2022PDM3 Abstract 01

Human Brody disease and novel therapeutic approaches of its animal model cattle pseudomyotonia

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Bovine "congenital pseudomyotonia" (PMT) is a genetic muscular disorder very similar to human Brody myopathy (Brody 1996)[1] for clinical signs. Exercise-induced stiffness and delayed muscular relaxation following even mild physical exercise, are the major clinical symptoms. A missense mutation in the ATP2A1 gene (Drögemüller et al 2008)[2], encoding sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA1), causes congenital PMT in cattle and Brody disease in human. Clinical symptoms genetic and biochemical findings clearly demonstrated that congenital PMT in Chianina cattle is the real analogue of Brody myopathy (Sacchetto et al 2009).[3] This is not unexpected given that human diseases have recently been discovered in a variety of domestic animal species. Our research team provided evidence that in bovine PMT, SERCA1 mutation generates a protein most likely corrupted in proper folding, which was ubiquitinated and prematurely degraded by the ubiquitin-proteasome system, although it retained its catalytic properties (Bianchini et al. 2014).[4] The treatment with proteasome inhibitors restores SERCA levels and Ca2+ homeostasis in a cellular model and in muscle fibres from PMT affected animals (ex vivo experiments) (collected in conformance with the institutional guidelines for the care and use of animals). At present, neither specific therapy nor mouse model for Brody myopathy exists. However, we have recently designed and proven *in vitro* a novel pharmacological approach based on the employment of protein folding correctors named CFTR (Cystic Fibrosis Transmembrane Regulator) exploited in Cystic Fibrosis. The treatment with CFTR correctors rescued the expression level of mutated SERCA1 in HEK293 cell model. These data have been confirmed in vivo by local treatments of bovine PMT muscle with the most effective in vitro CFTR corrector.

Key words: Brody disease; Bovine pseudomyotonia; SERCA1.

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2022PDM3 Abstract 02

The Venice Marathon 2007-2019 as a model for analyses of Master Athletes

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The number of marathon events increased during the last 30 years, and in particular increased those of the Master Athletes.[1,2] There are evidences that long-distance running could provide considerable health benefits for older runners, specifically risk reduction of cardiovascular diseases, cancer, diabetes, depression, and falls.[3,4] In this study we have analyzed data from several editions of the Venice marathon, a famous Italian race that attracts people from every corner of the world. The data analysis was done on age category distribution (left panel), nation (right panel) and performance in 13 editions (2007-2019) evaluating the performance of all the athletes vs. the top 10% fastest athletes (TOP 10%). The analysis has shown a steady increase in female participation, in particular for 40-50 years old, and a substantial reduction of performance in the younger category of 30-35 years old, both male and female, from 2003 to 2019. The intra-category differences of performances have shown that the trends in male and female were similar and a specific steeper decline in performance was identified at age 55 for the women and at 50 for the men with specific differences between the set of all athletes and the TOP10% one. The model of the Venice marathon can therefore be useful to analyze the gender participation and in particular the human model of successful aging .[5-7]

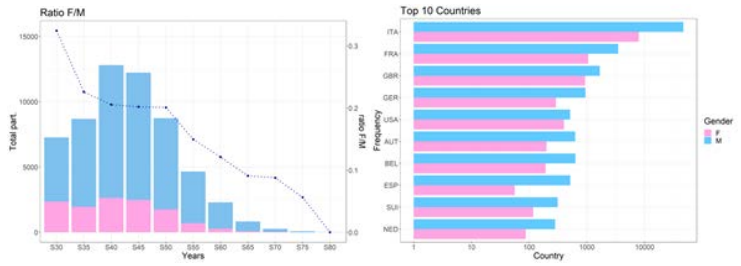

Fig 1. *. The data analysis was done on age category distribution (left panel), nation (right panel) and performance in 13 editions (2007-2019) evaluating the performance of all the athletes vs. the top 10% fastest athletes (TOP 10%).*

Key words: Aging performance decay; Masters; gender differences.

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2022PDM3 Abstract 03

Masticatory muscles guided orthodontic treatments

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The purpose of orthodontic treatment is to guide the development of the upper and lower jaw during childhood and adolescence to establish proper eruption and arrangement of teeth in the alveolar bone in balance with oral functions.[1] Centripetal forces are exerted on the dental arches by the perioral muscles and centrifugal forces by the tongue. These structures exert slight pressures at rest for prolonged periods of time and are therefore fundamental in determining the dental position. Alteration in these forces influence the orientation of the skeletal structures thus resulting in malocclusion.[2] The development of the masticatory system is mostly determined by hereditary and environmental factors. In this context the activity of masticatory and perioral muscles is determinant not only in the setting of malocclusion but also in the definition of the treatment plan and in the long-term retention phase.[3] During childhood the highest risk to develop malocclusion is linked to alterations in the normal pattern of oral functions (atypical swallowing, oral breathing, bad oral habits) with a consequent modification of the skeletal relations between the two jaws. In this period orthopedic and myofunctional treatment are pursued to restore a correct balance in the jaw's growth.[4] In adolescence patients presenting skeletal class II malocclusion (most frequent malocclusion in Europe) are mostly treated by using functional appliance to correct the discrepancy between the upper and lower jaw (increased overjet). To do so the removable appliance stress out the soft tissue and muscles fibers to allow a forward repositioning of the mandible.[5] In adults' patients the lack of residual growth affects the treatment toward a surgical procedure or a camouflage treatment. Even in this period, muscles activity represents a determinant element in the treatment planning and in the long-term stability.[6]

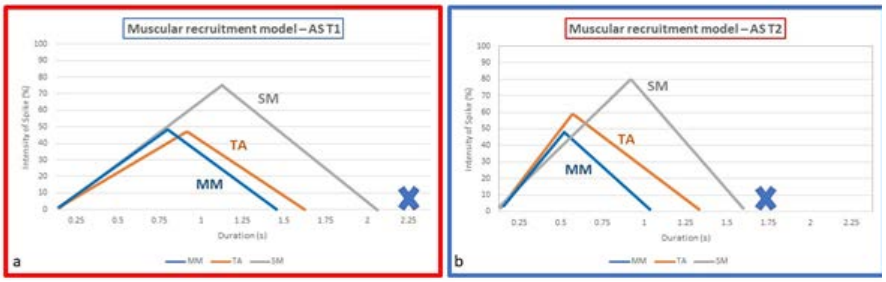

Fig 1. *Graphic representing the time and the intensity of the spike of activation of each couple of muscles during saliva swallowing in a sample of adolescent patients before the myofunctional treatment at T1 (fig a) and after the myofunctional treatment at T2 (fig b), considering mean values. MM, TA and SM are reported with different colors. Duration (s) on the abscissae axis and intensity of activity (%) on the ordinates axis. MM, masseter; SM, submental muscles; TA, anterior temporalis. "X" is located on the x axis at the time of the end of the swallowing act.*

Key words: Orthodontics; malocclusion; muscles activity; myofunctional therapy.

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2022PDM3 Abstract 04

The effect of direct muscle stimulation on denervated gluteal muscles and tissue composition in people with chronic spinal cord injury – preliminary results

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Electrical stimulation of denervated muscles with long pulses to convert connective and adipose tissue into contractile muscle tissue has been investigated in persons with chronic spinal cord injury.[1–3] A European funded project (RISE) has demonstrated that a stimulation period of two years led to significant changes in muscle growth and reduction in adipose tissue in the lower limb muscles.[4] It is still unclear after what time the transformation process from connective and fatty tissue to contractile muscle tissue begins in chronic paraplegia with damage to the lower motor neuron. Likewise, the process of this conversion and how it occurs remains unclear. In other words, is there an immediate visible increase in muscle mass or does connective and adipose tissue break down first and when do these changes in tissue composition start to occur? Kern and Carraro have visualized and emphasized on the importance of life-long stimulation in people with lower motor neuron lesion in order to maintain proper blood circulation and to produce a so-called "cushioning effect" in people with spinal cord injury (SCI) to prevent them from skin injuries.[5] The aim of the present study is to investigate the effect of electrical stimulation of chronic denervated gluteal muscles in the first six months after stimulation onset. Changes in muscle as well as adipose tissue thickness were assessed over the stimulation period of six months using standardized magnetic resonance imaging (MRI) measurements of the gluteal area on the projected line of the sacroiliac joint (SIJ), the acetabulum and the coccyx. The study participants (four participants with AIS A and one with AIS C) stimulated the denervated gluteal muscles five times a week during a period of six months. The stimulation was composed of a warm-up phase and a training phase lasting a total of 33 minutes per session. The electrical stimulation was administered with the Stimulette den2x (Schuhfried GmbH Vienna, Austria) with the sponge electrodes being placed on the buttocks to generate a horizontally oriented electrical field to cover the entire gluteal area.

A **B**

Fig 1*. Changes in tissue composition over a period of six months elicited through the application of direct electrical muscle stimulation.* ***A)*** *Changes in muscle thickness from baseline to three months after stimulation onset (light blue); from the third to the sixth month after stimulation onset (mid blue) and from baseline to six months after stimulation onset (dark blue).* ***B)*** *Changes in thickness of fat tissue from baseline to three months after stimulation onset (light green); from the third to the sixth month after stimulation onset (mid green) and from baseline to six months after stimulation onset (dark green). Abbreviation: SIJ=sacroiliac joint 2 results depicted in figures 1A and 1B show that from baseline to six months after stimulation onset, the muscle thickness increased at the level of the SIJ and the acetabulum. This change in muscle tissue was accompanied by a decrease in the thickness of adipose and connective tissue. In contrast, a rather small increase in muscle thickness and a gain in adipose tissue was assessed at the lowest of the three measuring points (coccyx). Based on these few preliminary results, the authors hypothesize that a gain in muscle tissue is already initiated within only six months of direct muscle stimulation in people with chronic SCI (>2 years). The differences in muscle thickness increase in the three measured regions might be explained by anatomical and simulation-based circumstances. However, these first results of the study highlight changes after only six months of direct muscle stimulation in people with chronic SCI. The preliminary results show an increase in muscle thickness and a decrease in adipose tissue. However, the results among the five participants were heterogeneous and the investigation of a higher number of participants is therefore required as anticipated in the current study (NCT02265042).*

Keywords: Denervation, Electrical stimulation, SCI, Tissue composition, Magnetic resonance imaging

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2022PDM3 Abstract 05

Activation of Akt-mTORC1 signaling reverts cancer-dependent muscle wasting

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Loss of muscle mass is a debilitating aspect in numerous forms of cancer. Here, we use various transgenic mouse strains to determine the role of Akt-mTORC1 signaling in skeletal muscle during cancer cachexia. We find that loss of Raptor only in muscle fibers does not aggravate cancer-related muscle wasting. On the contrary, activation of Akt-mTORC1 signaling is sufficient to completely revert muscle wasting, normalize the muscle transcriptome, and prevent protein degradation.

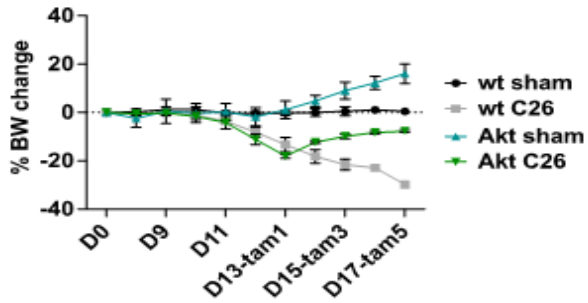

Fig 1. *Representation of body weight progression in wt and Akt mice with or without inoculation of C26: when mice lose about 10-12% of body weight, tamoxifen is administered daily for 5 days to activate Akt.*

Key words: Cancer cachexia, mTOR, Raptor, Akt, muscle growth.

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2022PDM3 Abstract 06

Our Discovery of new intracellular junctions: The calcium entry units (CEUs)

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In 2017, Boncompagni, Michelucci et al. demonstrated that during exercise the sarcotubular system of extensor digitorum longus (EDL) fibers undergoes a profound remodeling that leads to the assembly of new junctions between T-tubule extensions at the I band and sarcoplasmic reticulum (SR) stacks. As these junctions contain colocalized STIM1 and Orai1 and enhance store-operated Ca2+ entry (SOCE), they have been named Ca2+ entry units (CEUs). In addition, it has been more recently shown that (1) CEUs disassemble following recovery, with T-tubules retraction from the I band faster than SR stacks disassembly, and (2) lack of calsequestrin-1 (CASQ1) induces a constitutive assembly of CEUs, resulting in enhanced SOCE that counteracts the SR Ca2+ depletion. We have now analyzed (1) CEUs during postnatal maturation (at 2 wk of age) and (2) whether CEUs form in slow-twitch fibers (soleus). (a) Compared with adult (4 mo) EDL fibers of resting WT mice, at 2 wk of age we found a greater longitudinal disposition of T-tubules associated to SR membranes forming junctions virtually identical to CEUs in adult EDLs of exercised WT mice, which promote increased STIM1/Orai1-mediated SOCE. (b) We also compared structure and function of soleus (which also express the cardiac isoform CASQ2) from WT mice and from mice lacking either CASQ1 (CASQ1-null) or CASQ1/2 (dCASQ-null). In soleus from both genotypes, CEUs are constitutively assembled although they appear structurally smaller than those described previously in exercised WT or CASQ1-null EDLs. A detailed EM quantitative analysis revealed that CEUs were more abundant in dCASQ-null than CASQ1-null mice. The amount of CEUs strictly correlated with the ability of soleus fibers to recover extracellular Ca2+ via SOCE to support contractility during high-frequency stimulation. Molecular analysis of Western blots, showing that Orai1 expression was enhanced following ablation of CASQ, support these data.

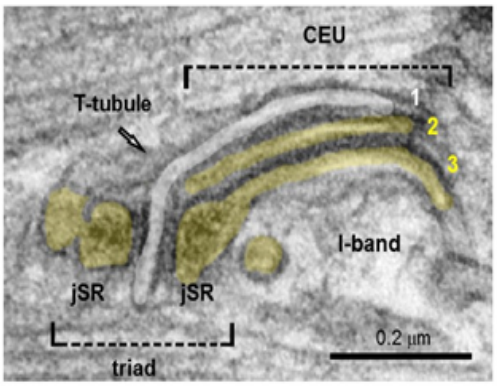

Fig 1. Representative EM image of a triad and a CEU assembled in an adult EDL fiber from wild type mouse*. During acute exercise, sarcotubular membranes are capable of significant remodeling that leads to assembly of CEUs. In this image, the CEU is composed of one T-tubule (in white)and two SR cisternae (in yellow. The continuity of a triadic T-tubule with one of these newly formed structures is clearly visible.*

Key words: Sarcotubular system; CASQ1-null; Ca^{2+} entry units; CEUs.

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2022PDM3 Abstract 07

Rehabilitation of peripheral nerve disorders by the use of physical agents. A multiperspective literature evaluation

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A large number of diseases can affect the peripheral nervous system, with different etiologies and different relative needed managements. The proper diagnosis is necessary for the correct indication of treatment bases on drugs and surgery. Besides these, rehabilitation plays a fundamental role to get the amelioration of functions and quality of life. Among the several approaches, physical agents represent an effective group of conservative methods, which may positively influence nerve recovery. These agents are based on different types of energies (mechanical, electrical, magnetic, thermal) with peculiar indications and targets aimed at pain relief, tissue restoration, neuromuscular stimulation, anti-inflammatory action. We performed a multiperspective literature evaluation to present the state of art about the use of physical agents in the rehabilitation of nerve injuries. We collected data about the major studied diseases and the relative effects, the most common outcome measures, the most involved countries in the world about this topic and the modality of the description of the physical agents. We searched in PubMed the terms “physical agents rehabilitation AND peripheral nerv*”, finding 131 papers. Our literature review showed the potential benefits of the physical agents even if further studies should be needed for confirmation.

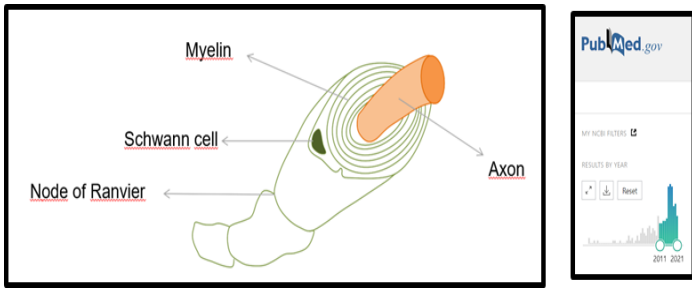

Fig 1. *Literature analysis of “physical agents rehabilitation AND peripheral nerv*”*

Key Words: Rehabilitation; peripheral nerve; physical agents; literature review.

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2022PDM3 Abstract 08

Connections between hyaluronan properties and fascial health

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Hyaluronan (HA) is the most abundant polysaccharide of the extracellular matrix of connective tissues and the key element that underlies the fascial gliding, lubricating the loose connective tissue between the densely packed collagen layers, between the deep fascia and muscle, and within the muscle itself[1]. It plays also an important role in cell proliferation and mobility, inflammation and angiogenesis, and it is involved in various diseases such as cancer and diabetes[2]. The mean amount of HA in muscular deep fasciae changes according to the various gliding functions to the anatomical site: we found about 43 µg/g of HA in aponeurotic fasciae, which are free to glide over the muscles; there was a drastic decrease (about 6 µg/g) in epimysial fasciae, completely adherent to the underlying muscles, and an increase in the retinacula surrounding joints (90.4 µg/g)[3]. Many physical, mechanical, hormonal and pharmacological factors can influence the production of the various fibrous and glycosaminoglycans components of the fascial extracellular matrix: we found that human fascial fibroblasts are able to produce HA-rich vesicles in vitro within a few hours of cannabinoid CB2 receptor agonist treatment, leading to greater tissue fluidity[4]. In the same way, extracorporeal shock-waves also produce a change in the extracellular matrix and an increase in the HA production, with a reduction in tissue stiffness. Furthermore, viscosity of HA is increased by changes in body temperature (cold) and immobilization. These data can have many clinical implications. For example, an increase in temperature, even of only 2 degrees centigrade, caused by a thermal care, sauna or a deep massage, brings a progressive break-up of the three-dimensional superstructure of HA chains, with a consequent decrease in viscosity. At the contrary, cold environment and immobility can increase viscosity of the loose connective tissue, leading to less flexibility of the fasciae and more friction among muscle bundles. Understanding the physicochemical characteristics of HA can help to understand which are the behaviors and the targeted treatments for a healthy fascia.

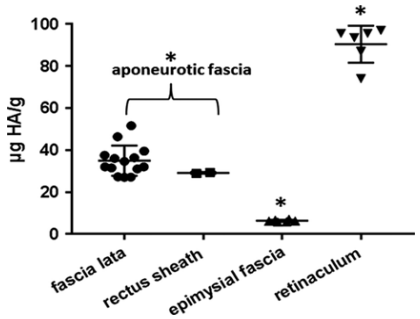

Fig 1. *Mean HA values (µg HA g−1 of wet starting tissue) and standard deviations, derived from fascia lata and rectus sheath (aponeurotic fasciae), epimysial fasciae, and retinacula. P > 0.05, t-test fascia lata vs. rectus sheath; *P < 0.01, t-test comparing aponeurotic fascia, epimysial fascia, and retinaculum.*

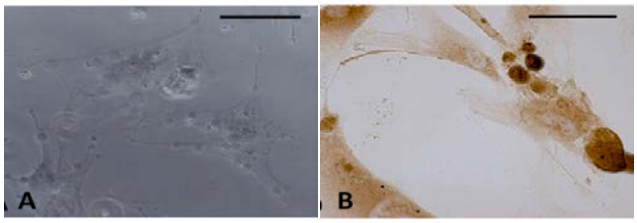

Fig 2: *Bright field image (A) and immunostaining with HABP (B) of fascial cells treated for 4 h with HU-308 2.5 µM. HA-rich vesicles are evident. Scale bars: 25 µm.*

Key Words: Hyaluronan; fascia; viscosity; gliding; stiffness

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2022PDM3 Abstract 09

Proteomic profiling of the aged diaphragm from the *mdx-4cv* model of dystrophinopathy

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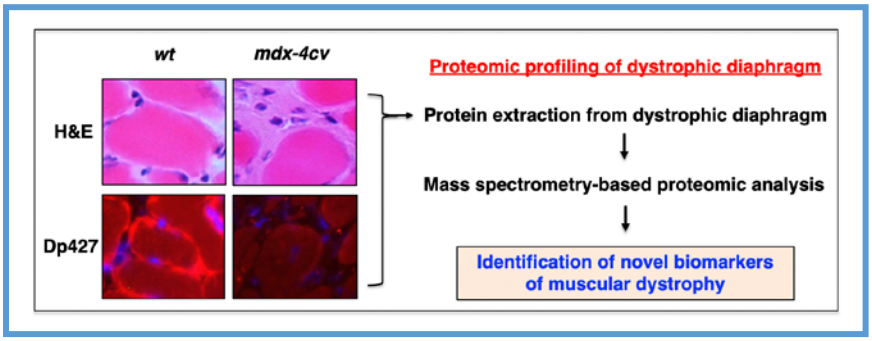
X-linked Duchenne muscular dystrophy is due to mutations in the *DMD* gene, which causes the almost complete loss of the membrane cytoskeletal protein dystrophin. The diaphragm is severely affected in dystrophinopathy and is characterized by progressive muscle fibre degeneration, chronic inflammation and reactive myofibrosis. The major goal of this new study was to identify novel proteomic biomarker candidates of muscle weakness and respiratory deficiency in dystrophinopathy. In order to investigate proteome-wide changes in the dystrophic and aged diaphragm muscle, our laboratories have analysed 15 months old wild type mice versus age-matched *mdx-4cv* mice, which represent an established genetic model of Duchenne muscular dystrophy. Mass spectrometry-based proteomics was combined with systems biological analyses and verification studies using comparative immunoblotting. A variety of changes in proteins involved in the maintenance and organization of both the extracellular matrix and the intracellular cytoskeleton were identified by mass spectrometry. These new disease-related protein markers could be helpful to improve diagnostic procedures, as well as prognostic and therapy-monitoring approaches.



Key Words: Diaphragm, Duchenne muscular dystrophy, fibrosis, mass spectrometry, proteomics.

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2022PDM3 Abstract 10

Gait analysis interplay with non-motor mental symptoms in Parkinson's disease

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Parkinson's Disease (PD) is a neurodegenerative disease which involves both motor and non-motor symptoms [1]. Non-motor mental symptoms are very common among patients with PD since the earliest stage. They include mood and affective symptoms like apathy, anhedonia, anxiet and depression, cognitive dysfunction, and psychotic symptoms such as hallucinosis and delusions. In this context, gait analysis allows to detect quantitative gait variables able to distinguish patients affected by non-motor mental symptoms from patients without these symptoms. In this research, 68 PD patients were grouped in subjects with and without non-motor mental symptoms based on MDS-UPDRS I (item 1-6). All patients performed gait analysis according to the Davis protocol [2] both in single and dual-task. Spatial and temporal parameters describing gait were extracted, as already done in previous researches [3]. The results of statistical analysis showed significant different gait patterns in patients with non-motor mental symptoms as compared with those without. Single-task variables showed that 9 out of 16 spatiotemporal features were statistically significant for the univariate statistical analysis ($p < 0.05$). In addition, a statistically significant difference was found in stance phase ($p=0.032$), swing phase ($p=0.042$) and cycle length ($p=0.03$) of the dual task. In conclusion, PD patients with non-motor mental symptoms versus PD patients without this cluster of symptoms showed a worse gait pattern in both single and dual task, thus supporting the notion of a more widespread disease involving neuro-transmitters systems other than dopamine in patients with neuropsychiatric symptoms.

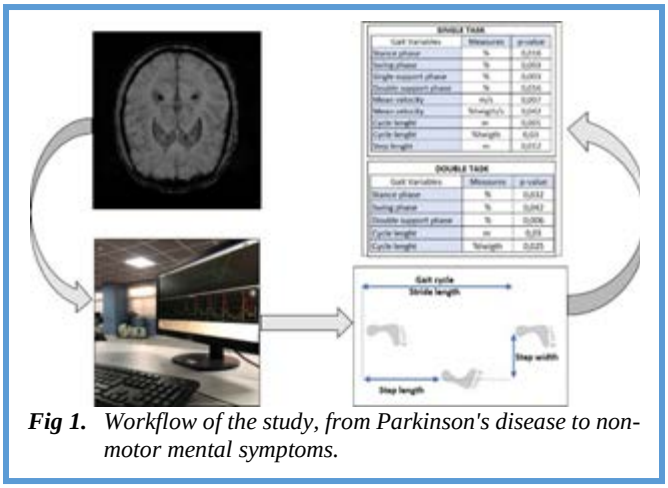

Fig 1. *Workflow of the study, from Parkinson's disease to non-motor mental symptoms.*

Key Words: Parkinson's Disease, non-motor mental symptoms, gait analysis

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2022PDM3 Abstract 11

A Novel Knee Bone and Cartilage Osteoarthritis Index Extracted from a Patient-Specific Image Feature Analysis

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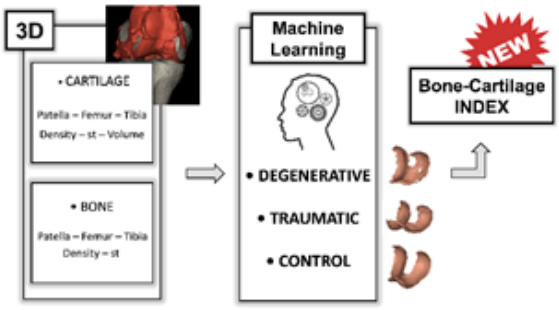

Fig 2. *Novel 3D features extracted from Knee CT Scan and MRI are used to classify degenerative, traumatic, and control subjects in order to create a new knee bone-cartilage index to assess osteoarthritis.*

Knee Osteoarthritis is one of the most common form of arthritis and affects a significant percentage of the world population.[1] Multiple indexes created to classify the cartilage status are present in the medical literature and are mainly based on physicians' image analysis and subjected to different and personal interpretations.[2,3] In the frame of the European Project RESTORE a database of MRI and CT-scan images of degenerative, traumatic, and healthy patients is developed. Approximately 100 objective features are extracted from both 2D, and 3D bone and cartilage images. Machine Learning is then performed to select the most important features which will define a novel patient-specific index able to classify the knee osteoarthritis status with high accuracy.

