284,285] We specifically targeted focal adhesion kinase (FAK) and Vinculin isoforms (gamma- and meta-), mechanosensitive costameric proteins, involved in the modulation of muscle remodelling and protein synthesis.[286,287,] The different adaptations to CON and ECC RT were reflected by a higher y397-FAK/FAK activation and meta-vinculin at the MTJ compared to mid-belly.[285] Furthermore, changes muscle architecture were positively correlated to y397FAK activity only after ECC RT and only at the MTJ site.[3] In the present study, we investigated, using Extended Field of View (EFOV) ultrasonography the regional changes in VL architecture after 8-weeks of ECC-RT or CON-RT at 60% one repetition maximum (1RM ECC and 1 RM CON). Sixteen males were randomized into 3 groups: ECC (n=6), CON (n=6), and non-training controls (CTRL). Longitudinal EFOV images were collected at baseline and at 8-wks to assess fascicle length (Lf) and pennation angle (PA) at 0-20%, 20-40%, and 40-60% of muscle length (Lm), 0 representing the distal MTJ. Changes in Lf were heterogeneous along the VL in the ECC RT group: 9.6±1.9% at 0-20% Lm ($P<0.001$), 6±1% at 20-40% Lm ($P<0.05$) 3.9±1% at 40-60% Lm ($P<0.01$), while PA showed little change. No changes were observed for the CON group. Instead, PA specifically increased after CON training and showed distinct regional variations: 9.4±1.4% at 0-20% Lm ($P<0.05$), 5±0.6% at 20-40% Lm ($P<0.05$) and 4.3±1% at 40-60% Lm (N.S.). The present data provide evidence that the architectural remodelling strategies of human muscle to ECC and CON loading are region-specific. Together with evidence of activation of region and contraction-specific mechanosensitive pathways, such data may suggest that pY397FAK and meta-vinculin may play a role in orchestrating the pattern of muscle remodelling.

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