Key words: Knee Osteoarthritis; machine learning; medical imaging; knee cartilage.

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2022PDM3 Abstract 12

Postural Control Assessment through Visual Induced Motion Sickness and a Moving Force Platform

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In this research a novel and unique Motion Sickness moving platform is used to study different postural control reactions.[1,2] The platform simulates a rough sea environment through virtual reality which visualize waves that coordinate the platform movements. Together with EEG, EMG, and hearth rate sensors a force platform is installed under the subject feet during the experiment. This research is focused on the extraction of multiple features from the force platform to study the postural movements of the different subjects before, during and after the sea simulation. These features are then selected using a machine learning approach focusing on the ones which allows to distinguish between motion sick and not sick subjects with higher accuracy. Then the different postural behaviors during the simulation are confronted between different classes of participants like elderly and young, sporty, and not and others. Finally, the selected features are correlated with the EMG data to study the relations between postural and muscle reaction to the induced motion sickness environment.

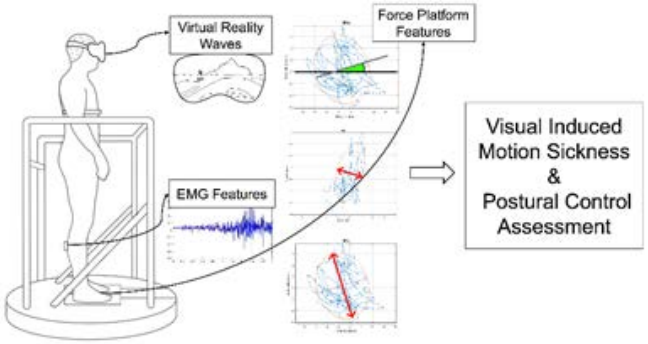

Fig 3. *Novel 3D features extracted from Knee CT Scan and MRI are used to classify degenerative, traumatic, and control subjects in order to create a new knee bone-cartilage index to assess osteoarthritis.*

Key words: Motion Sickness; posturalcControl; machine learning; force platform; virtual reality.

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2022PDM3 Abstract 13

Multi-scale Bone Remodeling Prediction in Patients Undergoing Total Hip Arthroplasty

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Total hip arthroplasty (THA) is the most widely used surgery in case of severe damage of hip joints. However, the implant produces significant variations of stress distribution in the operated femur, determining the host bone remodeling, which, in turn, influences the bone quality of THA patient. The aim of the study is to create a prediction model, which allows determining significant parameters for THA femur quality during the remodeling, starting from patient clinical data, diagnostic x-ray computed tomography (XCT) and 3D high resolution imaging. Patient clinical data as age, gender, body mass index, type of operation (unilateral or bilateral), prosthesis (cemented or uncemented) and diagnosed pathologies connected to bone tissue, are correlated to multi-scale parameters of femur. Information at macro level is obtained by the analysis of diagnostic X-ray computed tomography (d-XCT) scans of patient femurs. Data are segmented to collect the densitometry of regions of orthopedic interest (gruen zones). The densitometry of rectus femoris muscle is also considered, due to the fact that remains roughly recognizable also when it is degenerated. Micro and nano femur morphometric parameters are obtained by high resolution 3D imaging on femur biopsies, achieving information on bone microarchitecture and ultrastructure. After a learning, based on above-mentioned THA patients' data at 24 hours, 1 years and 6 years after the implant, the prediction model can predict the bone quality and the better-regenerated zones. The prediction model of THA femur quality works also as support of further clinical decisions on post-surgery treatments and rehabilitation, by providing a projection on patient mobility.

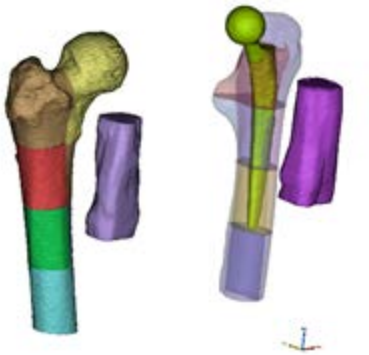

Fig 4. *The figure shows the healthy and operated femur of the same patient. In both are visible the Gruen zones highlighted cutting different planes of the bone, and the rectus femoris muscle. The Bone Mineral Density of those areas in the operated leg, is analyzed for predictions on bone remodeling due to variation of stress distribution after a THA surgery, while healthy leg is used as control.*

Key words:

References

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2022PDM3 Abstract 14

IGF-I from satellite cells is critical for skeletal muscle growth and regeneration.

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Satellite cells are an absolute requirement for postnatal muscle growth and muscle regeneration. Insulin-like Growth Factor-I (IGF-I) is well known to promote satellite cell function by expanding satellite cell proliferation and accelerating differentiation. We previously established that muscle fiber provides an important endogenous source of IGF-I for anabolic actions on the skeletal muscle(1); however, whether satellite cells require IGF-I from other cell types remains unclear(2). Much speculation determined that IGF-I diffuses into the satellite cell niche for its actions, although no direct evidence had been shown. Thus, we sought to determine the critical sources of IGF-I for muscle growth and regeneration. We generated novel mouse models with inducible tissue- and cell-specific ablation of IGF-I: SID (Satellite cell IGF-I Deficient), MID (Muscle IGF-I Deficient), SMID (Satellite cell and Muscle IGF-I Deficient), to test the hypothesis that satellite cells require an autocrine source of IGF-I for muscle growth and regeneration. Mice lacking the floxed exon 4 of *Igf1* (*Igf1fl/fl*) alleles but retaining the Cre drivers and Rosa reporters served as controls (CTRL) for doxycycline (DOX) and tamoxifen (TAM) treatment effects. Male pups at postnatal day 3 (P3) were treated with TAM/VEH(3) and/or DOX for 5 consecutive days to induce *Igf1* deletion, and hindlimb muscles were harvested at P28. SID and SMID pups that received TAM were 22% and 34% smaller in body weight when compared to VEH treated littermates. The absolute mass of major hindlimb muscles (TA, soleus, EDL, Gastrocnemius, and Quadriceps) for SID and SMID mice were significantly smaller than VEH treated littermates, suggesting the importance of IGF-I from satellite cells for muscle development. To determine if IGF-I from satellite cells is crucial for muscle regeneration, we treated 20-24-week-old mice (n=4/sex/timepoint/genotype) with TAM and/or DOX prior to unilateral Cardiotoxin (CTX) injection into the TA(3), while the contralateral TA served as an internal control. Muscles were harvested at days 3, 5, 7, 11, and 14 post-CTX. TA masses from all groups at days 3-7 post-CTX were significantly lower ($p<0.05$) compared to contralateral non-damaged muscles. All groups were able to recover to non-damaged mass levels by day 14 post-CTX except SID animals. There were no differences when comparing CTRL and MID muscle fiber areas, instead, SID muscle fibers were smaller when compared to CTRL muscles, suggesting that satellite cell IGF-I are crucial for muscle regeneration. Surprisingly, SMID mice were able to recover to CTRL levels in fiber area, indicating a potential compensatory IGF-I supply from non-muscle sources. Next, in-situ functional testing(4) was performed comparing damaged vs. non-damaged TAs. At day 14 post-injury, normalized forces were lower in damaged TAs of CTRL, MID, and SMID animals when compared to contralateral non-damaged TA; however, there was no difference in force for SID animals, suggesting the importance of muscle-specific IGF-I for muscle function post-injury. In conclusion, IGF-I generated by satellite cells is critical for efficient skeletal muscle regeneration, while both satellite cells and myofibers provide integral sources of IGF-I for muscle development.

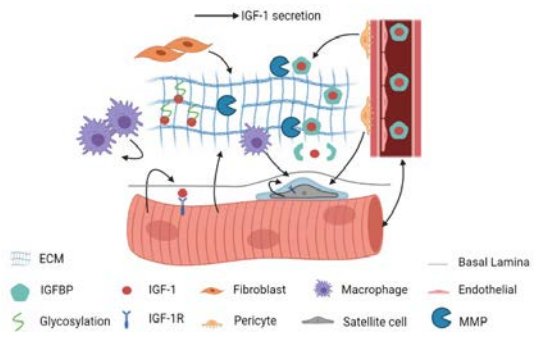

Fig 5. *Multiple sources of IGF-I in the muscle. IGF-I is secreted by various cell types within the muscle, including muscle fibers, satellite cells, fibroblasts, macrophages, pericytes, and from the circulation. IGF-I bind to its receptor for a downstream signaling cascade resulting in muscle growth.*[2]

Key words: Satellite cells; IGF-I; muscle regeneration; postnatal muscle growth.

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2022PDM3 Abstract 15

Micro-dystrophin-mediated utrophin displacement from cardiomyocyte sarcolemma in the D2.*mdx* mouse model of DMD

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Duchenne muscular dystrophy (DMD) results from mutations in the DMD gene and the subsequent loss of functional dystrophin protein.[1,2] In its absence, utrophin – the autosomal paralogue of dystrophin – is upregulated and compensates, albeit incompletely, for the loss of dystrophin.[3,4] Adeno-associated virus (AAV)-mediated gene delivery provides the means to restore the expression of missing proteins with the aim of reversing the pathology of such monogenic disorders. The size of the dystrophin gene, the largest in the human genome, however, makes it impossible to re-introduce full-length dystrophin. This limitation is partly overcome by the use of heavily truncated iterations of the dystrophin protein (micro-dystrophin, μDys) 5,6. AAVs containing versions of micro-dystrophin are now being delivered to patients with DMD in clinical trials. Such micro-dystrophins, however, are unlikely to fully compensate for the loss of dystrophin, a protein now recognized for its scaffolding/signaling roles in addition to its traditional role in mechanical force transmission. However, there has been no consideration of the fact that the cardiac disease progression is greatly slowed by the high degree of utrophin expression in the heart,[7,8] and that there could be a displacement of utrophin by micro-dystrophin expressed at high levels. Indeed, in examining the potential therapeutic efficacy of a micro-dystrophin, μDys ΔR3-R21_ΔCT (missing spectrin-like repeats 3 through 21 as well as the C-terminal end of the dystrophin protein) 5 in the D2.mdx, [9,10] mouse model of DMD, we find that AAV-mediated overexpression of micro-dystrophin leads to the displacement of utrophin from the cardiomyocyte sarcolemma. This appears to, in essence, phenocopy dystrophin/utrophin double mutants that exhibit an exaggerated cardiac dysfunction.[7]. As the heavily truncated micro-dystrophin, μDys ΔR3-R21_ΔCT, is unlikely to functionally replace full length utrophin, its overexpression may be detrimental to cardiac function.

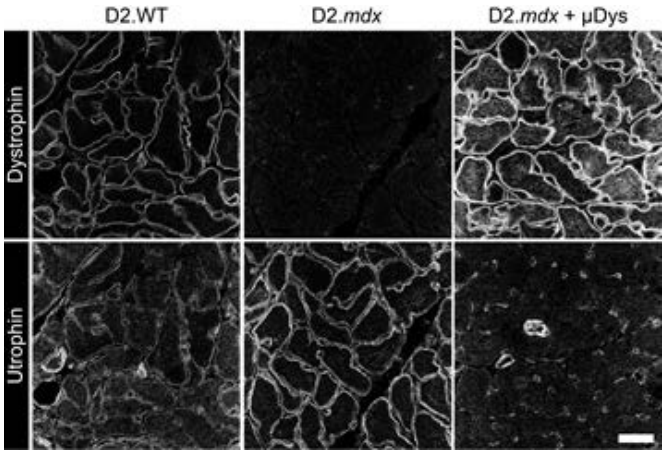

Fig 6. *Micro-dystrophin overexpression displaces utrophin from sarcolemma of cardiomyocyte and skeletal muscle fibers. Transverse sections of hearts from D2.WT, D2.mdx and micro-dystrophin (μDystrophin)-treated D2.mdx mice were labeled for dystrophin and utrophin. The increased sarcolemmal utrophin expression in absence of endogenous dystrophin is negated by μDystrophin overexpression in cardiomyocytes.*

Key words: Duchenne muscular dystrophy; dystrophin; utrophin; adeno-associated virus; microdystrophin; heart.

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2022PDM3 Abstract 16

Can home-based rehabilitation be effective to counteract skeletal muscle atrophy and to ameliorate physical functioning of elderly patients?

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Elderly subjects with musculoskeletal and neurological diseases spend less time training on a regular basis, worsening their mobility concerns and undergoing muscular atrophy. As a result of the pandemic, access to regular physical activity has reduced even in healthy older individuals, due to mobility restrictions, quarantine, and lockdown. It might be beneficial to provide all these individuals, both affected by chronic disorders and healthy, with rehabilitation program to be admistered at home or even in bed (Full-Body In-Bed Gym). Home-based exercise programs are increasingly being employed in order to improve physical functioning of elderly patients with orthopedic (e.g., hip and knee osteoarthritis) and neurological disorders (e.g., Parkinson's disease, spinal cord injury, etc.). Through a comprehensive review of the literature, we investigated what is the evidence of the efficacy of home-based exercise programs for different subjects and what are the most appropriate modalities to perform these programs. The evidence in literature seems to be promising: home-based treatment seems to be effective, safe and usable by elderly patients suffering from different diseases as well as healthy ones. In the future, home-based exercise programs may represent a valuable option to counteract muscle atrophy and reduced motor function in the elderly, taking also advantage of modern devices such as those employed in telerehabilitation.

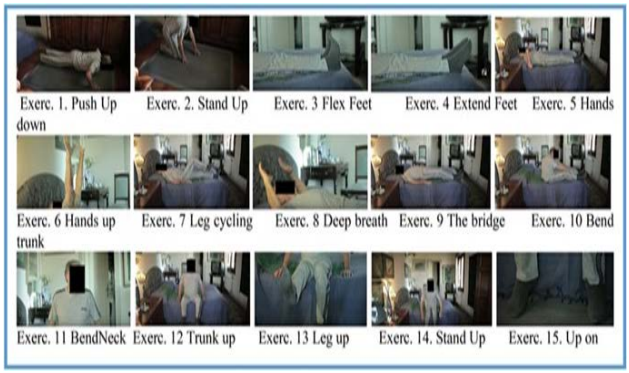

Fig 7. *Proposed sequence of Full-Body In-Bed Gym exercises to be practiced in hospital or at home to improve the motor function of bedridden patients.*

Key Words: Full-Body In-Bed Gym; Telerehabilitation; Muscle atrophy.

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2022PDM3 Abstract 17

Differential dysferlin expression in human fast and slow skeletal muscle

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A common form of limb girdle muscular dystrophy (LGMD) is dysferlinopathy presenting as LGMDR2/2B or distal Miyoshi myopathy, that show different clinical course. They are caused by mutations in the DYSF gene encoding the protein dysferlin, a transmembrane sarcolemmal protein which has a role in the repair of sarcolemma. Dysferlin is also a t-tubule protein. To the best of our knowledge (differential) dysferlin expression in skeletal muscle fibre types has not been studied so far. We investigated by western blotting human skeletal muscles, one proximal in the arm (biceps brachii) and the other distal in the leg (tibialis anterior) which are affected differently during the course of LGMDR2: usually first tibialis anterior (around the age of 20 years) and later on the biceps brachii, with clear involvement at the age of 45 years. Muscle specimen were collected after a few hours from sudden death or muscle biopsies of biceps brachii and tibialis anterior muscle of neuromuscular controls were analysed. Dysferlin to myosin heavy chain ratio differed in biceps brachii and tibialis anterior muscle. Preliminary results show a trend towards a higher "content" of dysferlin in fast skeletal muscle, i.e. biceps brachii, compared to slow, i.e. tibialis anterior (Figure1, Table1) which might be relevant for the different course observed in LGMDR2/2B and Miyoshi myopathy.

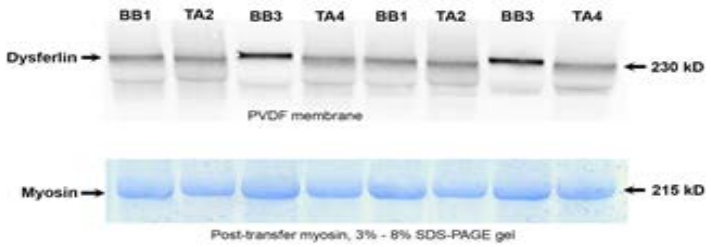

Fig 8. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stained by Coomassie brilliant blue (lower line). BB1 - biceps brachii muscle, a 63-year old male; TA2 - tibialis anterior muscle, a 60-year old male; BB3 - biceps brachii muscle, a 48-year old female; TA4 - tibialis anterior muscle a 52-year old female.*

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Muscle	Patient (n), Gender, Age (years)	Dysferlin/Myosin
BB1 - biceps brachii	1, Male, 63	2,36
TA2 - tibialis anterior	2, Male, 60	1,17
BB3 - biceps brachii	3, Female, 48	2,26
TA4 - tibialis anterior	4, Female, 52	1,56

Key words: Dysferlin; fast skeletal muscle; LGDM; slow skeletal muscle; human.

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2022PDM3 Abstract 18

Neutral Lipid Storage Diseases: a patient clinical follow-up and presentation of two novel cases

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Neutral lipid storage diseases (NLSDs) are rare inherited disorders caused by mutations in adipose triglyceride lipase (ATGL/PNPLA2) or in α-β hydrolase domain 5 (CGI-58/ABHD5). NLSD patients present defects in lipid droplets (LDs) metabolism resulting in accumulation of neutral lipids in these organelles that become larger and more numerous[1] (Fig.1). When the mutation is localized on *PNPLA2* gene, the main symptom is myopathy (NLSDM)[2]. The case report of a 29-year-old woman with NLSDM is presented. At 18 years, despite she was asymptomatic, hyperCkemia suggested NLSDM diagnosis and sequencing analysis revealed a retrotransposal insertion in *PNPLA2* gene[2]. In the following years, the patient has been experiencing increasing muscle weakness. Muscle MRI showed lipid accumulation in calves. At 27 years, she was put on MCT diet and CPK lowered[3]. Nevertheless, muscle weakness did not improve. In this patient, no beneficial effects were observed after treatment, probably due to complete loss of ATGL protein. When the mutation is on *CGI-58* gene, the main symptom is ichthyosis (NLSDI)[4]. Here we report the genetic and clinical symptoms of two young brothers 10.5 and 2 years old, with a new *ABHD5* gene mutation (p.W179Nfs22*). They presented generalized ichthyosis since birth, hepatomegaly and increased liver enzymes. The second child also had severe hypoplastic cerebellum, global developmental delay, generalized hypotonia, increased deep tendon reflexes and occasional attacks of dystonia. In conclusion, we focus the attention on NLSDs disorders, discussing the clinical follow-up of a woman affected by NLSDM and presenting two unreported cases of NLSDI.

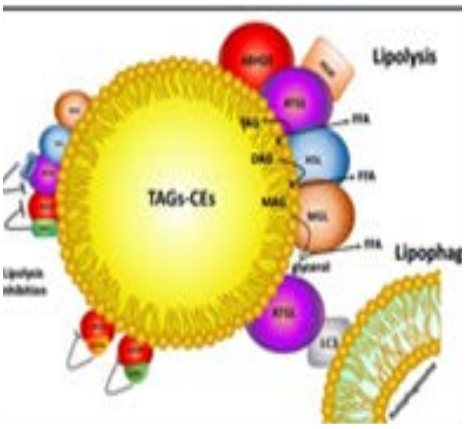

Fig 1. *LD schematic representation. LDs have a core composed of triacylglycerol (TAG) and cholesteryl ester (CE), surrounded by a phospholipid monolayer Some proteins binding to the phospholipid monolayer are involved in neutral lipid metabolism: abhydrolase domain 5 (ABHD5), adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), monoacyl-glycerol lipase (MGL), pigment epithelium-derived factor (PEDF), G0/G1 switch gene 2 (G0S2), hypoxia-inducible LD-associated protein (HILPDA), cell death activator CIDE-3 (CIDEC) and perilipin (PLIN). In particular, ABHD5 activates ATGL, and TAG is hydrolyzed sequentially by ATGL, HSL, and MGL to generate fatty acid (FA) and glycerol. In addition, ATGL can interact with LC3, an autophagic protein. In NLSDs there is a failure in lipolytic pathway resulting in an inhibition of neutral lipid catabolism. It seems likely that also lipophagic pathway is altered in NLSDs.*

Key words: Neutral lipid storage diseases, Lipid droplets, Myopathy, Ichthyosis, MCT treatment.

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2022PDM3 Abstract 19

Loss of MuRF1 prevents skeletal muscle wasting and weakness, and slows the rate of tumor growth, in mice bearing pancreatic tumors

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Skeletal muscle wasting and dysfunction in the context of cancer represents a major debilitating condition which contributes to a deterioration in physical function and reduced quality of life, and limits the ability of cancer patients to withstand anti-cancer treatments. Unfortunately, there are no currently available medical treatments for cancer-induced muscle pathology which is due, in part, to an incomplete understanding of the molecular mechanisms which are causative in the condition. The E3 ubiquitin ligase MuRF1 is required for muscle atrophy in several pathophysiological conditions which associate with muscle atrophy. Here we tested the requirement of MuRF1 for cancer-induced muscle atrophy using MuRF1 KO (MuRF1-/-) mice, or wild type (WT) controls, and an orthotopic pancreatic cancer (KPC) model. WT mice reached experimental endpoint 14 to 17 days following KPC cell injection, as defined by significant body weight loss (-12%) and deterioration of body condition score, with an average tumor mass of 1.9 ± 0.5 g. At this same time point, MuRF1-/- mice were weight stable (-0.6%), and showed ~84% lower tumor mass (0.3 ± 0.1 g) compared to WT mice. When allowing tumor growth to progress in MuRF1-/- mice, it took 21-36 days to reach a comparable tumor mass to that of WT mice at days 14-17. Moreover, when WT and MuRF1-/- mice presenting matching tumor sizes were compared, MuRF1-/- mice showed preserved muscle mass, with no muscle wasting observed in the triceps surae (-0.1% in MuRF1-/- vs. -9% in WT mice) and attenuated wasting observed in tibialis anterior (-5% in MuRF1-/- vs. -18% in WT mice). In addition to preserving muscle mass, MuRF1 deletion also protected against cancer- induced muscle weakness. Indeed, the soleus and diaphragm of WT mice showed a 32% and 46% reduction in specific tetanic force, respectively, whereas no such muscle weakness was observed in MuRF1-/- mice (+20% and +3% in soleus and diaphragm respectively). Skeletal muscle proteome and ubiquitinome analyses were conducted to examined the mechanisms through which MuRF1 deletion conferred its protection against cancer-associated muscle wasting and dysfunction. These analyses revealed that KPC-tumor-burden induces, in a MuRF1-independent manner, an early and sustained increase in the abundance of proteins involved in the acute phase response as well as in extracellular matrix remodeling, while proteins involved in protein translation and oxidative phosphorylation showed a MuRF1- dependent reduction in their abundance at time points reflective of cachexia initiation and progression. Identification of proteins ubiquitinated in a MuRF1-dependent manner further revealed an early and sustained enrichment in proteins related to muscle contraction, proteasome complex and glucose homeostasis. In conclusion, our work suggests that MuRF1 is required for the normal muscle wasting and weakness that occurs in KPC tumor bearing mice, and is also required for normal tumor growth. This suggests that MuRF1-dependent muscle protein breakdown may be required to supply the tumor with nutrients needed for its growth.

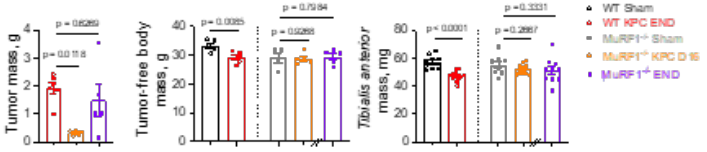

Fig 1. *MuRF1 deletion slows tumor growth and protects against cancer-associated body and muscle wasting.*

Key words: Cancer cachexia; muscle atrophy; E3-ubiquitine ligase; pancreatic cancer.

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2022PDM3 Abstract 20

Environmental and Exertional Heat Stroke: the role of skeletal muscle.

Feliciano Protasi

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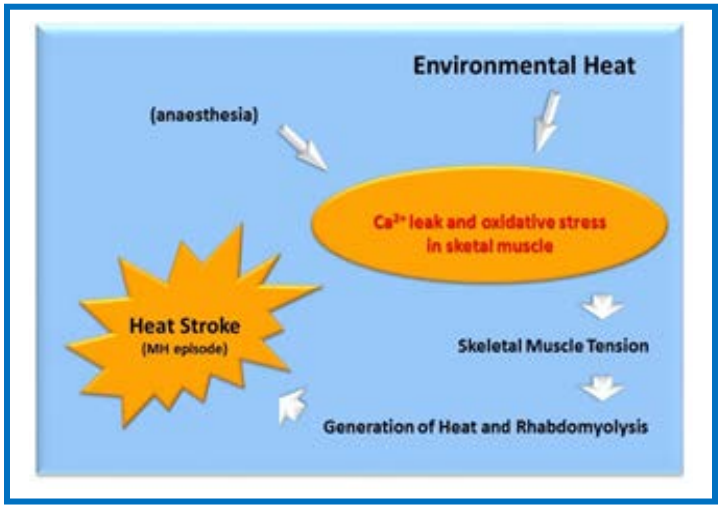
Climatic changes are increasing the incidence of heat waves, periods in which the temperature raise above the average temperatures for three or more days. Heat waves are very dangerous for human health, as it is now clear that the mortality rate increases dramatically during those days.[1] Even if several factors may contribute to sudden death in high environmental temperatures, the most common cause of death attributable to heat is dehydration, heat cramps and exhaustion, and hyperthermia, i.e. in one word heat stroke (HS).[2,3] The cellular and molecular mechanisms underlying HS are multiple and still debated; as a result: a) a cure for acute treatment of HS is still missing; b) patients are usually treated only with supportive measures (cooling and hydration); and finally c) clear guidelines for proper lifestyle habits before and during heat waves are not well defined. We have collected compelling evidence that HS shares common symptoms with malignant hyperthermia (MH) susceptibility (Figure), a life-threatening syndrome caused by mutations in proteins deputed to Ca^{2+} handling in muscle and triggered by administration of halogenated anaesthetics. There is general agreement that the main mechanism triggering MH crises is an abnormal SR Ca^{2+} leak through mutated RYR1, causing uncontrolled contraction and rhabdomyolysis. However, other factors must be taken in consideration: A) Ca^{2+} leak from the SR triggers a feed-forward mechanism leading to overproduction of reactive species of oxygen and nitrogen (ROS and RNS) by mitochondria, which in turns causes further Ca^{2+} release through RYR1-nitrosylation.[4] B) MH responses do not occur in the absence of extracellular Ca^{2+} (5) suggesting that Ca^{2+} influx from the extracellular space is an essential component to be considered. The *Central Hypotheses* guiding our recent work are: a) skeletal muscle plays a central role in HS; b) lifestyle habits may affect proper muscle function, hence cause either increased or diminished risk of the normal population to HS in challenging environmental conditions.



Key words: Heat-wave; malignant hyperthermia (MH); Environmental and Exertional Heat Stroke (EHS).

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2022PDM3 Abstract 21

High-fat diet increases the risk of environmental heatstroke in mice.

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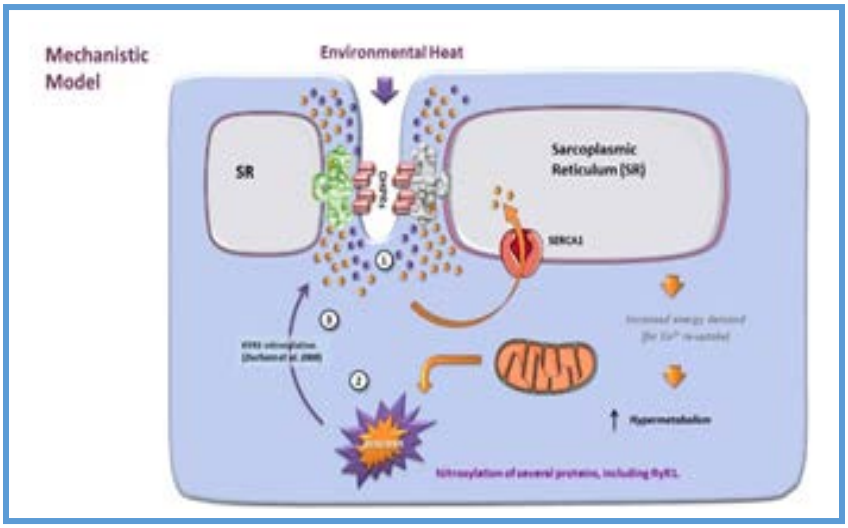
Heat-stroke (HS) is a life-threatening response to heat characterized by an abnormal increase in body temperature (>40°C) that causes dysfunction of organs, central nervous system and may end in death.[1] Environmental heat-stroke (EHS), often triggered by a hot and humid environment, is caused by excessive heat production in muscle, which in turn is the result of abnormal Ca2+ leak from the sarcoplasmic reticulum (SR) and oxidative stress.[2] See Figure for a Mechanistic Model. As high fat diet is known to increase oxidative stress,[3] the objective of the present study was to investigate the effects of high-fat diet in the heat-stroke susceptibility of c57bl/6 wild type (WT) mice of 4 months of age (adult). Our results show that, in comparison with mice fed with a control diet, mice after 3 months of high-fat: a) increased heat generation and energy expenditure (assessed by indirect calorimetry) during heat stress; b) elevated oxidative stress in both EDL and Soleus muscles; and c) enhanced sensitivity to caffeine and temperature of isolated EDL and Soleus muscles during in vitro contracture test (IVCT, the gold standard procedure to test in-vitro EHS susceptibility). Our data suggest that high-fat diet predispose mice to EHS, possibly as a result of increased oxidative stress and excessive release of Ca2+ from SR. This study may have important implications for guidelines regarding food intake during periods of intense environmental heat.



Key Words: high-fat diet, heat-stroke, oxidative stress.

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2022PDM3 Abstract 22

Exertional Heat Stroke: the possible role of external Ca^{2+}

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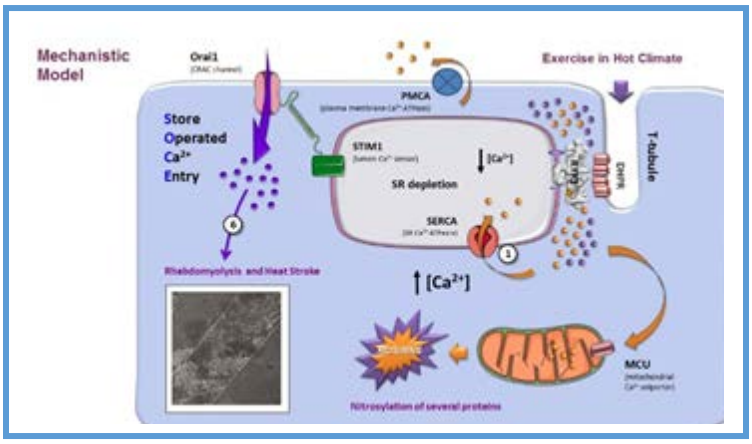
Exertional/Environmental Heat Strokes (EHSs) are hyperthermic crises triggered by strenuous physical exercise and/or exposure to environmental heat, which are caused by an altered intracellular Ca^{2+} homeostasis in muscle.[1,2] We recently demonstrated that a single bout of exercise on treadmill leads to formation of Calcium Entry Units (CEUs), intracellular junctions that promote interaction between STIM1 and Orai1, the two proteins that mediate Store-Operated Ca^{2+} Entry (SOCE). SOCE is a mechanism that is activated during muscle fatigue and that allows recovery of extracellular Ca^{2+} during prolonged activity.[3,4] The hypothesis underlying this project is that assembly of CEUs during prolonged exercise may predispose to EHS, when exercise is performed in challenging environmental conditions. To test this hypothesis, we used 4 months old WT mice that were: a) first, divided in 3 experimental groups: control, trained-1m (1 month of voluntary running in wheel cages), and exercised-1h (1 hour of incremental treadmill run); and b) second, subjected to an incremental treadmill run of 45 min at 34°C and 40% humidity. We then: i) measured the internal temperature of mice, which was higher in the pre-exercised groups (trained-1m: 38.9°C ± 0.33; exercised-1h: 38.7°C ± 0.40) compared to control (37.9°C ± 0.17); ii) applied an ex-vivo exertional stress protocol to isolated EDL muscles (tetanic stimulation performed at 30°C) and verified that samples from trained-1m and exercised-1h mice generated a tension significantly greater than control; iii) Analyzed CEUs by electron microscopy (EM) and verified that EDL muscles of exercised-1h ad trained-1m mice contained a greater number of elements forming CEUs. The data collected suggest that assembly of Calcium Entry Units during exercise could predispose to EHS when exercise is performed in challenging environmental conditions.



Key Words: Calcium entry unit (CEU); heat-stroke; store-operated Ca^{2+} entry (SOCE).

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2022PDM3 Abstract 23

The potential of eccentric training in older adults

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The interest in potential of eccentric training in older adults seems to be increasing in the recent years. Two very recent systematic reviewers focused on the effect of eccentric training in healthy older adults (Kulkarni et al., 2021; Molinari et al., 2019). Molinari et al. (2019) included five studies with muscle strength as outcomes, and reported similar effects of eccentric and concentric exercises, with the data slightly favoring the former. Kulkarni et al. (2021) examined 10 studies and reported that eccentric exercises can be as effective as conventional exercises in older adults for improving functional performance. We performed a broader review and included 18 studies that compared eccentric and concentric exercise interventions. Our analyses suggest that eccentric exercise is actually superior to concentric exercise in terms of the effect on functional performance. Specifically, larger improvements after eccentric compared to concentric exercise were seen for timed up and go test, 2-min stepping test and 30-s sit-stand test (moderate effect sizes). There was also a tendency for eccentric exercise to induce larger improvements in muscle strength and size, but the overall effects were not statistically significant. Together with previous reviews, our analyses suggest that eccentric training may evoke superior effects to conventional/concentric training, wherein functional performance outcomes were most illustrative.

ONE FIGURE, PLEASE

Fig 9. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stained by Coomassie brilliant blue (lower line*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: Flywheel training; eccentric exercise; elderly, functional outcomes.

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2022PDM3 Abstract 24

Skeletal muscle homeostasis in an experimental model of hind limb ischemia

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Low extremity arterial disease (LEAD) is the most common form of peripheral vascular disease and is often a direct consequence of the atherosclerotic process. Characterised by chronic decreases in lower limb blood flow, this progressive disease results in symptoms ranging from pain, muscle weakness, to severe exercise intolerance. Several animal models have been used to study the angiogenesis and muscle remodelling process in LEAD in order to progress novel therapies for patients. However, conflicting information is available on the effect of this condition on lower hind limb skeletal muscle remodelling, thus hindering the development of treatments to prevent muscle dysfunction in LEAD. Using a mouse model of severe lower hindlimb ischemia (femoral artery ligation), we found ischemic muscle at 7 days (n=5) demonstrated a loss of wet-mass and fibre cross-sectional area (CSA) ($t(8)=2.51$, $p<0.05$) alongside reduced type IIx fibre proportion (-20%) vs. non-ischemic muscle ($t(8)=2.159$, $p<0.05$; n=5). Muscle wasting was associated with excessive autophagy rather than other catabolic pathways, with autophagy and mitophagy markers significant upregulated. At 28 days following ischemia (n=5) vs control limb (n=5), muscle regeneration allowed increased muscle mass and fibre CSA ($p>0.05$) with markers of autophagy upregulated at 7 days returned to baseline values. Furthermore, expression of Sestrins was dysregulated at 7 days and normalised at 28 days following ischemic insult. In conclusion, these data indicate severe lower hindlimb ischemia is associated with early-activation of autophagy that may mediate early-onset muscle wasting. Whether hypoxia-associated ischemia triggers activation of Sestrin 2 and subsequent autophagy-dependent remodelling remains uncertain.

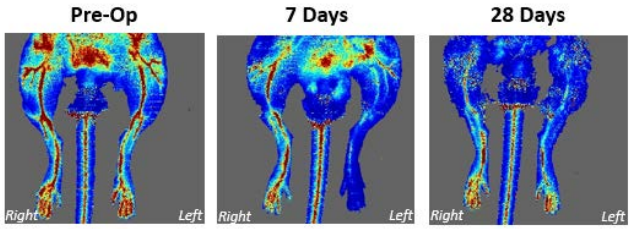

Fig 1. *Ischemia was induced in the left lower hind limb by ligation and dissection of the femoral artery. Representative images collected using Laser Doppler Flowmetry show the blood flow (in red) in the lower hind limb before and at different time-points (7 and 28 days) after surgery*

Key words: Ischemia; skeletal muscle; autophagy; Sestrins.

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2022PDM3 Abstract 25

The strength and the broadness of CFTR correctors for the treatment of sarcoglycanopathies

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Sarcoglycanopathies are recessive forms of muscular dystrophies in which the disruption of the sarcoglycan complex results in sarcolemma fragility and progressive muscle degeneration. Most of the reported cases are due to missense mutations originating a folding-defective sarcoglycan (SG) eliminated by the cells' quality control system, although potentially functional.[1] No treatment for sarcoglycanopathy is currently available. Cell and gene therapy-based approaches are under investigation, even if many challenges are present. Among others, there is the difficult of delivering the gene to skeletal muscle, which is the most abundant tissue of human body, the problem of immunogenicity of the viral vector and of the transgene (if the protein is totally absent in the patient), etc. An interesting alternative could be represented by a class of small molecules, called protein folding correctors, able to recover the mutants and avoid complex disruption. Furthermore, small molecules are easier to formulate, deliver and eventually optimize. Particularly, CFTR correctors are a subgroup of cystic fibrosis modulators able to enhance or even restore, through different mechanisms, the expression, function, and stability of type II CFTR mutants.[2]In α-sarcoglycanopathy, the effective rescue of different SG-mutants has been proved for a few of such molecules by using cell models and, importantly, myogenic cells from patients.[3,4] In addition, their pharmacological efficacy has been successfully tested in novel mouse models, ad hoc generated, expressing the human mutated α-SG sequence in both hind-limbs, delivered by early AAV injection. As the proof of concept of the strategy has been established for LGMDR3 both in vitro and in vivo, we checked the broadness of such approach by testing the effect of a panel of different CFTR correctors in LGMDR4 patient's myotubes, carrying a missense mutation on the SGCB gene. The analysis revealed the increased expression of the mutated β-SG protein upon treatment with the C17 molecule, the most promising corrector in our hands. In particular, C17 induced a two-fold increase in the β-SG membrane fraction in comparison to vehicle treated cells. The myotubes surface re-localization of the β-SG protein was further proven by immunofluorescence staining. These results confirm the hypothesis that CFTR correctors could be in principle applied to all the sarcoglycanopathies cases linked to missense mutations, regardless of the SGC gene. Remarkably, these data are the proof that CFTR correctors are effective not only in cystic fibrosis, but also in sarcoglycanopathy, suggesting a great potential for the protein conformational diseases, especially for those orphan diseases where no current therapeutics are available.

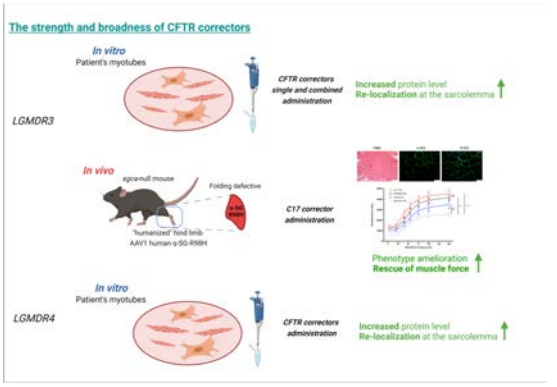

Fig. 1. Panel summarizing the strength and broadness of CFTR corrector treatments for sarcoglycanopathies*. These molecules are able to recover folding defective mutants of both α-SG and β-SG. Notably, the muscle force of the humanized mouse, model of LGMDR3, treated with CFTR correctors is indistinguishable from that of wild type animals. (Created with BioRender.com)*

Key Words: Sarcoglycanopathies; CFTR correctors; therapeutic approaches.

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2022PDM3 Abstract 26

Calf Muscle 3D Strain Imaging and Initial Results on Correlation with Histology

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Prior studies of isometric contraction in calf muscle using velocity encoded phase contrast (VE-PC) imaging have used either single slice imaging to extract 2D strain and strain rate tensors[1,2] or sequentially acquired single slice images which were concatenated to form a volume to extract 3D strain and strain rate tensors[3]. The advantage of 2D VE-PC is that it is fast but requires one to acquire the images in the plane of the fibers to leverage the fact that muscles exhibit planar deformation; a 3D acquisition circumvents the need for this positioning. A volume acquisition VE-PC sequence is usually too long for subjects to maintain consistent contractions through the scan duration. In this study, we present (i) sequentially acquired anatomical slices to extract 3D strain tensor and projections on the myofibrillar aggregate in a cohort of young and old subjects along with correlation of the strain indices to histology and (ii) initial results using a highly accelerated 4D Compressed Sensing flow sequence[4] to image the much smaller motion seen in muscle tissue. Significant age -related differences were identified in principal 3D strain (**E**) and strain rate (**SR**) indices (from sequential 2D slices) including in shear strain in the medial gastrocnemius (MG) and in the soleus (SOL). The tensor projections in the muscle fiber direction and cross-section (Figure 1) did not show any age-related differences but several principal and fiber-aligned strain indices showed correlation with collagen 3 from histology. The second part of the study establishes the feasibility of 3D volume acquisition with 3 directional velocity-encoding for calf muscle dynamic imaging to monitor sub-maximal isometric contractions using a 4D Compressed Sensing Flow imaging sequence originally developed for blood flow imaging (Figure 2).

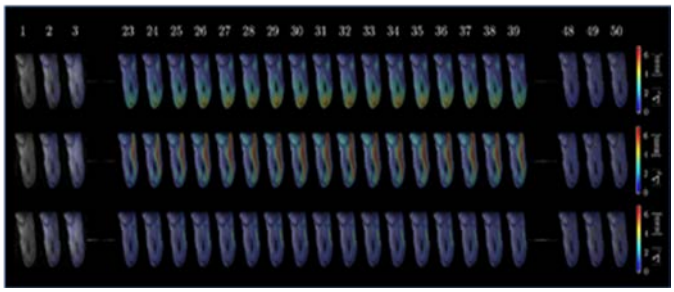

Fig. 1. *Temporal plots of the projection along the directions of the 3 diffusion eigenvectors of the Strain and Strain rate indices.*

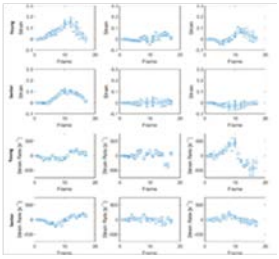

Fig. 2. *Sagittal images of the calf muscle through the dynamic cycle (50 temporal phases) showing colormaps of the displacement in the x-, y- and z-axis derived from the 4D CS Flow imaging sequence.*

Keywords: Aging muscle; dynamic MRI; compressed sensing; 3D strain imaging; fiber aligned strains.

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2022PDM3 Abstract 27

Fiber Strains and Strain Tensor Mapping of Medial Gastrocnemius at Sub-Maximal Isometric Contraction at Different Ankle Angles

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Prior studies of isometric contraction in calf muscle using velocity encoded phase contrast (VE-PC) imaging have revealed several interesting features in the medial gastrocnemius including strain heterogeneity along and across fibers, gear ratios decreasing with unloading, and constant in-plane areas during isometric contraction suggesting little or no deformation in the out-plane direction [1,2]. In addition to analyzing fiber strains, spatially localized strain and strain rate (SR) tensors can be computed from velocity encoded phase contrast images that have the potential to identify changes in tissue deformation along and perpendicular to the muscle fiber. Strain tensor analysis has revealed anisotropy of deformation in the cross-section of the muscle fiber, deviation of the principal strain direction from the muscle fiber orientation, heterogeneity of strains along the length of the muscle and across the muscle fiber and in study of age-related effects, maximum shear strain emerging as the most significant predictor of force loss with age [3]. Earlier studies identified fiber direction from fascicle directions on water suppressed MR images (fascicles appear bright due to fat infiltration at the fascicles). However, identification of fascicles in young subjects is difficult since the fascicles are relatively thin and do not have sufficient fat infiltration. We used the average of the lead DTI eigenvector in each region to extract the direction of fibers. Fiber strains, length and angle changes were calculated for three-foot positions and at two % MVC at each position. This study calculates regional fiber strains, and strain and strain rate (SR) tensor in the medial gastrocnemius muscle from 2D velocity encoded phase contrast (VE-PC) images acquired under isometric contraction at three-foot positions (representing different muscle fiber pennation angles/length) at two % MVC's at each position. The plantarflexed foot position had the highest normalized fiber strain while the dorsiflexed position had the lowest normalized fiber strains (to force and to torque) (Table 1). In the strain and strain rate tensor analysis, plantarflexed foot position had the highest normalized strain and strain rate (to force) and strains decreased with %MVC's compared to the neutral and dorsiflexed foot positions (Figure 1). Strain in the fiber cross-section showed radial expansion in the in-plane and radial contraction in the out-plane direction; radial deformation was lowest in the plantarflexed position.

Table 1. *Fiber strains for the three ankle positions: N(eutral), D(orsiflexed), P(lantarflexed) and two %MVC.*

position	nominal % MVC	MVC (N)	peak force (N)	peak torque (Nm)	initial angle (deg)	Δ angle (deg)	initial length (mm)	Δ length (mm)	peak strain	strain / force (N^{-1})	strain / torque (Nm^{-1})
D	25%	287.5	78.4	3.45	13.93	6.69	40.27	-7.13	-0.18	-0.0023	-0.0518
D	45%	290.6	132.6	5.89	13.93	10.11	40.27	-11.92	-0.30	-0.0023	-0.0509
N	25%	244.5	63.2	2.98	16.29	9.98	38.25	-9.26	-0.25	-0.0039	-0.0837
N	50%	244.3	122.8	5.79	16.29	14.32	38.25	-11.37	-0.30	-0.0025	-0.0524
P	25%	108.5	28.4	1.47	20.10	8.32	35.24	-9.97	-0.30	-0.0107	-0.2065
P	45%	110.3	46.6	2.48	20.10	13.58	35.24	-9.47	-0.28	-0.0059	-0.1112

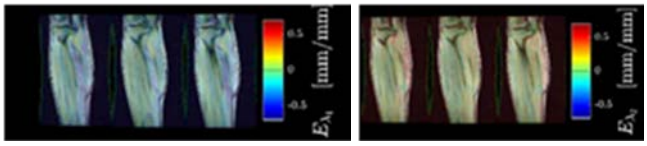

Fig. 1. *Shows the principal negative and positive strain values during the contraction peak for (L-R) N, D and P ankle positions..*

Keywords: Dynamic muscle MRI; fiber architecture and function; fiber strain; strain tensor mapping.

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2022PDM3 Abstract 28

Fascia lata alterations in Hip Osteoarthritis

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Osteoarthritis (OA) is a degenerative pathology of the joint,[1] characterized by progressive damage to articular cartilage, which currently represents an important cause of disability,[2] with an estimated prevalence ranging from 1 to 10% in the general population.[3,4] Despite several mechanisms having been proposed to explain its onset and progression, the aetiopathogenesis of osteoarthritis is still doubtful. This study hypothesizes an association between fascia lata alterations and hip OA; in particular, changes in the production of collagen and HA could result in altered fascial structure and behaviour in OA patients. The structure and composition of fascia lata in healthy subjects and in patients with hip OA were compared with the aim to evaluate any differences in the amount of Collagen type I (COL I), Collagen type III (COL III), and Hyaluronan (HA). Ten samples from healthy subjects and 11 samples from OA patients were collected. COL I was significantly more abundant in the OA group (p = 0.0015), with a median percentage positivity of 75.2 % (IQR 13.11), while representing only 67 % (IQR: 8.71) in control cases. COL III, with median values of 9.5 % (IQR 3.63) (OA group) and 17.10 % (IQR 11) (control cases), respectively, showed significant reduction in OA patients (p = 0.002). HA showed a median value of 10.01 % (IQR 8.11) in OA patients, denoting significant decrease (p < 0.0001) with respect to the control group median of 39.31 % (IQR 5.62). The observed differences suggest a relationship between fascial pathology and hip OA: the increase in COL I in OA patients, along with the reduction of COL III and HA, could lead to fascial stiffening, which could alter fascial mechanics and be linked to the development and symptoms of OA.

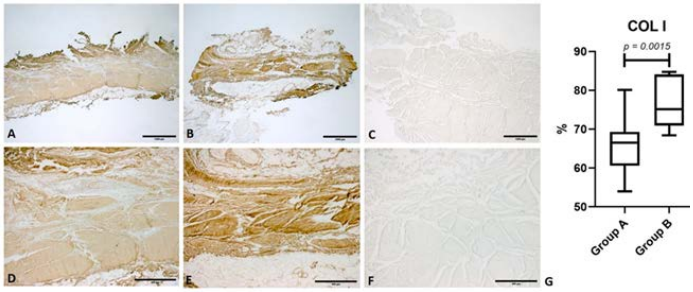

Fig 1. *Immunostaining for collagen I (COL I). Comparison between fascia lata samples from a healthy subject (Group A) and a patient with osteoarthritis (Group B). (A) Group A: fascia lata (1× magnification); (B) Group B: fascia lata (1× magnification); (C) Negative control: omission of the primary antibody (1× magnification); (D) Group A: fascia lata (5× magnification); (E) Group B: fascia lata (5× magnification); (F) Negative control (5× magnification); (G): comparison of the percentage antibody positivity for COL I.*

Key Words: Fascia; hip osteoarthritis; hyaluronan; collagen; stiffness; myofascial pain.

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2022PDM3 Abstract 29

Extracorporeal Shock Wave Therapy (ESWT) in muscular pathologies

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Extracorporeal Shock Wave Therapy (ESWT) is considered part of the Regenerative Therapy, that represents one of the most challenging branches of rehabilitation field and, more generally, in modern medicine. Basic science studies demonstrated the effectiveness of the ESWT in stimulating biological activities, by the mechanotransduction of the acoustic signal into a biological response, that involves both intra-cell and cell–matrix interactions. According to the Consensus Statement elaborated by ISMST (International Society for Medical Shockwave Treatment) in 2016 and subscribed by SITOD (Italian Society for Shockwave Therapy), some muscular pathologies are among the indications based on clinical experience. Indeed, despite the few studies in the literature, ESWs are widely used in clinical practice in many acquired muscle diseases. The rationale for the use of this treatment is related to its well-known anti-inflammatory, analgesic, anti- fibrotic and myorelaxant action. Moreover, ESWT could act on Muscular Satellite Cells, myogenic progenitors, by stimulating them to proliferate, and eventually differentiate through fusion with each other or to damaged fibers to reconstitute fiber integrity and functionality. This hypothesized mechanism may play an important role in helping muscle regeneration, if ESWT is properly inserted in a multimodal rehabilitative approach including therapeutic exercise. However, there is still a lot of confusion about the different types of shock waves (focal, radial), the different generators (electrohydraulic, electromagnetic, piezoelectric), the parameters and treatment protocols. Therefore, this presentation aims to provide an update on the efficacy of extracorporeal shockwave treatment for muscular acquired pathologies by focusing on the evidence.

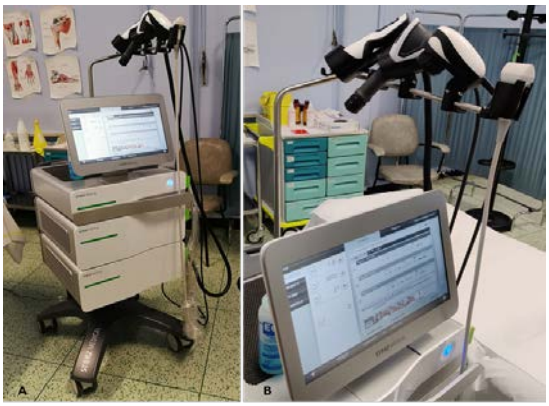

Fig 1. *(A) the device used for the administration of Extracorporeal Shock Wave Treatment (DUOLITH® SD1 »ultra«, STORZ Medical);(B) a detail of the different handpieces used for the muscular treatment.*

Key Words: Extracorporeal Shock Wave Therapy; ESWT; muscle; evidence; regenerative medicine.

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2022PDM3 Abstract 31

Beneficial effects of boosting skeletal muscle metabolism by SIRT1 activator in DMD mouse model

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Duchenne muscular dystrophy (DMD) is a severe and relentlessly progres- sive myopathy caused by out-of-frame or nonsense mutations in the X-linked DMD gene. DMD is a complex disease and multiple approaches are needed to target pathological processes, both the underlying genetic mutations and the secondary complications. Despite several therapeutic options have been developed with good results, more effective treatment options are essential and may be generated through the definition of novel therapeutic targets. A muscular metabolic dysregulation is an established DMD feature with mito- chondrial dysfunctions as one of the earliest deficits that arise from multiple cellular stressors. Among metabolic regulators, Sirtuin 1 (SIRT1) represents an intriguing candidate since it acts on different aspects of cellular metabo- lism**,** regulating energy homeostasis, mitochondrial biogenesis, and inflam- mation. SIRT1 overexpression represents an important counter-mechanism to alleviate the dystrophic phenotype and its pharmacological modulation could be relevant as well in DMD conditions. Consistently, SIRT1 activation by selective compound, i.e., SRT2104, has already been proven to reinforce muscular structure, mitochondrial functionality, and to reduce inflammation. SRT2104 has never been tested in muscular diseases; therefore, considering its metabolic and immunomodulatory effects, we tested SRT2104 as an at- tractive candidate for DMD treatment. Accordingly, in our preliminary data, long-term SRT2104 administration improved muscle force and stimulated oxidative capacity. This was paralleled by reduced fibrosis and inflammatory infiltrate and increased regeneration in mdx muscle. In conclusion our results demonstrate the efficacy of SRT2104 as a new SIRT1 activator in DMD and highlight that considering DMD also as a metabolic disease and treating it as such, could provide important therapeutic strategies additional to gene therapies.

ONE FIGURE, PLEASE

Fig 10. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stained by Coomassie brilliant blue (lower line*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: Duchenne Muscular Dystrophy; SIRT1 activators; mitochondria.

References

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2022PDM3 Abstract 31

Muscle clocks change with age: A potential contributor to sarcopenia?

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Skeletal muscle circadian clocks regulate a daily program of gene expression that is necessary for predictive homeostasis and tissue resilience. Aging is associated with progressive decline in skeletal muscle function. To date, our lab has found age associated changes in expression of the core clock gene, PER2, in mouse muscle. However very little is known about how aging alters the circadian transcriptome. To address this gap in the field, we collected skeletal muscle from Young (6mo), Aged (18mo), and Old (27mo) male C57BL/6J mice every 4h for 48h for RNA sequencing and circadian analysis. We identified that circadian expression of the core clock genes was unchanged from Young to Aged, however, from Aged to Old there were significant changes in several genes within the repressor limb (e.g. *Per1/2*, and *Cry1/2*). Consistent with age-associated changes in core clock genes we found an age-related decline in the circadian transcriptome that was evident by 18 months, with 941 oscillating genes in the Young mice, 699 oscillating genes in the Aged mice, and 474 oscillating genes in the Old mice. Comparing across the 3 ages there was less than 10% overlap in the circadian transcriptome between any age and only 3% of genes shared across all three ages, and these shared genes were composed primarily of the core circadian clock factors. These are the first data to demonstrate a progressive age-related decline the skeletal muscle clock and the skeletal muscle circadian transcriptome. Functional cluster analysis of the transcriptomes suggest that altered circadian clock output with aging likely contributes to changes in skeletal muscle function and adaptability with age.

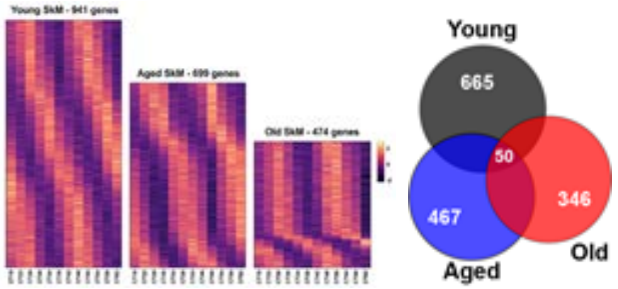

Fig 1. *Skeletal Muscle Circadian Transcriptome Across the Lifespan. Left panel: z-scored heatmap of the circadian genes over 48h for young (6mo), Aged (18mo), and Old (27mo) male C57BL/6J mice. Right panel: venn diagram of circadian genes that overlap across the three age groups.*

Key words: Muscle clock; Circadian rhythms, Clock output.

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2022PDM3 Abstract 32

Skeletal muscle Nox4 knockout prevents the loss of maximal diaphragm force in mice with heart failure with reduced ejection fraction

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Patients with heart failure with reduced ejection fraction (HFrEF) experience diaphragm weakness that contributes to the disease pathophysiology, and diaphragm strength is strongly associated with adverse clinical outcomes in this patient population [1]. Skeletal muscle function is influenced by the cellular redox balance [2]. An oxidative shift, which can result from an increase in the production of reactive oxygen species (ROS), can cause muscle weakness and dysfunction through excessive oxidation of proteins involved in muscle contraction and activation of proteolytic pathways [2]. Diaphragm biopsies from patients and animal models of HFrEF reveal markers of an oxidative shift in the redox tone contributing to muscle weakness, but the exact source of ROS remains unknown. NAD(P)H Oxidase 4 (Nox4), located within the mitochondria and sarcoplasmic reticulum, is an important source of ROS in skeletal muscle [3] and recent reports have observed increased diaphragm Nox4 protein content with HFrEF [4]. In the current study, we examined the role of skeletal muscle-specific Nox4 (skmNox4) on diaphragm contractile properties, fiber size, and mitochondrial function in a mouse model of HFrEF resulting from myocardial infarction. HFrEF caused a 20% loss in maximal and submaximal specific force. skmNox4 KO protected against the loss of maximal specific force but not submaximal force. Diaphragm weakness was not accompanied by changes in muscle fiber type, cross sectional area, or mitochondrial respiration. skmNox4 KO increased mitochondrial respiration measured..

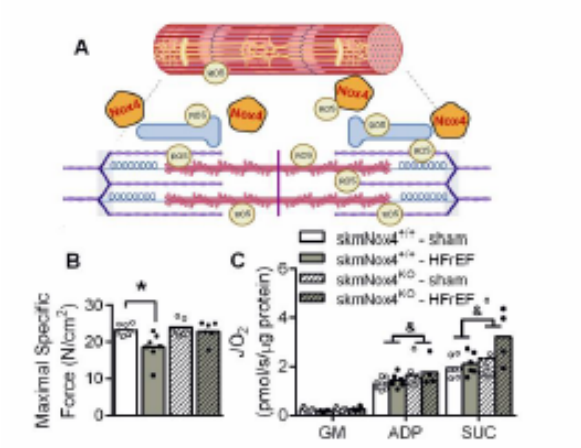

Fig 1. *Skeletal Muscle Circadian Transcriptome Across the Lifespan. Left panel: z-scored heatmap of the circadian genes over 48h for young (6mo), Aged (18mo), and Old (27mo) male C57BL/6J mice. Right panel: venn diagram of circadian genes that overlap across the three age groups.*

Key words:

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2022PDM3 Abstract 33

Cancer cell-derived IL-8 and CXCL1 mediate cachexia in mice bearing human pancreatic tumors

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Pancreatic Cancer Cachexia is a multifactorial wasting syndrome, where extent of muscle loss predicts survival and response to therapy in patients. Among the molecular network of factors that may be responsible for initiating pancreatic cancer cachexia are tumor-secreted factors, and in recently published work we demonstrated that human pancreatic cancer cells secrete high levels of both CXCL8 (IL-8) and CXCL1, and these factors are sufficient to induce muscle wasting. In the current study we therefore determined the requirement of pancreatic cancer cell-derived IL-8 and CXCL1 for the cachexia phenotype in mice bearing human L3.6pl pancreatic tumors. CRISPR-Cas9 technology was utilized to generate L3.6pl cells containing single or co-deletion of IL-8 and CXCL1. Genetically modified L3.6pl cells (L3.6pl$^{IL8-/-}$, L3.6pl$^{CXCL1-/-}$, L3.6pl$^{IL8-/-CXCL1-/-}$) or their Cas9 controls (L3.6pl^{Cas9}) were injected into the pancreas of 20 week old NSG mice, and tissues harvested approximately 3 weeks later, upon L3.6pl^{Cas9} mice reaching experimental endpoint. Compared to Sham, mice with L3.6pl^{Cas9} tumors showed significant cachexia, as indicated by reduced tumor-free body mass and reductions in gonadal fat, skeletal muscle and heart mass. Although L3.6pl$^{IL8-/-}$ and L3.6pl$^{CXCL1-/-}$ tumors induced comparable cachexia in mice to that of L3.6pl^{Cas9} controls, mice with L3.6pl$^{IL8-/-CXCL1-/-}$ tumors showed marked protection against cachexia, based on preservation of tumor-free body mass, fat mass and heart mass, and attenuated skeletal muscle wasting, despite comparable tumor burden. RNA-sequencing of TA muscles revealed 1293 genes differentially expressed in L3.6pl^{Cas9} mice compared to Sham, of which only 176 (13%) were similarly changed in L3.6pl$^{IL8, CXCL1-/-}$ mice, demonstrating significant normalization of the molecular atrophy signature. Analyte profiling of serum confirmed significantly elevated levels of IL-8 and CXCL1 in L3.6-pl ^{Cas9} mice, but not in the respective knockouts. We further found that elevations in tumor-derived (human) IL-6 and MCP-1, factors previously implicated in cachexia, were also attenuated in serum of L3.6pl$^{IL8, CXCL1-/-}$ mice, suggesting a dependency of IL-6 and MCP-1 production and/or secretion on IL-8 and CXCL1 in pancreatic tumors. In summary our data indicate that combined, but not individual, targeting of IL-8 and CXCL1 in human pancreatic tumors significantly blocks cachexia.

Key words:

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2022PDM3 Abstract 34

Succinate impairs skeletal muscle isometric and isotonic contractile function

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Mitochondrial and metabolic disorders such as heart failure with preserved ejection fraction (HFpEF) cause skeletal muscle contractile dysfunction through mechanisms not fully understood. Abnormal mitochondrial metabolism causes cytosolic accumulation of metabolites of the tricarboxylic acid (TCA) cycle, such as succinate and its derivatives (1). Therefore, we investigated whether succinate impairs skeletal muscle contractile function. We exposed mouse diaphragm bundles to dimethyl-succinate (0.5 to 10 mM, membrane-permeable) ex vivo for 1h to 4h at 37°C and measured contractile properties. Dimethyl-succinate (0.5 and 1 mM, 4 h) decreased diaphragm twitch force (~15%), tetanic force (~10%), and peak power (~25%; Figure 1.A). The greater decline in peak twitch force compared to maximum tetanic force allude to impairments in the excitation-contraction coupling (ECC) machinery, whereas the notable decrease in peak power implicates dysfunction in sarcomeric proteins. Myofibrillar and calcium- handling proteins are susceptible to hypersuccinylation at lysine residues, suggesting that elevated lysine succinylation contributes to the contractile impairments in conditions associated with succinate accumulation. We performed post-translation modification proteomics in diaphragm bundles exposed to dimethyl-succinate (10 mM, 1h: 75-90% decrease in force and power) and found increased lysine succinylation in nebulin (6-fold), titin (5-fold) and troponin-I (2-fold). Myosin heavy chain (fast), myosin-binding protein C (fast), troponin C, myomesin, and calsequestrin were among the proteins with lysine succinylation detected only in bundles exposed to dimethyl-succinate. Inhibition of complex II activity (dimethyl-malonate, 10 mM) had no effect on contractile function and did not prevent the loss of force and power induced by dimethyl-succinate. Exposure of permeabilized single fibers to dimethyl-succinate (10mM, 1h at 15°C) revealed a ~20% decrease in maximum isometric force in fast-twitch fibers and a ~20% decrease in submaximal force and calcium sensitivity in slow-twitch fibers. The high concentration used in single fibers accelerates mass action and elicits non-enzymatic effects (2), suggesting direct effects of succinate on sarcomeric proteins. Our study shows that membrane-permeable succinate causes isometric and isotonic contractile dysfunction via impairments in excitation-contraction coupling and sarcomeric protein function. Moreover, several myofibrillar proteins responsible for sarcomere structure and function emerged as being susceptible to lysine succinylation. The data suggest that cytosolic accumulation of succinate is a potential mediator of contractile dysfunction in mitochondria and metabolic disorders. 1

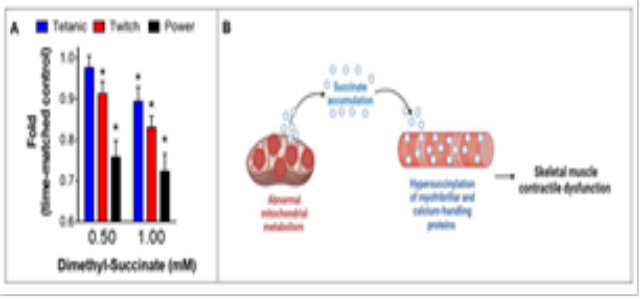

Fig 1. *(A-B). Succinate impairs isometric and isotonic contractile function. (A) Mouse diaphragm incubated in dimethyl-succinate (0.5 – 1 mM) for 4 h at 37°C. Maximal tetanic force (blue), peak twitch (red) and peak power (black) were normalized to time-matched control. * P-value <0.05 versus time- matched control. (B) Illustration of our hypothesis that abnormal mitochondrial metabolism promotes skeletal muscle dysfunction through succinate accumulation. Impairments in mitochondrial respiration causes accumulation of succinate.[1] Myofibrillar and calcium-handling proteins are susceptible to lysine succinylation upon succinate exposure, which suggests that hypersuccinylation of these proteins contributes to skeletal muscle dysfunction in mitochondrial and metabolic disorders associated with succinate accumulation.*

Key words: …… ……; …… ; ……; ……; …… .

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2022PDM3 Abstract 35

Skeletal muscle specific rescue of Bmal1 is sufficient to extend the lifespan of the Bmal1 KO mouse

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Several studies across different model organisms have indicated that age-associated changes in skeletal muscle may be a critical contributor to healthspan and lifespan of the organism. In this study we tested whether skeletal muscle specific rescue of the core clock gene, *Bmal1*, is sufficient to extend the lifespan of the *Bmal1* knock out mouse (*Bmal1* KO). The *Bmal1* KO mouse exhibits 50% mortality by 37 weeks of age and features of aging have been described across multiple organ systems, including skeletal muscle. Prior studies from other labs have performed liver and brain-specific rescue of *Bmal1*, however neither of these approaches were sufficient to extend survival *of the Bmal1* KO. We used systemic delivery with an AAV construct expressing *Bmal1* behind a muscle specific promoter (CK6). We tracked a variety of physiological and behavioral parameters in the mice. We found that *Bmal1* rescue in skeletal muscle of about 20-30% of control was sufficient to significantly extend lifespan assessed by Kaplan-Meier and log-rank survival tests (n=36-45 mice/group). In addition, we found that the Bmal1 KO+AAV mice exhibited improved total cage activity, motor coordination (rotarod) and measures of *ex vivo* muscle force. RNA-seq analysis of gastrocnemius muscle of these mice identified a significant cluster of genes related to oxidative phosphorylation, insulin signaling, and protein homeostasis that were rescued in the Bmal1 KO+AAV group. Ongoing studies are in progress to better define both muscle specific and systemic mechanism(s) through which survival is improved.

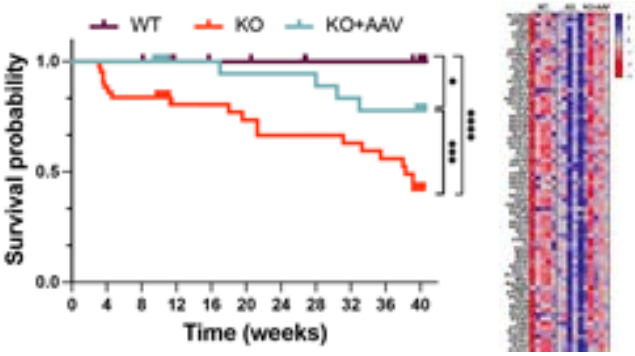

Fig 1. *Left panel: Kaplan–Meier survival curves of WT, KO, and KO+AAV mice..*

Key words: Bmal1; survival; adeno-associated virus.

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2022PDM3 Abstract 36

The role of muscle IGF-I after a single bout of exercise on AMPKα in mouse skeletal muscle

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Insulin-like growth factor I (IGF-I) contributes to glucose homeostasis and may glucose handling in skeletal muscle through activation of hybrid receptors, which contain insulin and IGF-I hemireceptors. Our previous work showed that loss of IGF-I in murine muscle can have negative effects on glucose metabolism and exercise tolerance in addition to muscle mass. AMP-activated protein kinase (AMPK) has a key role in maintaining the balance between anabolic and catabolic programs for cellular homeostasis in response to metabolic stress.Increased AMP/ATP ratio in muscle cells during repetitively contraction activates phosphorylation at AMPKα^{Thr172}, and this induces downstream signaling cascades to promote glucose uptake. Phosphorylation of AMPKα$^{Ser485/491}$ can inhibit AMPK activity and insulin may be one of its upstream triggers in skeletal muscle. The influence of IGF-I in glucose uptake and its interplay with AMPK activation during exercise is not well understood. The aim of this study is to determine the acute response of AMPK signaling pathway after a single bout of aerobic exercise in muscle loss or overexpression of IGF-I (Figure 1). We used two different mouse models in our study to determine the acute exercise response in muscle deficient or overexpressed IGF-I: Muscle IGF-I Deficient (MID) and mice harboring the rat Igf1a cDNA under the control of the fast myosin light chain promoter (mIgf1$^{+/+}$, MLC/mIgf). We induced Igf1 deletion with chow containing doxycycline at postnatal day 21 for 5 days in MID and the controls without floxed exon 4 of Igf1(Igf1$^{fl/fl}$). Littermates without the transgene were used as wildtype controls for mIgf1$^{+/+}$ mice. We used 16-week-old MID and mIgf1$^{+/+}$ transgenic male mice, and relevant controls in our study. The mice were randomly assigned to No Exercise control (NO) or to exercise groups with three time points (0-hour, 2-hour, or 24-hour after exercise). For the exercise groups, mice ran at 20 m/min with 5% inclination for 30 minutes. Tibialis anterior muscles from each mouse were used to examine AMPKα phosphorylation. In our preliminary data, the phosphorylation of AMPKα$^{Ser485/491}$ was higher at rest in mIgf1$^{+/+}$ transgenic muscle compared to wild type muscle, and the trend of phosphorylation was different immediately after exercise and 2-hours later. In contrast, phospho-AMPKα$^{Ser485/491}$ seemed unchanged in MID mice. This suggests that high IGF-I levels may be needed to modulate the inhibitory AMPKα phosphorylation state, whereas normal or diminished IGF-I has no effect.

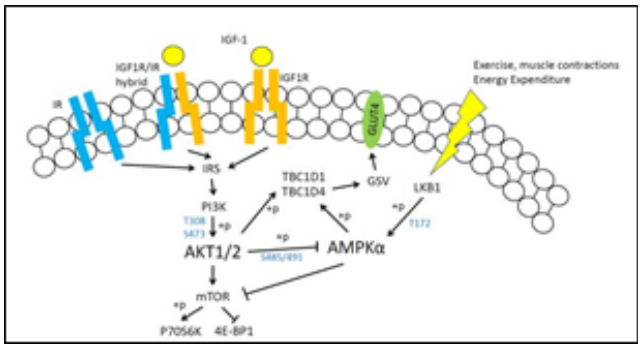

Fig 1. *Acute response of AMPK signaling pathway after a single bout of aerobic exercise in muscle loss or overexpression of IGF-I.*

Keywords: Senescent skeletal muscle; IGF-I; exercise; AMPK.

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2022PDM3 Abstract 37

The role of age on neuromuscular performance decay induced by a maximal intensity sprint session in a group of competitive athletes.

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† These authors contributed equally to this work
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Age-related changes in the neuromuscular system functions may affect profoundly high-level athletes' performance across their career, and potentially determining its length. Therefore, the present study aimed to explore the role of age on neuromuscular functions in a group of competitive athletes, and in particular, to analyse the fatiguing effect of a maximal intensity sprint session (MISS). Thirty-one competitive endurance athletes completed a knee extensors and flexors' maximal-voluntary-isometric-contraction (MVC) test (3x5s, 60s recovery) before and after a maximal intensity sprint session (MISS) consisting of 4x15s Wingate-tests. The data have been stratified considering three age categories (18-28, n=11, 29-38; n=10; 39-43, n=10). Non-significant differences in neuromuscular performance markers emerged between the three age groups at baseline ($p>.05$). Overall, both quadricep and hamstring muscles early and late RTD dropped significantly more than the MVT ($p<.05$). Age had a significant effect on early RTD with older athletes exhibiting greater RTD drop compared to younger athletes ($p<.05$). A significant effect of age emerged also for the changes in surface electromyography (sEMG) variables, in which the frequency spectrum variables (MDF and MNF) dropped significantly more than the sEMG amplitude (RMS) ($p<.05$). In addition, age was largely to very largely and significantly correlated with early RTD drop. On the contrary, no significant effects of age emerged for the MVT and late RTD drop ($p>.05$). The dynamics of changes in neuromuscular performance markers after a MISS suggested that getting older, competitive athletes may potentially experience greater lost in early explosive strength compared to maximal or late explosive strength, due to neuromuscular fatigue.

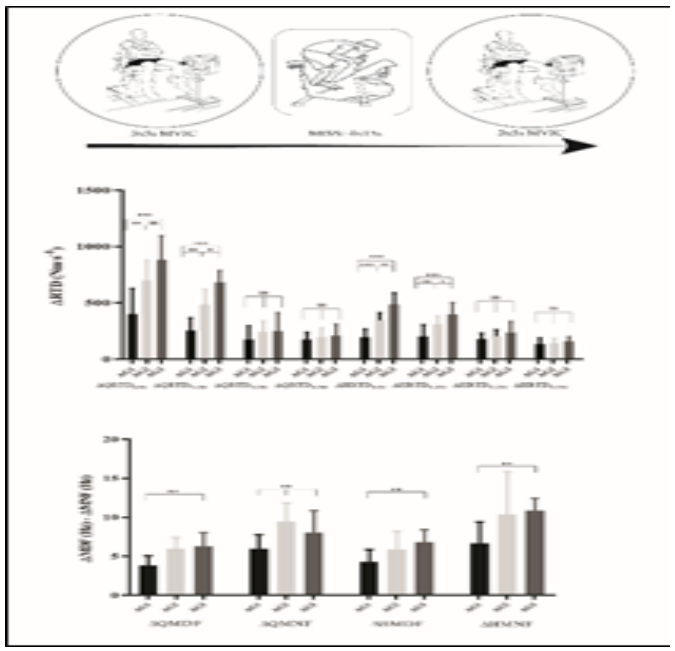

Fig 11. *From top to bottom: graphical representation of the testing protocol, differences between age groups in ΔRTD, differences between age groups in ΔMDF and ΔMNF.*

Key words: Ageing; physical performance; maximal strength; explosive strength.

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2022PDM3 Abstract 38

Establishment of models for mechanical and oxidative stress based on tissue-engineered skeletal muscle

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Models adequately mimicking skeletal muscle injuries and diseases are important tools to understand and study mechanisms underlying initiation and progression of diseases as well as tissue regeneration and remodeling after injuries.[1] 3D tissue models help increase significance of generated results due to their similarity to *in vivo* conditions compared to conventional cell culture without the obvious drawbacks of animal tests. Herein, we present how an established tissue engineering approach to create biomimetic skeletal muscle-like tissue can be used to model pathologies. Healthy tissue constructs created through mechanical stimulation of murine myoblasts with a bioreactor,[2] and subsequently subjected to different types of stress present the basis of this study. Stress was induced either by 1.) *mechanical overstimulation* with high frequency or high tensile stress with the same bioreactor;[2] 2.) complete *deprivation of mechanical stimulation* or 3.) by exposure to *oxidative stress* with another bioreactor system based on reactive oxygen species generated through hydrostatic pressure.[3] Mechanical overstimulation resulted in an immediate stress response (Fig. 1) as well as prolonged downstream signaling activity. Furthermore, high frequency overstimulation led to increased gene expression of markers of proliferation and early- and mid-stage myogenesis, and changed morphology of myotubes (Fig. 2). Deprivation of mechanical stimulation for seven days led to severely impaired integrity of myotubes on a morphological level (Fig. 2), but also to downregulation of signaling pathways involved in stress response and myogenic development. Oxidative stress induced decreased viability Ffig. 3) and loss of structural integrity of myotubes. Thus, we conclude that the first steps towards the establishment of a biomimetic tissue-engineered model for skeletal muscle pathologies were successful. Nevertheless, further refinement and investigation of the mechanisms involved are still needed.

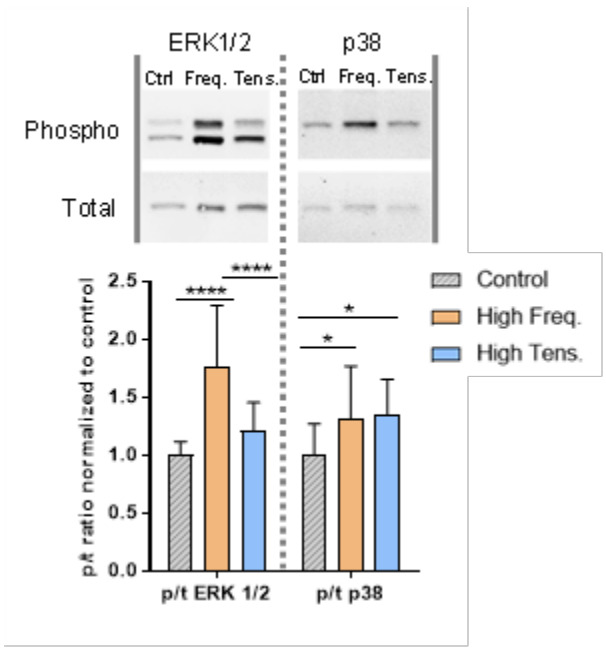

Fig 1. *Western blot of stress transducer activation in biomimetic skeletal muscle-like tissue after mechanical stress*

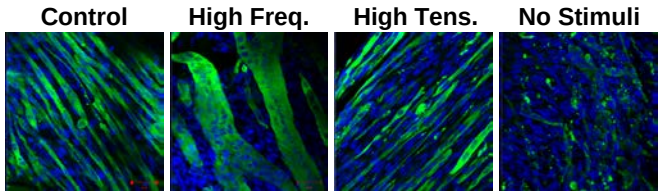

Fig 2. *MHC stainings of biomimetic skeletal muscle-like tissue after mechanical stress*

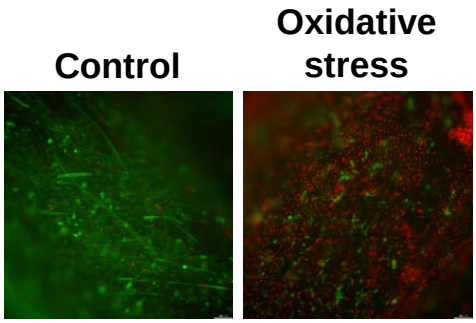

Fig 3. *Live (green) / dead (red) staining of biomimetic skeletal muscle-like tissue after oxidative stress.*

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2022PDM3 Abstract 39

A Dual-AAV gene therapy strategy for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a severe rare pediatric disease that affects the skeletal and cardiac muscles, leading to progressive muscle wasting and premature death. DMD is caused by X-linked mutations in the dystrophin gene that results in lack of dystrophin, a crucial protein involved in the biomechanical support of the muscle fibers. As a consequence of the membrane instability, DMD muscle fibers are progressively more fragile, highly susceptible to damage and fibrotic. Up to date there is no cure for DMD. Although DMD is a suitable disease for gene replacement therapy, the extent of Dystrophin transcript exceeds by far the AAVs cargo capacity and only short versions of dystrophin can be accommodated and are currently being investigating in clinical trials. However, is still not clear whether some domains of dystrophin are really dispensable for its function and stability. Our strategy consists in the generation of a larger version of dystrophin that includes additional domains required for interactions with signaling and structural proteins. To overcome the AAV limit capacity, we developed a dual system dystrophin vector, encoding for a larger dystrophin protein (Quasidystrophin) after homologous recombination of the overlapping region of the 2 dystrophin vectors. We analyzed the therapeutic efficiency of dual-AAVs gene transfer by systemic injection in DBA2-mdx mice, an aggressive DMD mouse model. Our data show high transduction efficiency in the cardiac and skeletal muscles with significant reduction in fibrosis and calcification and overall amelioration of the dystrophic phenotype using the dual-AAV vector gene transfer.

Key words: Duchenne muscular dystrophy, Dual-AAV, Dystrophin.

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2022PDM3 Abstract 40

Dysregulation of heme synthesis-export axis in skeletal muscle reshapes energetic metabolism and results in impaired motor performance

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Heme metabolism plays an essential role in the maintenance of skeletal muscle health [1,2]. Feline leukemia virus subgroup C receptor 1a (FLVCR1a) is a plasma membrane heme exporter that, by removing the intracellular excess of heme, limits the feedback inhibitory effects of heme on its own production, thus sustaining heme biosynthesis. Being part of the heme synthesis-export functional axis, heme export by FLVCR1a has been reported to sustain the tricarboxylic acid cycle (TCA) flux and the electron transport chain (ETC) activity in tumors[3]. Here we generate skeletal muscle-specific *Flvcr1a*-null mice and analyze the impact of disrupted heme synthesis-export axis in this tissue. Metabolic data obtained in gastrocnemius homogenate show that, upon deletion of *Flvcr1a*, the activity of TCA cycle enzymes and ETC complexes is increased, along with glutaminolysis and fatty-acid beta oxidation. Conversely, the activity of glycolytic enzymes is reduced. *Flvcr1a* deletion also affects muscle morphology. The distribution of cross-sectional fibers' area (CSAs) is compromised in *Flvcr1a* knockout mice, showing a prevalence of fibers with small area. Along with these data, increased number of PAX7+ cells was also observed. In motor behavior tests, *Flvcr1a* knockout mice show worse performance compared to controls, a phenotype exacerbated by aging. Collectively, these results hint that FLVCR1a is involved in the regulation of energetic metabolism in skeletal muscle, and that the heme synthesis export system is important to maintain proper muscle function.

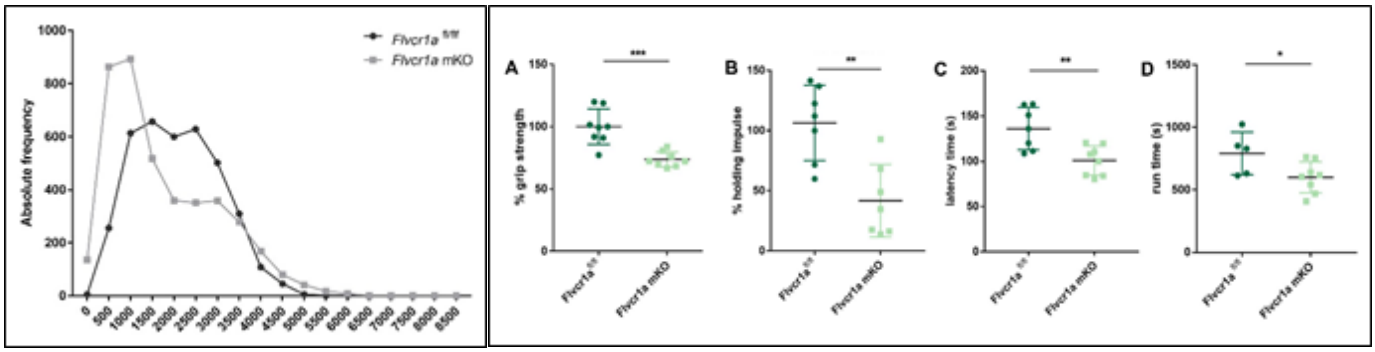

Fig 1. *Left panel: Cross-sectional area (μm2) distribution. Right panel: (A) Grip strength test, (B) Kondziela's inverted screen test, (C) Rotarod performance test, (D) Treadmill performance test.*

Key words: block of heme export, reduced fibers' area, worse muscle performance, energetic metabolism.

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2022PDM3 Abstract 41

The p97/Nploc4 ATPase complex plays a role in muscle atrophy during cancer and amyotrophic lateral sclerosis

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The p97 complex participates in the degradation of muscle proteins during atrophy upon fasting or denervation interacting with different adaptors. We investigated whether and how it might also be involved in muscle wasting in cancer, where loss of appetite occurs, or amyotrophic lateral sclerosis (ALS), where motoneuron death causes muscle denervation and fatal paralysis. The mRNA levels of p97 were induced in tibialis anterior (TA) of three distinct cancer cachectic mouse models but not in the non-cachectic 4T1 tumor. Similarly, p97 was high both in mRNA and protein in muscles from SOD1G93A mice. Electroporation of a shRNA for murine p97 into muscle reduced the fiber atrophy caused by colon adenocarcinoma C26 and ALS. When we interrogated a microarray we had previously generated for the expression of p97 adaptors, we found few of them induced in cachectic TA from C26-mice. By qPCR, we validated their inductions in TA of cachectic and ALS models and selected Nploc4 as the one most induced. Electroporation of a Crispr/Cas9 vector against Nploc4 into muscle reduced the fiber atrophy caused by C26 and ALS. Since Disulfiram (DSF) uncouples p97 from Nploc4, we treated atrophying myotubes with DSF, and found accumulated polyubiquitinated proteins and reduced degradation of long-lived proteins. DSF halves Nploc4 in the soluble muscle fraction and given to C26-mice limited the body and muscle weight loss, with no effect on tumor. The p97/Nploc4 complex plays a crucial role in muscle atrophy during these disorders and disrupting this complex might serve as a novel drug strategy.

ONE FIGURE, PLEASE

Fig 12. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stained by Coomassie brilliant blue (lower line*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: 3 to 5

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2022PDM3 Abstract 42

The timecourse of adaptive change in gene expression across 30d of daily programmed resistance exercise in rats

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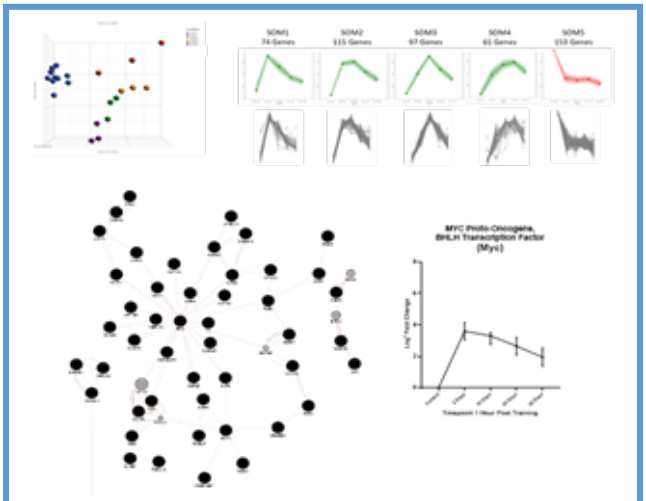

Fig 1. (A) Principal component analysis (PCA) of control (blue), 2 days (red), 10 days (yellow), 20 days (green) and 30 days spillover training (purple). (B) The top 500 differentially expressed genes were characterised into 5 distinct clusters following self-organising map (SOM) temporal analysis. 5 were selected based on the inflection point of the minimum centroid distance between clusters. (C) GeneMANIA multiple association network identifies MYC Proto-Oncogene, BHLH Transcription Factor as the master regulator of the transcriptional response

Despite data over several decades showing that resistance exercise induces hypertrophy, the transcriptional mechanisms responsible for the process are still not fully understood. Recent meta-analyses (1,2) have attempted to identify time-dependent patterns in the expression of genes that regulate the transcriptional response to resistance exercise. The objective of this study was to perform a detailed transcriptomic analysis through RNA-sequencing of the acute response to resistance exercise (one hour after an exercise session), across a 30 day period of daily resistance training. We have developed and implemented a highly controllable resistance training model that uses implantable pulse generators in free-living rats to induce hypertrophy in the tibialis anterior by co-contraction of the dorsiflexors and plantarflexors (3). We assessed the skeletal muscle transcriptome (1-hour post exercise) after 2, 10, 20 and 30 days of our exercise training paradigm and found 5 temporal clusters across our timecourse (Self-organizing maps (SOM) 1-5, Figure 1B). SOM1 and SOM2 included known resistance-exercise responsive pathways including the ribosome, proteasome, and protein processing in the endoplasmic reticulum as well as genes associated with inflammation/macrophages. These genes were highly responsive while the muscles were growing, but their expression was blunted as muscle mass reached a new steady state. GeneMANIA network association analysis (Figure 1c) found that the genes within these clusters were centrally controlled by the transcription factor Myc, a well-known regulator of cell cycle, metabolism, growth, RNA polymerase activity and ribosomal biogenesis (4). However, the expression of many of these genes in skeletal muscle is not well understood. We propose that it will be informative to study the group of genes whose timecourse of expression is associated with the period of growth and whose response declines as the muscle adapts to a daily stimulus to achieve a new steady state.

Key words: Transcriptomics; resistance exercise; Myc.

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2022PDM3 Abstract 43

PCM1 labelling reveals myonuclear and nuclear dynamics in skeletal muscle across species

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Myonuclei transcriptionally regulate muscle fibres and are key regulators of muscle plasticity. Their cellular location and quantity are important when characterising phenotypes of myopathies, the effect of treatments and to understand the roles of satellite cells in muscle adaptation and muscle 'memory'.[1] We aimed to determine whether PCM1 is, as claimed,[2] a reliable specific marker of myonuclei in-vitro and in-vivo. Single isolated myofibres and transverse sections from mouse, rat and humans were studied from several models including Wild-type and Lamin A/C mutant mice after functional overload,[3] resistance training in rats,[4] and damage and recovery in humans following forced eccentric contractions.[5] Fibres were immuno-labelled for PCM1, Pax7 and DNA. C2C12 myoblasts and human primary muscle cells were also studied in vitro to investigate changes in PCM1 localisation during differentiation and fusion into myotubes. PCM1 labelled the nuclear envelope of myonuclei in mature myofibres and in newly formed myotubes but also labelled centrosomes in proliferating myogenic precursors which may or may not fuse to join the myofibre syncytium. It also labelled non-myogenic nuclei near the sarcolemma especially in regenerating areas of the LMNA+/ΔK32 mouse and damaged human muscle. Such nuclei were found in satellite cells, macrophages, and other interstitial cells. PCM1 is therefore not completely specific to myonuclei, and especially in damaged or regenerating muscle the impact of false-positive identification of interstitial cells on myonuclei counts would tend to cause overestimation of myonuclei per fiber compared with classical counting based on myonuclear positioning relative to the sarcolemma. PCM1 may further prove useful as a marker of satellite cell dynamics due to the distinct change in localisation during differentiation, revealing satellite cells in their quiescent (PCM1-), proliferating (PCM1+ centrosome), and pre-fusion states (PCM1+ nuclear envelope).

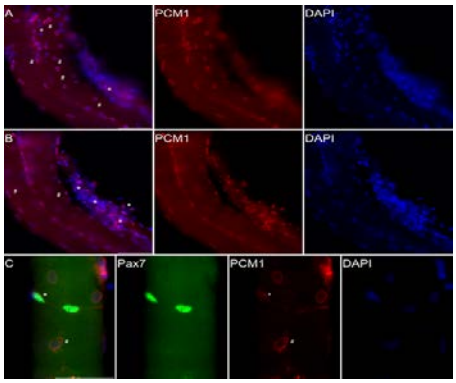

Fig 1. *Single human myofibres extracted 7 days post eccentric damage. (A, B) shows 3 myofibres at different positions in the z-stacks. The right most fibre is necrotic, indicated by infiltration of a dense population of nuclei belonging to proliferating macrophages with PCM1+ centrosomes (*). (C) Two Pax7+ cells in proximity one of which displays PCM1 positivity (*) adjacent to PCM1+ myonuclei (#). Scale bars indicate 100µm.*

Key words: PCM1; hypertrophy; regeneration; myonuclei.

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2022PDM3 Abstract 44

The HERG K^+ Channel Increases Intracellular Calcium Concentration in Myotubes by Modulation of IP3 Signaling

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The HERG K^+ channel is upregulated in skeletal muscle atrophying in response to disuse[1], cancer[1], and denervation[2]. Using C2C12 myotubes transduced with HERG-encoded virus, we have shown that over-expression of the HERG channel increases basal intracellular calcium concentration and calpain activity[3]. However, the mechanism by which HERG modulates intracellular calcium levels is not known. To explore this further, we increased intracellular calcium levels by depolarization with 100 mM KCl and used Fura2 dyes and immunoblot to show that HERG does not alter myotube calcium levels by affecting L-type calcium channels (i.e., Cav1.1). Instead, using the SERCA blocking agent thapsigargin with our Fura2 assay, we discovered that the HERG-mediated increase in calcium occurs through modulation of intracellular calcium stores[4]. Therefore, we hypothesized that HERG may be modulating the phospholipase C (PLC)-PIP2-IP3 pathway. To investigate this, we produced an increase in intracellular calcium using bethanechol to activate muscarinic receptors, which activate the PLC-PIP2-IP3 pathway. Using single cell calcium imaging, we reveal that, relative to myotubes transduced with an appropriate control virus, bethanechol treatment of HERG-expressing myotubes produces a significant 2-fold increase ($p<0.05$) in intracellular calcium levels. The data suggest that HERG may indeed modulate the PLC-PIP2-IP3 pathway. Interestingly, however, this HERG-mediated increase in bethanechol-induced calcium levels is not a result of increased PLC activity as demonstrated by IP1-Gq HRTF kit assays, which show no increase in the concentration of IP1 (an IP3 degradation product) in HERG-expressing myotubes relative to controls. However, single cell calcium imaging reveals that the HERG-mediated increase in calcium is inhibited by treatment with the IP3 receptor antagonist Xestospongin-C. The data suggest that HERG enhances calcium release through IP3 receptor activation without affecting PLC activity.

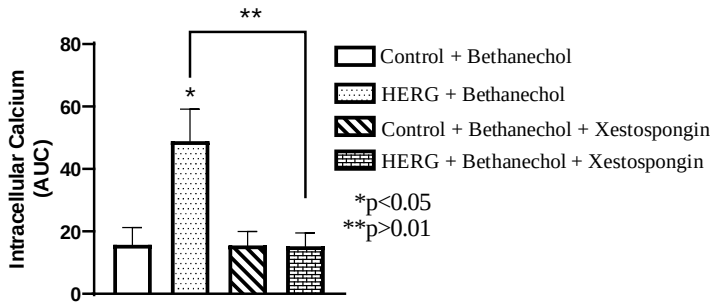

Figure. HERG increases calcium release by IP3R activation.

Fig 13. *HERG increases calcium release by IP3R activation.*

Key Words: HERG; IP3 signaling; intracellular calcium; myotubes.

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2022PDM3 Abstract 45

Uncovering the epigenetic control of paracrine crosstalk between motor neurons and skeletal muscles in SMA.

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Spinal muscular atrophy (SMA) is a genetic disorder characterized by motor neuron degeneration and progressive muscle weakness, atrophy and premature death. It originates from the homozygous mutation of the survival of motor neuron 1 (SMN1) gene with an incidence of 1 in 10,000 live births representing the most common genetic cause of infant mortality. Loss of motor neuron function is thought to be the initial event which triggers muscle atrophy due to the lack of physical nerve-muscle contact at the neuromuscular junction (NMJ). But, recent findings reveal that muscle atrophy in SMA precedes motor neuron death. However, the crosstalk between the muscle and the motor neuron in the pathogenesis of the disease has not yet been deciphered. We aim to clarify the motor neuron function and its contribution to skeletal muscle pathology using the multi-omics analysis of DNA epigenetics and transcriptomic profiles. Our data intriguingly supports an alternative paradigm where paracrine crosstalk between motor neuron and skeletal muscle plays a key role in the survival and function of both tissue compartments. We found that (1) spinal cord tissue of the SMAdelta7, a severe SMA mouse model, exhibited a genome-wide decrease in the DNA demethylation mark 5hmC; (2) reduced 5hmC mark supresses the expression of a subset of genes, identified to play an important role in paracrine signalling regulating muscle function. Our data support the crucial role of modulating DNA epigenetics in MN's; demonstrating that the effects of altered 5hmC levels in SMA go beyond the MN compartment to impair muscle function, by affecting the crosstalk signals independent of NMJ function.

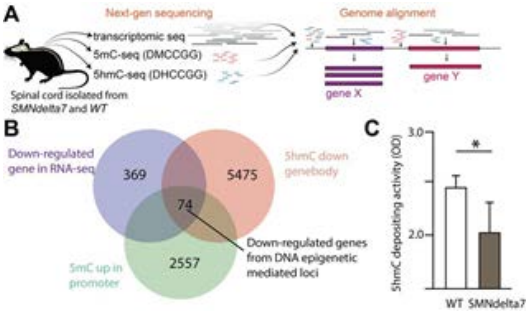

Fig.1 *Caption of the figure A: ……..;*
B: ……
C: ……

Key words: 3 to 5

References 3 to 5

2022PDM3 Abstract 46

Targeting ER stress to resolve aggresome accumulation in oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a rare, late-onset genetic myopathy (Trollet et al., 1993)[1] affecting mainly the eyelid and pharyngeal muscles, resulting in ptosis and dysphagia, respectively. OPMD is due to an alanine expansion in the Poly-A Binding Protein Nuclear 1 (PABPN1) gene which encodes a regulator of poly-A tail length of RNA. This mutation results in PABPN1 nuclear aggregates in OPMD myonuclei that sequester essential RNA and proteins and thus induce muscle alterations. Therefore, human OPMD muscles present a global proteostatis impairment (chaperones, ubiquitin-proteasome and autophagy-lysosomal processes) (Abu-Baker et al., 2003; Anvar et al., 2011; Raz et al., 2017).[2-4] Endoplasmic reticulum is also a key actor of cell proteostasis assuring folding of one third of the cellular proteome. Interestingly, OPMD mouse model exhibits muscle endoplasmic reticulum (ER) stress (accumulation of misfolded proteins in the ER) and restoration of the homeostatic unfolded protein response (UPR) by guanabenz acetate treatment decreases PABPN1 nuclear aggregates and improves muscle function in OPMD mice (Malerba et al., 2019).[5] To extend these results to humans, we analyzed muscle biopsies from OPMD and healthy donors. We found that UPR genes were all upregulated in human OPMD muscles, as revealed by RNAseq analysis, strongly indicating ER stress. Consistently, GRP78 protein, the key ER chaperone, was reduced in OPMD muscles. GRP78 protein was also trapped in PABPN1 nuclear aggregates, further reducing its amount available for ER protein folding. Consequently, OPMD muscles showed an accrual of large β-sheet protein aggregates (called aggresomes) in the fibers, beside to the classical PABPN1 nuclear aggregates. Consistently, in culture, OPMD human muscle cells were more likely to develop aggresomes than healthy cells following ER stress specifically. Finally, in mouse cells overexpressing PABPN1, we found that ER stress induction leads to accumulation of aggresomes and aggravation of PABPN1 nuclear aggregates. Overall, our results demonstrated an ER protein folding disruption leading to ER stress and aggresome accumulation in human OPMD muscle, that could reinforce pathogenesis of OPMD. Thus, restoration of ER folding and activation of protein aggregate clearance sounds promising therapeutic strategies against OPMD.

Key words: Oculopharyngeal muscular dystrophy; protein aggregation; endoplasmic reticulum; proteostasis; skeletal muscle.

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2022PDM3 Abstract 47

Peroxisomal-mitochondrial interaction impinging on muscle homeostasis

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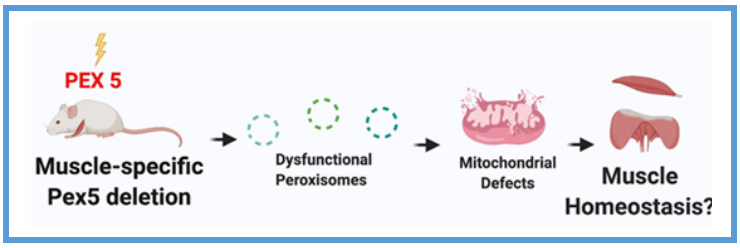
The skeletal muscle tissue is a primary metabolic activity site controlling almost 80% of insulin-dependent glucose uptake and storage. It is also the largest protein reservoir (75%) of our body, supplying aminoacids to support energy production in other organs during catabolic conditions. Thus, skeletal muscle is crucially important to control health and disease progression in distant tissues both for its role in balancing other organs' metabolic needs and its reserves to use in energy production. Its dysfunction is associated with poor prognosis and reduced quality of life. Peroxisomes are ubiquitous dynamic metabolic organelles that play an essential role in a variety of cellular events such as reactive oxygen species clearance and lipid metabolism. However, their role in muscle metabolism and function has largely been overlooked. To investigate the consequences of peroxisomal dysfunction in muscle we have generated a mouse model with impaired peroxisome biogenesis by disrupting specifically in skeletal muscle the Peroxisomal biogenesis factor 5 (PEX5), which serves as a shuttle receptor for the import of peroxisome matrix protein. We found the peroxisomal dysfunction in muscle causes several mitochondrial defects, and increased ROS production resulting in muscle ultrastructural alterations and reduced running performance. Thus, peroxisomal integrity is critical for skeletal muscle homeostasis.



Key words: Peroxisomes; skeletal muscle; ROS.

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2022PDM3 Abstract 48

Mitochondrial dysfunction in skeletal muscle promotes precocious sarcopenia, degeneration of multiple organs, and premature death through inflammation and metabolic changes

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A healthy mitochondrial network is essential for post-mitotic tissues as muscles. Mitochondria-shaping machinery is downregulated in sarcopenia and is maintained by lifelong exercise. OPA1 is a profusion protein that plays an important role in mitochondrial dynamics we have demonstrated. Interesting, OPA1 protein is significantly decreased in sedentary humans and old mice compared to young controls and, exercise is sufficient to restore the protein level. In conditional tissue-specific Opa1 ko is sufficient to induce a sarcopenic-cachectic phenotype in just 3 months of deletion and Fgf21 is a central myokine in this process. This work aims to find new specific players involved in aging sarcopenia. Particularly, that synergistically promotes the aging of the whole body and the secretome related. we are comparing different genetic mouse models to dissect the specific role of the different genes in the sarcopenia scenario. Mitochondrial dysfunction in muscle tissue is sufficient to directly drive metabolic changes and systemic inflammatory by increasing the expression and secretion of the myokines FGF21 and IL6. Despite the key role of FGF21, we have new evidence that is connecting mitochondrial dysfunction, autophagy alteration, muscular IL6, and aging. Opa1 is a "sensor" for the health of the muscle, the reduction of the protein level recapitulates the acute sarcopenia process. The changes related to mitochondrial dysfunction are specifically correlated to the shape of these organelles. Different alteration induces some common but mainly specific metabolic and stress responses directly correlated to sarcopenia development.

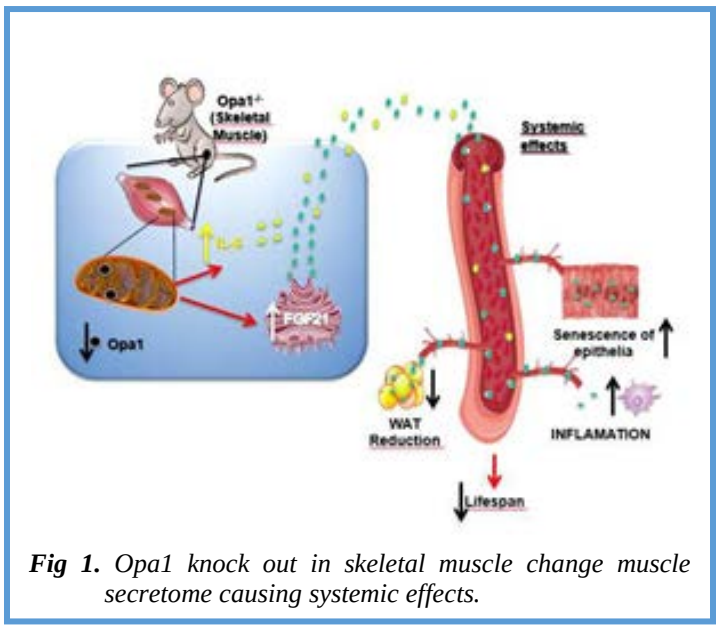

Fig 1. *Opa1 knock out in skeletal muscle change muscle secretome causing systemic effects.*

Key words: Skeletal muscle secretome; inflammaging; metabolism; sarcopenia; precoscious aging.

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2022PDM3 Abstract 49

Identification of a novel TFEB and exercise dependent gene

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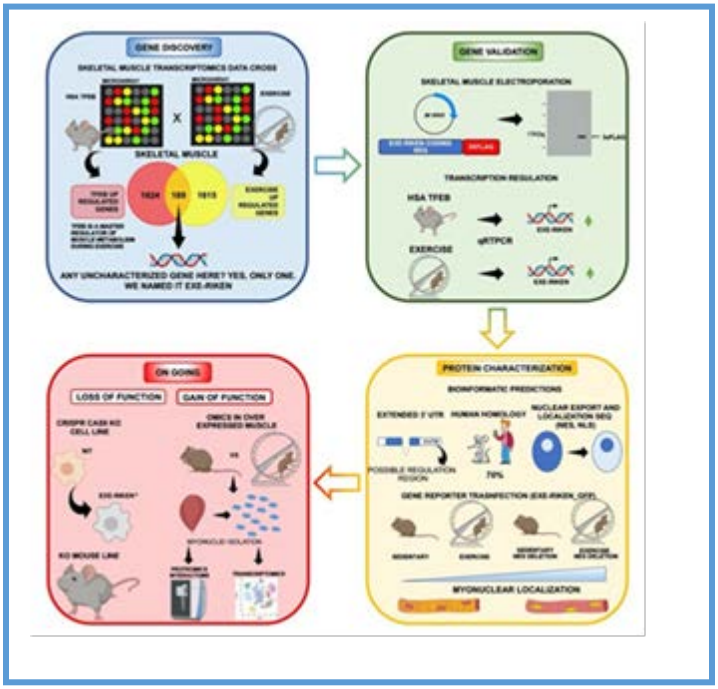
While the benefits and adaptations to regular physical activity are long known, molecular networks, signalling pathways and regulatory molecules that coordinate adaptive responses to exercise are still matter of debate. Transcription Factor EB (TFEB) is a master metabolic regulator in skeletal muscle, finely tuning fuel uptake to energy production during exercise. These findings strongly support TFEB activity as crucial for mediating the beneficial effects of exercise1. Starting from this evidence we decided to discover new uncharacterized genes that may mechanistically explain adaptive effects of TFEB on skeletal muscle. By crossing gene expression data of TFEB transgenic and trained muscles, we found a gene of unknown function belonging to the Riken cDNA collection2 that we called Exe-Riken. We showed that Exe-Riken is a real protein coding gene. We also confirmed its transcriptional dependence from TFEB and exercise, with a fine tuning in different muscles; interestingly, its transcription induction does not depend on extra muscular factors. Moreover, we showed its differential localization in muscle fibers, with a positive correlation between cross sectional area and immunostaining reactivity. Oxidative muscles such as soleus did not present cytoplasmic but more nuclear Exe-Riken localization. Thanks to overexpression and NES deletion experiments, we showed that exercise is an Exe-Riken cytosol-to-nucleus shuttling stimulus. Finally, qRT-PCR experiments suggest the presence of at least two transcript variants that differ on the 3'UTR sequence. So far, all the findings emphasize Exe-Riken as a novel but compelling molecular player mediating metabolic adaptions to physical training.



Key words: TFEB, exercise, adaptation, RIKEN.

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2022PDM3 Abstract 50

Uncovering a novel mechanism for intramuscular fat formation

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Adipose tissue can accumulate between muscle fibers, known as intramuscular fat (IMAT) and is strongly correlated with loss in muscle mass and function. IMAT infiltration is a hallmark of ageing and a diverse set of diseases such as muscular dystrophies, diabetes, and spinal cord injuries. No therapies exist currently that limit IMAT formation. We and others have shown that exogenous activation of the Hedgehog (Hh) pathway is beneficial for overall muscle regeneration and improves muscle health in a Duchenne muscular dystrophy (DMD) model (mdx mouse). We have also demonstrated that pharmacological and genetic activation of the Hh pathway blocks the formation of IMAT after an adipogenic injury and in mdx mice. To define the endogenous function of the Hh pathway during acute muscle regeneration, we evaluated which of its 3 possible ligands (Sonic [SHH], Indian [IHH] or Desert Hedgehog [DHH]) is expressed. Exploring single cell RNA sequencing data of dissociated muscle tissue pre and post injury, we found that DHH is expressed by endothelial and Schwann cells and strongly upregulated after injury. To test the functional requirement for DHH, we evaluated the regeneration of Dhh knock-out (Dhh KO) mice compared to controls. For this, 21 days post cardiotoxin (CTX) injury, we assessed IMAT and found a significant increase compared to control littermates. This suggests that the Hh pathway, activated endogenously through DHH, acts as an adipogenic break, that when lost leads to increased IMAT. To further evaluate the pathway's physiological role, we sought to compare and manipulate Hh activity in mouse strains with differing adipogenic predisposition. When comparing IMAT 7 days after a CTX injury between CD1 and 129S1/SvlmJ mice strains, CD1 mice have higher levels of IMAT compared to 129S/1SvlmJ. To test if this difference in IMAT formation is due to differences in Hh activity, we compared the expression levels of the pathway's downstream gene target Gli1 by RT-qPCR. Excitingly, our results demonstrate that, compared to CD1 mice, 129S1/SvlmJ mice display higher Hh activity. As ectopic Hh activation acts as a potent adipogenic brake, endogenous Hh activity levels may explain the differences in adipogenic predisposition between mouse strains. Confirming our hypothesis, inactivating endogenous Hh activation through the small molecule Vismodegib in 129S1/SvlmJ mice resulted in an increase in IMAT. Together, our data identify DHH as the key Hh ligand during muscle regeneration, and that Hh signaling, via DHH, acts as an endogenous adipogenic break. In addition, our results also indicate that endogenous differences in Hh activity may explain the differences in IMAT predisposition. Thus, the Hh pathway represents an exciting and novel therapeutic target to control IMAT formation.

ONE FIGURE, PLEASE

Fig 14. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: Intramuscular Fat; FAPs; Muscle Regeneration

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2022PDM3 Abstract 51

Understanding BMP signaling in cancer cachexia

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Cancer cachexia is a multi-factorial metabolic syndrome characterized by excessive body weight loss due to progressive fat and lean mass catabolism in spite of nutritional support,[1] occurring in more than half of cancer patients.[2] The frailty experienced by cachectic patients increases morbidity, reduces tolerance to treatments, complicates patient management and accounts for up to 30% of cancer deaths.[3,4] The loss of skeletal muscle mass and strength are considered as the most relevant features of cancer cachexia and predictors of poor outcomes.[5,6] Bone Morphogenetic Protein (BMP)/Smad1/5/8 pathway is a positive regulator of muscle mass repressing the transcription of MUSA1, an atrophy-associated E3 ubiquitin ligase that facilitates proteasome-dependent protein breakdown.[7,8] We demonstrated that diminished BMP (Bone and Morphogenetic Protein)/Smad1/5/8 signalling in muscles plays a critical role for the onset of cancer cachexia. Re-activation of BMP signaling in muscles is sufficient to prevent tumor-induced muscle atrophy, and to extend the lifespan in tumor bearing mice despite tumor growth. Importantly, these protective effects are prevented by knocking down Smad1/5.[9] We aim to understand how the cancer stimulates transcriptional changes leading to BMP-Smad1/5/8 inhibition in skeletal muscles to favor cachexia onset in order to identify new potential therapeutic targets.

ONE FIGURE, PLEASE

Fig 15. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: Cancer cachexia; gene regulation; BMP signature.

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2022PDM3 Abstract 52

D2.*mdx* mice undergo a transient period of left ventricular restriction prior to heart failure

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Duchenne muscular dystrophy (DMD) is the most frequently inherited neuromuscular disease of childhood and cardiopulmonary failure is the leading cause of death for this patient population. In this study, we used electrocardiograms (EKGs) and ultra high frequency echocardiography to longitudinally monitor the heart function of a severe mouse model of DMD, the D2.mdx mouse. By 10 months of age, D2.mdx mice have a left ventricular end diastolic volume (LV EDV) that is ~32% less than age-matched wild- type mice. This restricted LV results in a significant decrease in stroke volume (SV) and cardiac output (CO); however, parameters of systolic function, such as ejection fraction (EF) and fractional shortening (FS), are preserved. In line with this impaired relaxation, EKGs of D2.mdx mice reveal they have a prolonged QT interval, due to an increase in T wave duration and indicative of impaired repolarization. Further evidence of the diastolic dysfunction of D2.mdx hearts was found using pulsed-wave doppler of the blood flow through the mitral valve (MV), visualized using color doppler. D2.mdx mice have a reduced MV E/A ratio, and in agreement with the EKG findings, an increased isovolumic relaxation time (IVRT). In conclusion, like DMD patients and a canine model of DMD, the D2.mdx mouse displays left ventricular restriction prior to the progression to a dilated cardiomyopathy (DCM) and is a useful small animal model for the cardiomyopathy associated with DMD.

Pre-DCM Left Ventricular Restriction

Echocardiographic M	Murine		Canine		Human[1]	
	DBA2/J	D2.*mdx*	Normal	GRMD	Normal	DMD
End Diastolic Volume (µL or mL)	75.77±5.99	52.10±4.15*	66.53±3.24	51.57±3.35*	64.8±11.6	41.8±7.7*
End Systolic Volume (µL or mL)	19.97±3.64	8.25±1.69*	26.87±2.68	23.51±2.43*	21.6±4.4	18.7±5.12
Stroke Volume (µL or mL)	55.81±3.07	43.85±2.98*	39.65±1.85	28.06±1.58*	43.3±10.4	29.1±5.4*
--SV Deficit (%)	--	-21.43	--	-29.23	--	-32.79
Ejection Fraction (%)	79.53±2.36	84.82±2.62	60.47±2.49	56.88±2.28	66.3±7.2	61.2±7.1

Volumes are in µL for murine values and mL for canine and human values; SV = stroke volume;
*p < 0.05 vs. control values. [1]Panovský et al. 2021

Key words: Duchenne muscular dystrophy; restrictive cardiomyopathy; mouse model.

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2022PDM3 Abstract 53

Molecular Antagonism between DUX4 and DUX4c Highlights a Potential Pathomechanism in Facioscapulohumeral muscular dystrophy

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Aberrant expression of the transcription factor DUX4 from D4Z4 macrosatellite repeats on chromosome 4q35, and its transcriptome, associate with pathogenesis in Facioscapulohumeral muscular dystrophy (FSHD)(1, 2). In skeletal muscle cells, forced DUX4 expression halts cell proliferation and induces cell death (Figure 1). DUX4 binds DNA via homeodomains that have high sequence similarity to those of DUX4c, a closely related transcriptional regulator encoded by a single, inverted, mutated D4Z4 unit located centromeric to the D4Z4 macrosatellite array(3). However, DUX4c function and contribution to FSHD pathogenesis is unclear. To explore interplay between DUX4, DUX4c and DUX4-induced phenotype, we investigated whether DUX4c interferes with DUX4 function in human myogenesis. Constitutive expression of DUX4c rescued the DUX4-induced inhibition of proliferation and blunted the onset of cell death in human myoblasts (Figure 1). Functionally, DUX4 forces the nuclear translocation of β-CATENIN and disrupting canonical WNT signalling. However, concomitant overexpression of DUX4c restores β-CATENIN cytoplasmic/nuclear shuttling and the downstream transcriptional program (Figure 1). We identified a subset of genes involved in the β-CATENIN pathway that are differentially regulated between DUX4 and DUX4c and can separate FSHD from healthy muscle biopsies based on the expression pattern. Constitutive expression of DUX4c robustly suppresses expression of DUX4 target genes confirming molecular dueling. In line, FSHD myoblasts display upregulation of DUX4 and downregulation of DUX4c compared to aged-matched unaffected myoblast (Figure 2). Finally, blockade of WNT/β-CATENIN pathway rescue proliferation defect of FSHD myoblasts (Figure 2). Together, our study demonstrates that DUX4 alters cell viability via β-CATENIN activation and that DUX4c can counteract aspects of DUX4-mediated toxicity in human muscle cells, informing future interventions to ameliorate FSHD pathology..

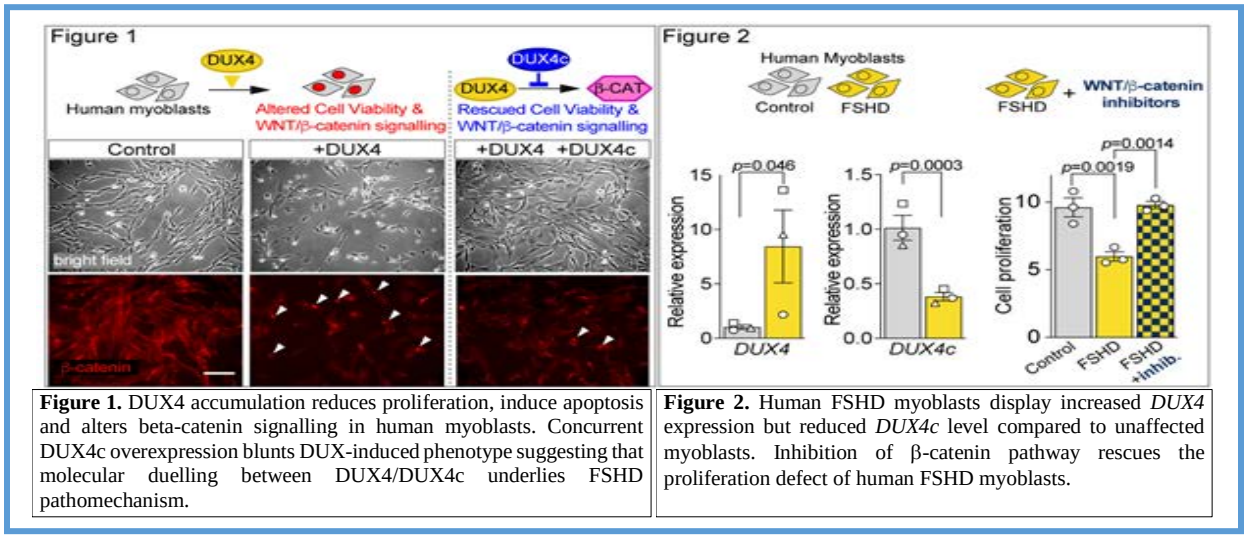

Figure 1. DUX4 accumulation reduces proliferation, induce apoptosis and alters beta-catenin signalling in human myoblasts. Concurrent DUX4c overexpression blunts DUX-induced phenotype suggesting that molecular duelling between DUX4/DUX4c underlies FSHD pathomechanism.

Figure 2. Human FSHD myoblasts display increased *DUX4* expression but reduced *DUX4c* level compared to unaffected myoblasts. Inhibition of β-catenin pathway rescues the proliferation defect of human FSHD myoblasts.

Key words: FSHD; DUX4; DUX4c; β-CATENIN; apoptosis; myoblast, proliferation.

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2022PDM3 Abstract 54

NOX4 inhibition reduces skeletal muscle fibrosis in a severe murine model of Duchenne muscular dystrophy

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The muscular dystrophies (MDs) are a class of genetic muscle diseases often characterized by progressive degeneration and replacement of muscle with fibrotic tissue. Limited treatment options exist for MD patients, and no therapeutics specifically target the progression of intramuscular fibrosis. NAD(P)H oxidase (NOX) 4 has been previously implicated in the development of fibrosis in other organs, but not skeletal muscle. The specific aim of the current report was to investigate NOX4 as a potential anti-fibrotic target in dystrophic skeletal muscle, using the severe D2.mdx mouse model of Duchenne muscular dystrophy (DMD). It is shown that NOX4 is increased in the muscles of D2.mdx mice and localizes primarily to interstitial regions between muscle fibers. In agreement with the hypothesis that this increase in NOX4 contributes to muscle fibrosis, genetic ablation of Nox4 prevents the progressive accumulation of fibrosis in skeletal muscle. Furthermore, it is demonstrated that pharmacological targeting of NOX4 using the small molecule clinical candidate NOX1/4 inhibitor, GKT137831, also prevents the progression of muscular fibrosis in the diaphragm and gastrocnemius of D2.mdx mice. Mechanistically, NOX4 loss-of- function results in decreased accumulation of periostin and periostin-associated interstitial cells within dystrophic muscle. These data indicate NOX4 is an effective anti-fibrotic target to combat progression of muscle fibrosis in DMD and, potentially, other MDs.

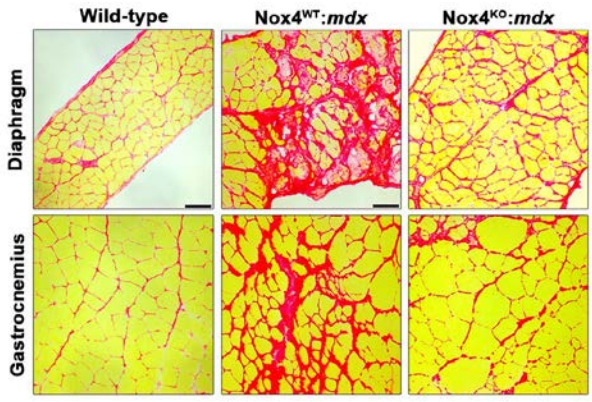

Fig 1. NOX4 is an antifibrotic target in skeletal muscle. Representative picrosirius red-stained images from wild-type DBA/2J, NOX4-WT D2.mdx, and NOX4-KO D2.mdx mice demonstrating reduced muscle fibrosis via Nox4 ablation.

Key Words: Muscular dystrophy; fibrosis; NADPH oxidase; extracellular matrix; regeneration; pharmacology; myogenesis

References
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2022PDM3 Abstract 55

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Since the pioneering work of Mosso (1906),[1] physiologists have recognized that fatigue comprises two series of phenomena: one related to the decline in the force capacity of muscle and the other involving the sensations that arise during sustained physical activity. Despite intense international effort over the intervening 100 years, it has proven impossible to identify a single underlying mechanism responsible for 'muscle fatigue'. Recent attempts to resolve this impasse have focused on three issues: (1) defining fatigue as a symptom; (2) distinguishing between fatigue and fatigability (work capacity); and (3) encouraging precise and consistent use of terminology. The result has been an evolving taxonomy that is inclusive of the many stakeholders with an interest in fatigue, yet contains sufficient detail to identify specific therapeutic targets for various groups of individuals (Enoka et al. 2021).[2] The utility of this approach will be illustrated with work we are performing on the use of transcutaneous electrical nerve stimulation (TENS) to reduce fatigue—a key disabling symptom—in persons with multiple sclerosis and the functional benefits that result from such treatments (Alenazy et al. 2021).[3] The consequences of this approach are that fatigue, similar to pain, can only be measured by self-report and should not be used as a metric for a reduction in work capacity.

ONE FIGURE, PLEASE

Fig 16. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Keywords: State fatigue; trait fatigue; fatigability; interoception; transcutaneous electrical nerve stimulation.

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2022PDM3 Abstract 56

Adaptive Force in Patients with Long-COVID

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After 6 month 68% of COVID-19 survivors, and still 49% after 12 months, show one or more long-COVID symptoms.[1] The symptoms persist regardless of COVID-19 severity.[2] The public attention to Long-COVID contributed to shed light on a similar but neglected syndrome. Chronic fatigue syndromes after acute infections (e.g., Ebstein-Barr virus) are known since decades, but mostly are without adequate recognition from medical professionals.[3] Based on established diagnostics, there is still no satisfying approach to understand the phenomena of post-infectious syndromes and, consequently, to apply purposeful therapies. In view of the immense and increasing number of Long-COVID patients, diagnostical approaches are urgently needed. The most common symptoms of post-infectious diseases are fatigue and muscle weakness.[1] In clinical practice, a considerably reduced ability to resist external forces is regularly seen in those patients. Therefore, it is questioned whether the newly inaugurated Adaptive Force (AF) could be a promising approach to measure post-infectious states. The AF reflects the capacity of the neuromuscular system to adapt adequately to external increasing forces in an isometric holding manner. Healthy persons show a high maximal isometric AF (AFisomax (N)) assessed during manual muscle test (MMT) objectified by a handheld device.[3,4] Thereby AFisomax is close to the maximal AF (AFmax (N)) reached in the same measurement, reflecting a proper adaptability of the neuromuscular system. AF pilot measurements of Long-COVID patients gathered in clinical routine (no clinical study) will be presented. The AF of the hip and elbow flexors of one side was assessed manually (MMT) each three times (alternating) by well-skilled examiners objectified by the handheld device. The maximal holding capacity (AFisomax) and the total maximal AF (AFmax) were evaluated (Fig. 1) as well as the ratio of AFisomax/AFmax (%). Long-COVID patients show a substantial and significantly lower AFisomax and AFisomax/AFmax compared to healthy subjects. After successful treatment the holding capacity is immediately improved and the ratio is significantly higher compared to the initial measurements. This suggests the holding capacity is essentially lower in post-infectious conditions and could possibly be a sensitive biomarker for post-infection fatigue. These preliminary data need to be confirmed by future research (clinical study). In case of verification it could be used to optimize the individual diagnostics and to apply purposeful treatments.

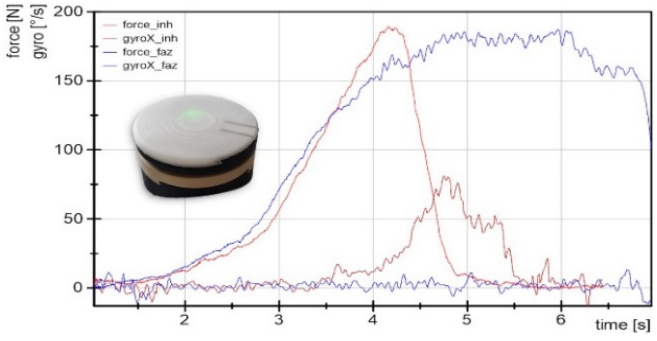

Fig 1. *Measuring device and AF curves of the hip flexors in stable (blue) and unstable (red) condition.*

Key Words: Adaptive Force; holding isometric muscle function; long-COVID; post-COVID.

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2022PDM3 Abstract 57
Dental rehabilitation from a muscular point of view

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Dental occlusion changes may be needed for several reasons. Osseous and dental static and dynamic relationships alterations often result from severe tooth wear, crowding, loss of masticatory units and oncologic surgical resections that require reconstruction of dental and/or skeletal occlusal relationships. Any dental work that changes the occlusal surfaces and/or the tooth position could potentially modify oral proprioception. Kogawa., et al.[1] demonstrate how the interdental perception thresh-old in healthy subjects is less than 2 hundredths of a millimeter. Neuromuscular system could be forced to develop functional adaptations to dental treatments involving occlusal surfaces that change oral proprioception. It has been clearly demonstrated how dental afferents play a role in the masticatory muscles recruitment. Occlusal modifications could change masticatory muscles global contraction intensity but also their functional relationship.[2] Moreover muscle imbalances may originate from several factors including interference in the working or balancing side and loss of posterior vertical support.[3] Indeed, it has been demonstrated how proprioceptive dental alterations due to iatrogenic occlusal disturbances (200 µm thick) may require also neck muscles functional adaptations.[4] The relationship between abnormal muscle recruitment and symptoms such as pain or signs of dysfunction like movement limitation does not appear linear, underlining that a great number of subjects has a wide functional adaptability.[5] The absence of clinical symptoms (mainly pain) following an occlusal intervention does not correspond directly to a procedure free from imperfections and/or anomalies. The nervous tissue can adapt the muscles rectruitment to new oral conditions (without causing symptoms such as pain),[6] "masking" changes in other structures, for example, teeth, bones and joints. Occlusal proprioception requiring (asymptomatic) muscle adaptations could causes changes in the distribution of the occlusal forces causing the following major complications: 1) Functional. The adaptation capability is not constant between individuals (some patients may develop symptoms as a result of altered occlusion) and over time; 2) Biological. Mechanical forces play a role on bone biology; the bone apposition and resorption mechanisms are managed by chemical processes initiated by mechanical stimuli. It has been demonstrated how the bone modifies in order to support functional loading needs; 3) Mechanical. The prosthetic (crowns and implants) reliability is the result of the stress developed in artificial products. In this context the use of masticatory function instrumental evaluations (before and after therapies that modify dental occlusion) are suggested to support the clinician to quantify the effect of occlusal changes on the oral biology. In fact, the biomechanical studies show that each muscle has its own specific action vectors (or more than one) and that changes in the muscle forces distribution cause alterations of the mechanical stresses on the hard structures.[7] In conclusion, a reliable dental treatment include masticatory functional evaluation to ensure greater reliability and durability of homogeneously stressed oral rehabilitations.

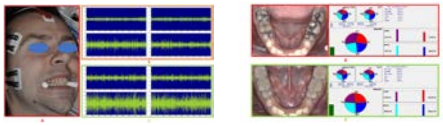

Fig.1.(Left) Masseter and Temporal sEmg analysis. A) electrode positioning, B) sEmg reference signal clenching on cotton rolls C) Maximal voluntary clenching sEmg signal.

Fig.2. (Right) Oral rehabilitation respecting masticatory muscles recruitment. A) Clinical situation and standardized muscles activity before treatment B) After treatment.

Key words. 3 to 5

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2022PDM3 Abstract 58

Back muscles training and balance therapy in rehabilitation of patients with osteoporotic vertebral fractures

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Medical rehabilitation of patients with osteoporotic vertebral fractures (VF) remains an insufficiently developed topic and requires additional research. Aim of the study was to assess the efficiency of back muscles training and balance therapy in rehabilitation of patients with osteoporotic vertebral fractures. Prospective, interventional, open-label, controlled study in two parallel groups, performed in inpatient department settings at "National Medical Research Center of Rehabilitation and Balneology" during 2018. The study involved 120 patients (11 men and 109 women) aged 40-80 (mean age 65.4±9.1 years) who were admitted for medical rehabilitation for systemic OP and VF. The rehabilitation program in the main group included: 1) Mechanotherapy on the Back-Therapy-Center Dr. Wolf complex with biofeedback (Germany); 2) Balance therapy on a double unstable COBS platform, with biofeedback (Germany); 3) Hydrokinesiotherapy in a pool; 4) Gymnastic exercises (Gorinevskaya-Dreving method). Results. The use of the three-week program of physical rehabilitation using mechanotherapy, balance therapy and special complexes of physiotherapy exercises in the gym and in the pool in patients with osteoporotic VF significantly increases the strength of the muscle corset, helps to eliminate the existing muscle deficit in TE and TF and results in a more physiological distribution of the strength ratio between TE and TF. The rehabilitation program improves the function of static and dynamic balance, both with closed and open eyes, which can be observed in the return of the center of gravity to a physiological position and in improved reaction speed to changes in body position. Usage of mechanotherapeutic methods in rehabilitation of patients with osteoporotic VF is effective for basic motor function improvement and disability reduction.

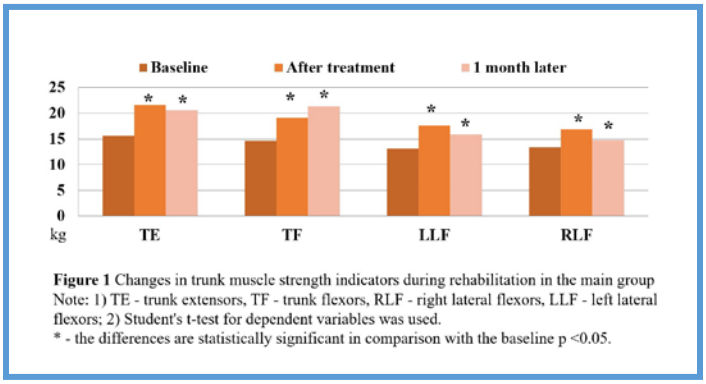

Figure 1 Changes in trunk muscle strength indicators during rehabilitation in the main group
Note: 1) TE - trunk extensors, TF - trunk flexors, RLF - right lateral flexors, LLF - left lateral flexors; 2) Student's t-test for dependent variables was used.
* - the differences are statistically significant in comparison with the baseline p <0.05.

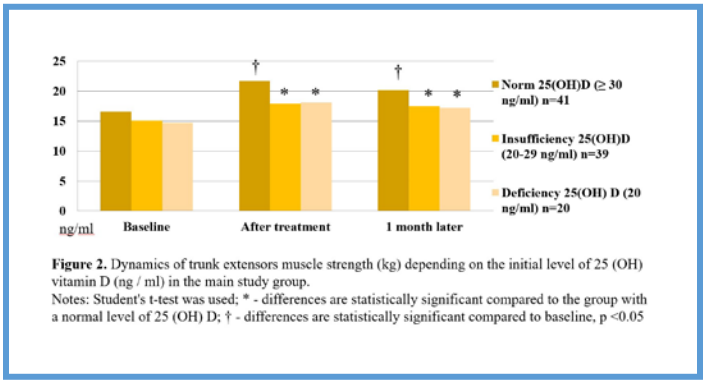

Figure 2. Dynamics of trunk extensors muscle strength (kg) depending on the initial level of 25 (OH) vitamin D (ng / ml) in the main study group.
Notes: Student's t-test was used; * - differences are statistically significant compared to the group with a normal level of 25 (OH) D; † - differences are statistically significant compared to baseline, p <0.05

Keywords: Rehabilitation; balance therapy; osteoporosis; active aging; physical therapy; muscle strength.

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2022PDM3 Abstract 59

Pathogenetic mechanism of Limb Girdle Muscular Dystrophy D2: functional characterization of Transportin 3 in cellular and animal models of disease

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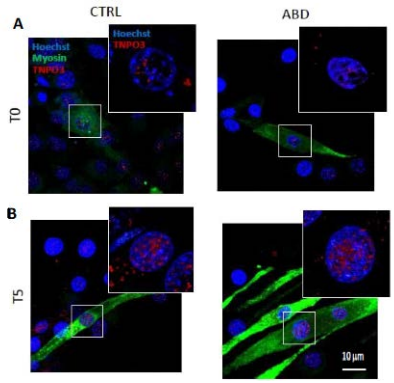

Fig.1 *Double immunoflorescence staining for TNPO3 (in red) and Myosin (in green) of C2C12 (CTRL left column) and C2C12 ABD (clone CRISPR/Cas9 right column) at different time of differentiation. A: T0 (undifferentiate cells) B: T5 (intermediate differentiation).*

Limb Girdle Muscular Dystrophy D2 (LGMD D2) is caused by a heterozygous mutation in the termination codon of the TNPO3 gene. This mutation gives a protein which is 15-aminoacids longer in its C-terminal domain. TNPO3 gene encodes for TNPO3, which normally mediates the translocation to the nucleus of SR proteins, a family of splicing factors and other proteins related to RNA metabolism. The goal of this work was to investigate the pathogenetic mechanism of LGMD D2 through a dual approach, in vitro and in vivo, focusing mainly on the role of TNPO3 in the myogenic process and in possible muscle-specific molecular pathways. In vitro murine C2C12 myoblasts were treated with CRISPR/Cas9 genome editing of TNPO3. Preliminary data suggest morphological, proliferative and expression changes of genes involved in myogenic differentiation in comparison to the C2C12 control line (Fig.1). For the in vivo approach, the microinjection of mRNA encoding human wild type (WT) and mutated TNPO3 in zebrafish embryos allowed to study its effects during embryonic development. We monitored the gene expression profiles of endogenous transportin 3, of myogenic regulatory factors (MRFs) and muscle-specific proteins, suggesting morphological and gene expression changes in embryos miscroinjected with the WT form and particularly in microinjected embryos with the mutated TNPO3 form. The approaches used are a first step to understand the role of TNPO3 in muscle physiology and in the pathogenetic mechanism underlying LGMD D2 which is still unknown.

Key words: LGMD D2; TNPO3; Zebrafish; myogenesis.

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2022PDM3 Abstract 60

Diaphragm ABCB6 overexpression preserves respiratory function following doxorubicin chemotherapy treatment

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Doxorubicin (DOX) is a highly effective chemotherapeutic agent used to treat a wide variety of cancers. However, its clinical use is associated with respiratory muscle weakness, resulting in fatigue, dyspnea and exercise intolerance.[1] While no therapeutic countermeasures exist to prevent the development of DOX-induced respiratory dysfunction, previous preclinical work has demonstrated that acute endurance exercise preconditioning prior to DOX administration was sufficient to preserve diaphragm muscle force production, maintain fiber cross-sectional area and prevent ventilatory deficits during a hypercapnic challenge. These protective effects were associated with the exercise-induced upregulation of the mitochondria-localized ATP-binding cassette transporters in the diaphragm, including ABCB6, which is hypothesized to play a role in maintaining mitochondrial redox balance and heme homeostasis.[2] To determine if ABCB6 overexpression, independent of exercise, is sufficient to prevent DOX-induced respiratory dysfunction, female Sprague-Dawley rats were randomly assigned to receive either rAAV9-MHCK7-rABCB6 to overexpress ABCB6 in the diaphragm or saline. These groups were further divided to receive saline or DOX (20mg/kg i.p.) treatment. Performance of an exercise tolerance test forty-eight hours following treatment revealed significant reductions in run time to fatigue in both DOX-treated groups. However, ventilatory function in response to hypercapnia was only reduced in the saline-saline rats compared to the saline-DOX rats. Additionally, ABCB6 overexpression attenuated the DOX-induced reduction in diaphragm muscle force production. These findings support a role for ABCB6 in the preservation of respiratory function following acute exposure to DOX.

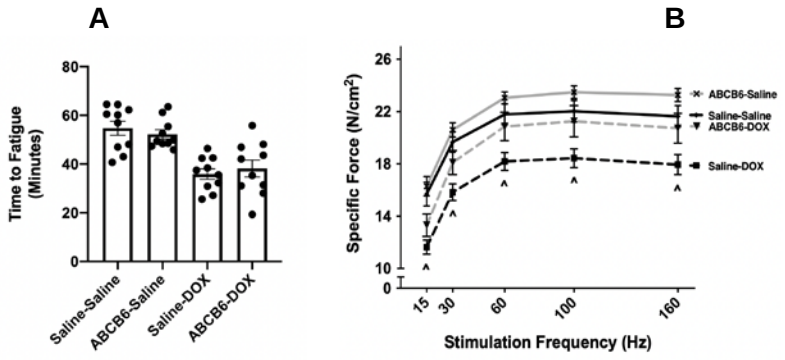

Fig 1. *Experiential animals received either Saline or rAAV-MHCK7-ABCB6-GFP (ABCB6). 4-weeks following administration, rats received an acute dose of either Saline or doxorubicin (DOX). An exercise tolerance test to fatigue (A) was performed and following euthanasia the diaphragm muscle force frequency response was assessed (B). Data is presented as mean +/- standard error. ^ Saline-DOX significantly different versus Saline-Saline and ABCB-Saline (P<0.05)*

Key words: Chemotherapy; diaphragm; respiratory; ABCB6; mitochondria.

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2022PDM3 Abstract 61

Study of correlations between neuromuscular occlusion and posturographic parameters in the elderly for falls prevention: a pilot study

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Previous researches have investigated the influence of occlusion on body posture for falls prevention reporting conflicting results. However, a few studies have explored the impact of the occlusion considering the neuromuscular component. Hence, the aim of this study was to investigate any correlations between EMG parameters of masseter and temporal muscles and posturographic parameters in elderly. We enrolled a number of 68 elderly people (16 female and 52 male) without fall history from the geriatric ambulatory clinic of the Department of Internal Medicine and Geriatrics of the University Hospital of Palermo (Italy). All participants were administered a surface EMG assessment of masseter and temporal muscles during a maximum natural intercuspation through a wireless device capable of measure the balancing of dental occlusion (Teethan, Garbagnate Milanese, Milano, Italia) and a baropodometric evaluation to assess plantar pressure in orthostatic position using a baropodometric platform (FreeStep, Sensor Medica, Guidonia Montecelio, Roma, Italia).

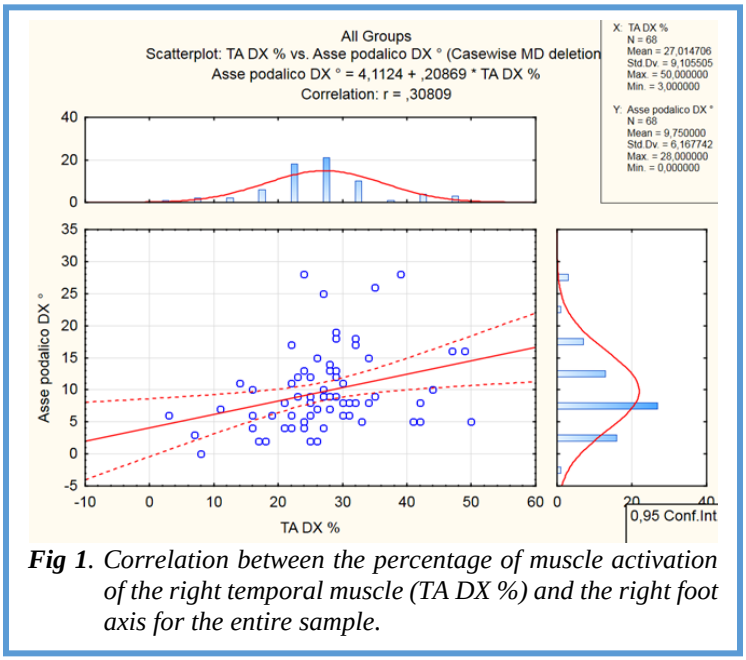

***Fig 1**. Correlation between the percentage of muscle activation of the right temporal muscle (TA DX %) and the right foot axis for the entire sample.*

Our results showed a positive correlation between the percentage of muscle activation of the right temporal muscle and the percentage of pressure on the right forefoot in male participants ($r=0.57$, $p<0.05$) and a positive correlation between the percentage of muscle activation of the right temporal muscle and the percentage of pressure on the right rearfoot in female participants ($r=0.29$, $p<0.05$). Moreover, for the entire sample, we found a positive correlation between the percentage of muscle activation of the right temporal muscle and the right foot axis ($r=0.31$, $p<0.05$). Although further studies are needed, our findings suggest a relationship between plantar pressure and neuromuscular occlusion in elderly without fall history and these may have an implication on falls.

Key Words: Neuromuscular occlusion; temporal muscle; masseter muscle; body posture; plantar pressure.

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2022PDM3 Abstract 62

Masters studies in Padua to honor the legacy of Paolo Gava

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Aging behaviors differ in females and males. Females are weaker, but survive longer. Paolo Gava described the gender decay of muscle performance by comparing the world records of Masters athletes. Masters compete in age groups of 5 from 35 to 100 or older. After normalizing - that is, taking the all-time world record of young sports athletes to 100% - the women's and men's world records are lists of 16 data showing declining trends in muscle performance as aging, indicating only minimal gender differences in the process. All trend lines tend to zero at around 110 years. The decline of short running disciplines is very similar. It is smooth from 30 to 50 years, it is almost linear from 50 to 70 years, then the decay is progressively steeper. Since gender hormones appear to have little influence on the decay of aging performance, other key bioregulators, such as those of cellular energy metabolism, appear to be more relevant.

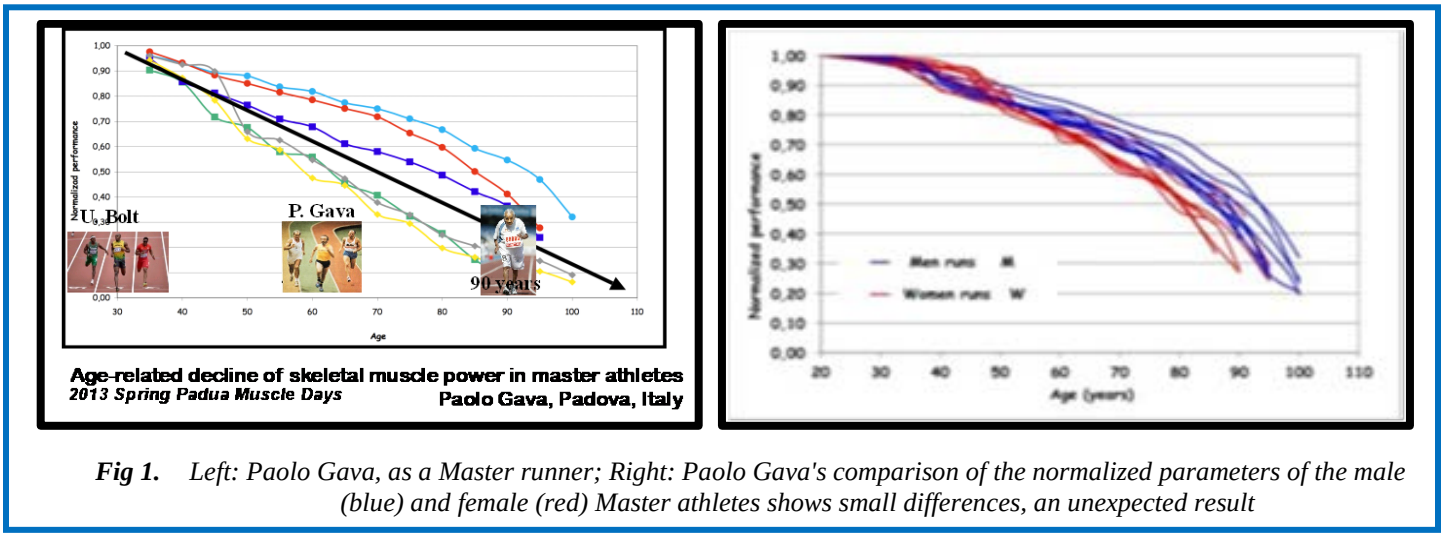

Fig 1. *Left: Paolo Gava, as a Master runner; Right: Paolo Gava's comparison of the normalized parameters of the male (blue) and female (red) Master athletes shows small differences, an unexpected result*

Key Words: Aging performance decay; Masters world records; gender differences.

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2022PDM3 Abstract 63

Cytosolic calcium as intracellular signal: local and average concentrations and their variations in relation to release from SR

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Calcium signaling is the most important pathway which regulates muscle contraction and relaxation. The knowledge of its spatial and temporal distribution among the major compartments in the muscle cells, namely cytosol, sarcoplasmic reticulum, but also mitochondria and T tubular extracellular space, is crucial to drive our understanding in physiological and pathological behavior in muscle mechanics. Despite a general agreement in the fluxes of calcium in skeletal muscle at the qualitative level, there are several open questions in the literature regarding a quantitative assessment of its concentration both in average and temporally/spatially resolved. We approach this problem through a three-dimensional diffusional model of calcium fluxes inside a half sarcomere, based on experimental data on FDB in WT mice.[1] We first estimate the citosolic [Ca2+] comparing the signal of our Fura-2 indicator during a twitch and during a 60Hz stimulation, accounting for the kinetic term through the Schneider equation,[2] with the simulated ratio of occupation of the two Ca-binding sites of the Troponin in the model, and the experimental tension developed by intact muscle fibers. Based on this data, we used the model to assess the relative amount of calcium which enter the mitochondria, which enter from T tubules through SOCE and which exit from the cell through PMCA and NCX. We extended our model to study the calcium fluxes in the parvalbumin knock-out mice, considering two different sets of experimental data recently published.[3,4] In particular we estimated the possible fate of the calcium released from the sarcoplasmic reticulum in the absence of the major cytosolic buffer and showed the contribution of the export to extracellular space and the uptake into mitochondrial matrix to stabilize the cytosolic concentration.

ONE FIGURE, PLEASE

Fig 17. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Keywords: ……..; ……; …… ……..; …… ……..; ……. .

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2022PDM3 Abstract 64

Higher abundance of deletions and strand break damage within specific mitochondrial ETC genes are associated with functional performance in older adults.

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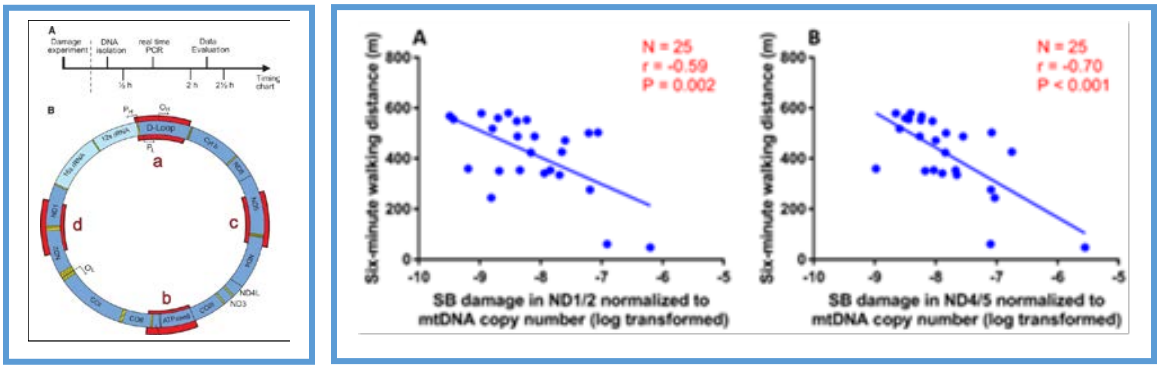
The goal of this study was to investigate if higher abundance of deletions and strand break damage within specific Mt ETC genes are associated with functional performance in older adults. Healthy older adults (65.2 ± 6.7 years; n = 25) were enrolled in this study(1). The participants completed a 6-minute walk test with an average 6-minute walking distance of 420 (± 149) meters and a usual-paced walking speed over 4 meters of 0.86 (± 0.28) meters per second. We quantified in muscle-biopsy specimen the abundance of mtDNA deletion and SB damage and associated these with the recorded walking speed. Specifically, mtDNA regions were selected based on previous findings from our group as potential hotspots for damage (2). We determined (a) the abundance of the 4977 bp deletion, a common deletion present in human mtDNA, which results in loss of a mtDNA fragment that includes genes coding for ATPase6, ATPase8, cytochrome oxidase III, NADH dehydrogenase subunit 3 (ND3), ND4, ND4 subunit L (ND4L), and ND5; and (b) the abundance of mtDNA strand break damage within specific mtDNA regions that code for subunits of Complex I (ND1, ND2, ND4, ND5), Complex III (Cytochrome B6), Complex IV (Cytochrome Oxidase subunit II), and Complex V (ATPase subunit 6/8), as well as SB in the regulatory non-coding D-loop region. Please note that the highly sensitive mtDNA strand break method spans through regions coding for several ETC genes. We plotted the robust correlations of mitochondrial SBs in specific mtDNA regions with walking performance in 25 older adults (Fig). For example, a greater abundance of density of mtDNA SB damage (SB damage normalized to mtDNA copy number) within NADH dehydrogenase subunit 1/2 (ND1/2) and 4/5 (ND4/5) was strongly associated with poor walking performance (r = -0.59 and r = -0.70). Our data showed the powerful association and sensitivity of mtDNA SB in specific Mt ETC regions with walking performance. Conclusio n: This set of data indicates that mtDNA mutations in ETC components (strand breaks to ND1/2 and ND4/5) are strongly related to slower walking speed in older adults.



Keywords: Sarcopenia, Mitochondria, Mutations, Deletions Mt genome.

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2022PDM3 Abstract 65

Epidermic Langerhans cells in upper or lower motor neuron traumatic SCI: Effects of NMES or h-bFES

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Innate and adaptive immune responses are deeply intertwined in the epidermis, that represents the first line of defense of our body against outsid agents, and numerous cell populations work together to achieve an effective and protective immunity. This study investigated changes in Langerhans cells, inpatients with traumatic injuries of lower and upper motor neurons in the spinal cord (SCI). In a previous work we shown that the use of functional electrical stimulation at home (h-bFES) for two years was able to improve muscle and epidermis atrophy of patients with lower motor neuron lesions (LMN SCI), however the number of Langerhans cells did not show substantial improvement. In this study, the analysis is extended to patients with upper motor neuron injuries (UMN SCI). To evaluate the possible variation in Langerhans cell density and epidermis atrophy in patients with long-term traumatic SCI, before and after two years of superficial electrical stimulation in two groups of patients suffering from complete lesions of the lower motor neuron or upper motor neuron. Skin biopsies were analyzed, before and after two years of h-bFES. In both cases, it was performed through application of anatomically shaped electrodes on the anterior surface of the thigh, with current intensity suited to the patho-physiological condition of the muscles and their innervation. Specifically, by neuromuscular electrical stimulation (NMES) in UMN SCI and in LMN SCI by high current h-bFES for chronically denervated muscles. By specific histological and immunohistochemical stains it was possible to evaluate number of Langerhans cells together with epidermis atrophy. At the end of the stimulation period, there was no statistically significant change in the number of Langerhans cells and in the thickness of the epidermis in the three patients with UMN SCI treated with NMES. However, comparing the data of UMN SCI with those of LMN SCI patients even within one year from trauma, the number of Langerhans cells in LMN SCI patients is nearly half than of those of UMN-SCI. despite the epidermis thickness being almost the same. These findings suggest that LMN SCI lesions can induce an early decay of the epidermis' immune defenses, which can persist for up to ten years after trauma. In future, studies of other cellular components of the innate and adaptive immune responses may better investigate the clinical implications of the present preliminary results.

ONE FIGURE, PLEASE

Fig 18. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: Epidermis; Langerhans cells; lower or upper motoneuron; spinal cord injury; effects of NMES or h-bFES.

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2022PDM3 Abstract 66

Heatwaves in a warming climate: overview and impacts.

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One of the effects of the global warming is the increase of heatwaves in terms of intensity, frequency, and duration. While global warming is a worldwide concern, heatwaves is a local issue depending on land use change, urbanization, orography, and meteorological conditions.[1,2] Even the local specificity of heatwave, in almost all regions a measure of cumulative heat shows significant increases since the 1950s, mainly driven by heatwave days.[3] Understanding past decades heatwave trends and future projection have strong implications to know the impact on human health, on ecosystems and on infrastructure planning.[4] Here we will show how temperature is changing at different scales from global to local,[5] focusing on the formation and evolution of urban heat islands. The analysis will cover the last 200 years of data and projections of future trends. Finally, will be discuss the impacts of heatwaves on ecosystems and human health pointing out drivers, mitigation, and adaptation strategies.

ONE FIGURE, PLEASE

Fig 19. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: ……..; ……..; ……..; …… ……..; …… .

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2022PDM3 Abstract 67

Deciphering the cachexia-inducing signature

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More than half of cancer patients are facing with a multifactorial syndrome characterized by excessive body weight loss, due to fat and lean mass loss, named cachexia (1). The debilitating frailty experienced by cachectic individuals impairs quality of life increases morbidity and complications from cancer surgery, reduces both tolerance and responsiveness to cancer treatment regimes, complicating patient management, and ultimately accounting for up to 30% of deaths associated with advanced cancer (2, 3). As the underlying mechanisms of this multifactorial syndrome are incompletely defined, effective therapeutics have yet to be developed. The loss of muscle mass and strength is considered the most important clinical event in cancer cachexia, and a key predictor of poor outcomes (4, 5) However, cachexia is not an inevitable consequence of cancer. Variation has been noted with regards to the prevalence and severity of cachexia and the causes of such variation are unknown. Cachexia onset is the result of the interaction between cancer cells and the organism that hosts cancer growth. Tumor's secreted pro-inflammatory factors depend from the type of cancer cells and from the interaction with organisms' cells. The combinatorial action of soluble secreted mediators (secretome) by cancer cells and cells within the tumor microenvironment, activates different catabolic signaling pathways within different tissues, including skeletal muscle. The signatures present in one organism could explain inter-cancer and inter-individual variations in susceptibility to cachexia.

ONE FIGURE, PLEASE

Fig 20. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key Words: Cancer cachexia; muscle wasting; secreted mediators; signaling pathways.

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2022PDM3 Abstract 68

Neuromuscular control in the neck muscles in patients suffering from whiplash associated disorders and traumatic brain injury

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Neck pain is an increasing healthcare problem throughout the developed world and is the fourth leading cause of disability globally. It can be difficult to diagnose and treat effectively because of the complex pathophysiology and variable presentation between patients. Misdiagnosed and improperly treated neck pain can develop into chronic conditions that last for years after initial onset. Despite a rise in the incidence of neck pain (21% globally between 2005 and 2015),[1] there is no standardized methodology to objectively define the underlying pathology that is the cause of the pain. The Icelandic startup company, NeckCare, had developed a system, measuring the kinematic variables of the head and neck.[2] The three main pillars of clinical assessment are: i) **Proprioception**: Measures the deviation in degrees from the initial neutral position of the head after a blindfolded subject has rotated the head to the left/right/flexion/extension and back to find again the initial position of the head; ii) **Neuromuscular control (The Butterfly test)**: Measures the movement control of the head. The subject controls a cross on the screen and the objective is to chase a moving target. The outcome measures are average distance away from the target, the percentage of time spent on the target and the smoothness of the movement. The Butterfly trajectories are divided into three difficulty levels that come up in random order and the patient will only see a single point moving and not the overall trajectory. The trajectories along the measurements for the two groups can be seen in Fig. 1; iii) **Range of motion**: Measures the overall range of motion in each plane. The outcome measures are in degrees. Measurements were taken of a control group and patients suffering from whiplash associated disorders (WAD). The measurements are carried out so that the subject was sitting in a chair 90cm in front a computer monitor that was adjusted so that the middle of the screen was at eye level. A movement sensor was placed on the head that connected via Bluetooth to a computer. The movement sensor used an IMU sensor which calculates the 3D Euler angles of the head and projects them onto the monitor for a visual feedback. The results demonstrated that there was a significant difference between the neuromuscular control of the control group and the whiplash group in the Butterfly test. The whiplash group was on average further away from the moving target and spent less time in the vicinity of it than the control group. The whiplash group had less range of motion in all planes and poorer proprioceptive control. These tests can help to identify the muscular impairment for patients suffering from whiplash associated disorders and can be used in the rehabilitation of these patients to activate the deep neck flexor muscles rather than using the larger superficial muscles of the neck.

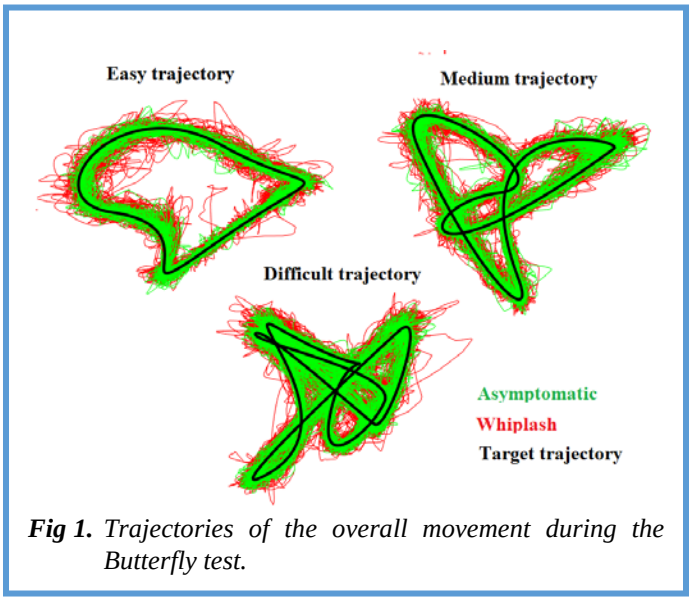

Fig 1. *Trajectories of the overall movement during the Butterfly test.*

Keywords: Neck muscles, Neuromuscular control, Rehabilitation, Head kinematics.

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2022PDM3 Abstract 69

Can aqua exercises in fresh water improve the gait stereotype function in patients with a neurological disease?

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An open descriptive study was carried out, which involved 12 patients (7 men, 5 women) aged between 40 and 62 years, with disorders of maintaining the vertical posture, which correspond to the functional diagnosis coded according to ICF “Gait Stereotype Function” b770.1 – minor disorders (5–24%). The test group (n = 12), in addition to basic therapy and exercises with a physiotherapy instructor, did aqua exercises in fresh water for two weeks (30 minutes, 6 days a week). Evaluation technique: the study before and after the rehabilitation course was carried out using “Habilect” video analysis system. To assess the significance of differences between the groups of qualitative variables $\chi 2$ test was used. To assess the significance of differences of quantitative variables of the two test groups Wilcoxon rank sum test was used. The significance of differences is considered to be established at $p<0, 05$. The study calculated the amplitudes of deviations of the body and the head along X, Y, Z axes before and after the exercises, as well as deviations of the body motion vector before and after the exercises. While calculating the increase of deviation (deviations of the main body axes from the reference value) using Wilcoxon rank sum test, statistically significant deviations along the X-axis were found (an increase by 306,5%, p=0,0504), along the Z-axis (an increase by 112,68%, p=0,0225) and the Body Angle parameter (an increase by 1973,86% p=0,0323). While calculating the increase of deviations of the head axis from the reference value using Wilcoxon rank sum test, statistically significant deviations along the X-axis were found (an increase by 163,04%, p=0,0280), along the Y-axis (an increase by 85,71%, p=0,0199) and Z-parameter (an increase by 173,34% p=0,0292) Thus, aqua exercises, due to a decrease of the deviations amplitude along the three axes (Z, Y, X), contribute to the adjustment of disorders of maintaining the vertical posture, a statistically significant decrease in the amplitude of head and neck movements.

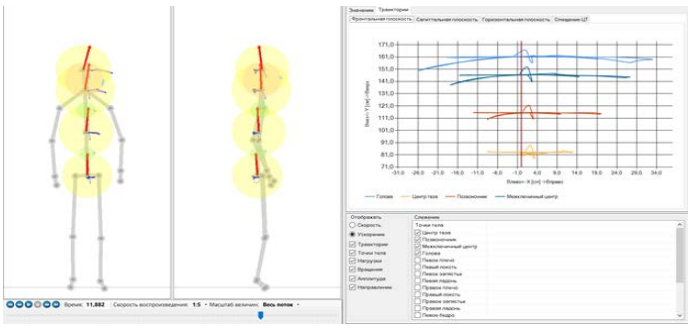

Fig 1. *Schematic representation of measurement points, size and deviation of the main directions (vectors) of movements (left), trajectory of movement of the main parts of the body (right) [https://habilect.com/hmotionlab].*

Key words: Movement; exercise therapy; gait; posture.

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2022PDM3 Abstract 70

Head and neck functional analysis: the Functional Anatomy Research Center experience

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The physiological functions of the human body represent the expression of biological phenomena easily observable, but hardly quantifiable in their entirety as they result from the dynamic interaction of multiple factors. Each phenomenon may present in different ways through a different combination of the same parameters that characterize it at different times. Therefore, the evaluation of a biological phenomenon implies the possibility of measuring only ONE or more aspects, and not the whole complexity. The same stimulus can in fact elicit different responses in the same individual in relation to time and environment. It is essential to associate the measurement of the phenomenon with a series of numbers that allow its interpretation, temporal evolution and comparison with other phenomena. In the scientific field, the numerical description of biological functions needs reliable instruments that provide accurate and repeatable values of one or more characterizing parameters. The response to the stimulus is not solely determined: the input-output relationship can lead to the same result by exploiting different paths. Hence, despite the knowledge of the input and the output, the biological phenomenon is not necessarily known. Each individual owns a specific adaptability to the different stimuli that undergoes fluctuations over time. When an input exceeds the adaptability threshold, unexpected and previously undetectable problems may be generated. In the clinical dental field, it is necessary to quantify the function and/ or dysfunction and the need of intervention to restore the functional alteration, by means of objective measures and not only of patient subjective symptoms. It is crucial to set instrumental tests with dedicated protocols that allow to measure the stability of the occlusal relationships both in static and dynamic conditions and to uniquely study the experimentally-induced variations. The evaluation should integrate the functional aspects with the morphological ones at micro and macrostructural levels. The research activity of the Functional Anatomy Laboratory of the stomatognathic apparatus (Functional Anatomy Research Center- FARC- University of Milan) has been developed aiming at defining standardized indices that measure the overall activity of the chewing muscles. Strength, recruitment and coordination of muscles are gathered in order to identify the impact of various occlusal conditions on the masticatory synergism and possible effects on other districts, primarily on the neck. The FARC 30-years-experience applied innovative non-invasive technologies (electro magnetic and electromechanical digitizers, optical morphometric methods, surface electromyographs, dynamometers, etc.) for the creation of protocols that clinician could use to measure the chewing function in each individual patient in a simple way, testing its fluctuations over time.

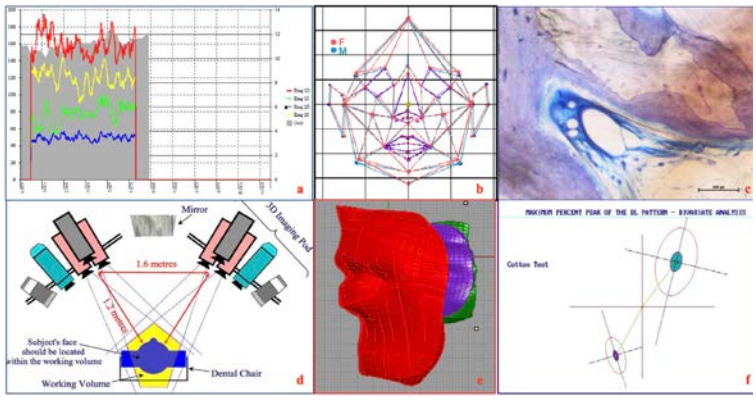

Fig 1. *Trajectories of the overall movement during the Butterfly test.*

Key words: Physiological functions; scientific instrumentation; biological measures; masticatory muscles activity.

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2022PDM3 Abstract 71

Integration of Motion, Forces, and the Central Nervous System

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Movement is encoded by spinal motor units. The motor unit is formed by a motoneuron, and the muscle fibres innervated by its axon. The motoneuron receives efferent and afferent synaptic inputs that modulate the force output of the muscle. There is very little knowledge on the changes in motor unit behaviour during natural dynamic movements consisting of changes in muscle length and neural drive. Moreover, the associations between kinematics, kinetics and motor unit dynamics are largely unknown. Here we aimed to explore these associations with a linear and nonlinear method. With the linear method, we decomposed high-density EMG signals into constituent motor unit action potentials during dynamic and isometric hand movements. With the nonlinear method, we built a framework with digital cameras that recorded the movements of the hand as well as recording isometric forces with an instrumented circular object. Deep neural networks were trained to understand the exact position of the hand from the digital cameras as well as from the high-density EMG signals. The movements consisted of more than 20 degrees of freedom, ranging from individual and combined finger flexion/extensions, and different hand and wrist gestures at different speeds (0.5 and 1.5 Hz). Four hundred EMG channels recorded the activity of the forearm muscles. The non-linear method was able to identify with 99.6% accuracy the exact movement of the fingers. The decomposition of the electromyogram revealed unique motor unit firing patterns that were significantly different than the control during isometric contractions. The delays between the onset of neural activity and movement kinematics were similar across all fingers and decreased with the speed of force contraction suggesting that the central nervous system compensates the muscle dynamics with unique control strategies for each finger. Because the hand digits were flexed and extended at various speeds, our interface shows that the dynamical output of the hand is fully explained by the neural strategies and that the musculotendinous unit has only a passive role for the rate of speed production

ONE FIGURE, PLEASE

Fig 21. *Western blot of dysferlin (upper line) and post-transfer myosin heavy chains (not separated) stain*

AND / OR ONE TABLE

Table 1. *Content" of dysferlin in fast skeletal muscle, i.e. biceps brachii (BB), compared to slow, i.e. tibialis anterior (TA)*

Key words: 3 to 5

References

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2022PDM3 Abstract 72

Mental health disorders of relatives of oncohematological patients

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The aim of the study is to study the features of the mental health of the closest relatives of oncohematological patients. The sample consisted of 274 subjects aged 18 to 65 years. The experimental groups were patients and their closest relatives at different periods of the course of the disease: at the onset, at three and six months. The number of people in the sample under consideration progressively decreased due to the mortality rate of patients with the underlying disease (92 at the onset of the disease to 70 people on each side at the end of the study). The following research methods were used: clinical and psychological (a conversation aimed at collecting psychological anamnesis and identifying biosocial characteristics of patients, assessment of the current mental status, assessment of the severity of the condition-the Karnovsky index); testing (methods SF-36, MFA-20, 4DSQ, coping behavior in stressful situations by Norman S.); instrumental studies (assessment of the Kerdo vegetative index); mathematical and statistical. The co-dependent relationships and the influence of cancer patients and their close relatives on the mental health of the latter during the six months of the development of hemoblastosis were studied. First identified predictors of mental health of the next of kin hematooncology at different stages of the disease of acute leukemia. New data on the structure and dynamics of the influence of various factors on the mental health of relatives of oncohematological patients through latent variables were obtained. The variants of disadaptation of the closest relatives of oncohematological patients during the first six months of the disease course are indicated. For the first time, a comprehensive approach to the problem of providing medical and psychological care is justified, including the mental health of the closest relatives of oncohematological patients in relation to the psychoemotional status of patients with hemoblastoses. The mechanisms of functioning of relatives of oncohematological patients, which have an impact on their mental health, are revealed, which is of differential importance in correctional and psychotherapeutic practice.

Key words: Relatives of cancer patients; distress; coning strategies; mental health; anxiety; depression; codependent relationships; quality of life; oncology; psychotherapy.

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2022PDM3 Abstract 73

The state of helplessness in preschool children with mental retardation and its correction

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The article deals with the problem of helplessness in preschool children with mental retardation (ANW). Became widely known studies of so-called "learned helplessness" Seligmena, in the national psychology studies "of personal helplessness" Ciring, etc., but work in assessing the role and place of helplessness (just helplessness, learned, personal) in preschool children in general and delayed mental development in particular is virtually non-existent. At the base of the research based on the ideas of Vygotsky about the features of development of children, as well as whole-value approach and the biopsychosocionoetic model of human nature, its health and development, proposed by one of the authors of the article. The authors put forward a hypothesis to test done quite voluminous study of the phenomenon of helplessness, which brought together 140 children and their families (mothers).Of these, 90 children with CRA and 50 without such a diagnosis. 45 children with CRA entered the pilot group, which on the second stage of the study, participated in the psychocorrective program proposed by the authors of the study. The monitoring group comprised 45 children with CRA and 50 children without the CRA. Based on the quantitative and qualitative analyses of the results obtained was differentially 3 groups of children with varying degrees of severity, the condition of helplessness: no signs of the condition of helplessness, with moderately expressed signs of helplessness and the lack thereof. The results of the second cut after the implementation of the program of psychocorrective indicate the possibility, under certain conditions, absorb any movement state of helplessness.

Key words: Helplessness; learned helplessness; personal helplessness; children of pre-school age; development; techniques; attitude; family; parents; psychological support.

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2022PDM3 Abstract 74

New molecular network identified in Amyotrophic Lateral Sclerosis reveals microRNAs involved in the neuromuscular junction development

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Amyotrophic lateral sclerosis (ALS) is a neuromuscular disorder of unknown aetiology, orphan of effective therapies (Kiernan, et al 2021)[1]. Previous studies demonstrated that aberrant gene expression of myofibers can be involved in the cause of this disease (Scaricamazza et al, 2021; Fischer et al, 2004).[2,3] Thus, to understand gene expression regulation can provide new insights for more efficient treatments. We analyzed gene expression in gastrocnemius muscle of SOD1(G93A) ALS mouse model during pathology progression. We identified 669 mRNAs and 85 miRNAs differentially expressed. Gene Ontology analysis revealed that different cellular functions are altered in ALS such as: apoptotic processes, negative transcription regulation and nervous system development. We focused our attention on this last function identifying 3 miRNAs (miR-193a-3p, 193b-3p and 152-3p) that regulate NMJ function targeting Chrna1, Chrnb1, Chrnd, Chrne, Chrng, Emb, Musk and Prima1 (Fig. 1). After the in vivo modulation of these miRNAs we evidenced a reduced expression of their targets and a reduction of gastrocnemius and tibialis anterior muscle mass. In association with these observations we evidenced a fast to slow fiber shift with a reduction in the total number of NMJs (Fig. 2) and in the amplitude of evocated potentials. These results support the role of 3 miRNAs in the maintenance of NMJ function and in the regulation of skeletal muscle atrophy.

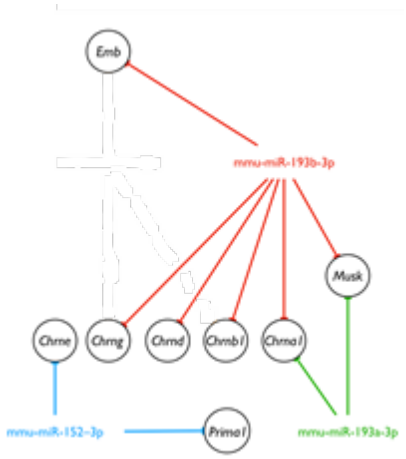

Fig 1. *Network of miRNAs and their targets altered in ALS. These genes are involved in the regulation of neuromuscular junction functions.*

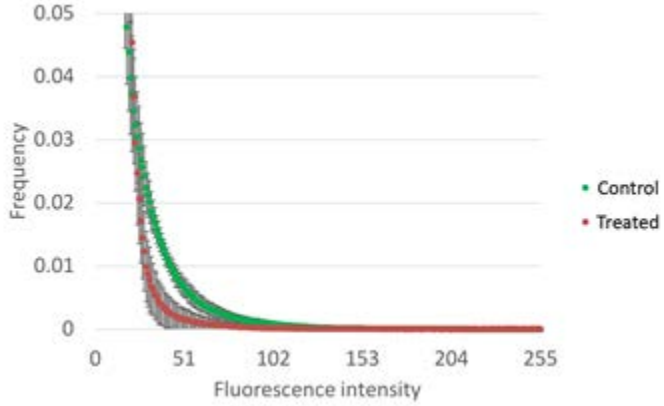

Fig 2. *Fluorescence intensity associated to NMJs. NMJs were labelled with alpha-bungarotoxin associated with a fluorophore and scanned under the microscope. Mice where miRNAs were upregulated showed a decreased labeling of NMJs, associated with a decreased expression of acetylcholine receptors.*

Key words: Amyotrophic Lateral Sclerosis; miRNAs; neuromuscular junction; Acetylcholine receptor.

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2022PDM3 Abstract 75

Skeletal muscle tissue restoration using functionalized biomaterials

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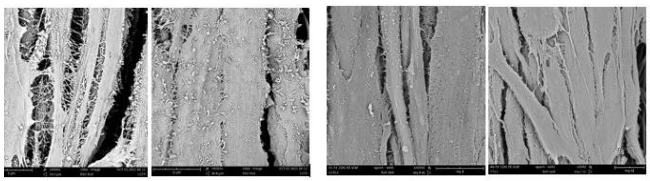
Skeletal muscle (SKM) loss is considered to be largely irreversible and therapeutic options to restore muscle mass have been limited. Frequent reasons for SKM loss are injuries, surgery, aging, metabolic diseases, as diabetes, and inherited genetic diseases. SKM has good regenerative capacity due to the presence of resident stem cells, the satellite cells, located between the cell membrane and the basal lamina, however *in vitro* studies demonstrated their poor ability to migrate and to self-renewal in situ: that means their intrinsic proliferative capacity is lost in normal or suitable cell culture. New strategies are required to overcome these limitations; recently, various studies have focused on recreating the niche-like environment combining biomaterials (tissue engineering) and cells (cell-based therapy). Tissue engineering (TE), in particular, has been recognized as a promising tool and a new therapeutic approach for the renewal of SKM tissue. Biomaterials for SKM TE designed in this work are able to persist long enough to support functional SKM tissue formation, while degrading gradually. The device developed is a biocompatible scaffold composed of aligned nanofibers obtained through the electrospinning technique. The alignment provides the anisotropy of SKM tissue mimicking its natural morphology. Moreover, the scaffold acts as carrier for cell vitality, proliferation and myogenic differentiation. Nanofibrous scaffold endows SKM tissue morphogenesis into parallel-oriented myotubes, promoting early cell cycle exit and contributing to an early myoblast differentiation and fusion into myotubes in comparison to conventional monolayer myoblast culture.



T 1 ***T 5*** ***T 7*** ***T 10***

Fig 22. *SEM analysis performed on myoblast cultured on nanofibrous scaffold at different time points T1 (early differentiation), T5 (intermediate differentiation), T7 (late differentiation), T10 (late differentiation).*

Key words: Skeletal muscle; Myogenesis; Tissue engineering; Regenerative Medicine

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2022PDM3 Abstract 76

The co-administration of simvastatin does not boost the benefit of gene therapy in the mdx mouse model for Duchenne muscular dystrophy

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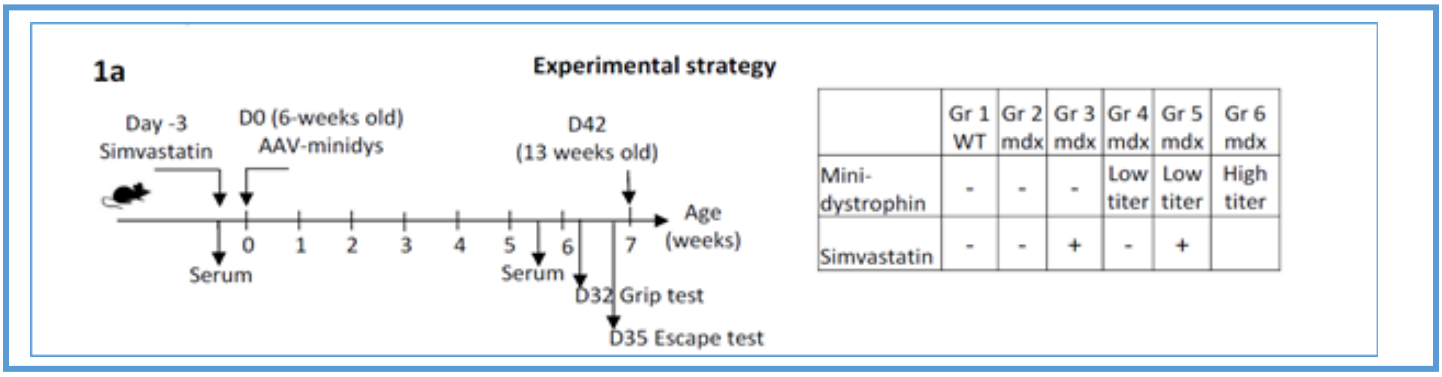
Duchenne muscular dystrophy (DMD) is the most common and a cureless muscle pediatric genetic disease, which is caused by the lack or the drastically reduced expression of dystrophin. Experimental therapeutic approaches for DMD are mainly focused on attempts to restore the expression of dystrophin. While significant progresses were achieved, therapeutic benefit of treated patients is still unsatisfactory. Efficiency in gene therapy for DMD is hampered not only by incompletely resolved technical issues, but likely also due to the progressive nature of DMD. It is indeed suspected that some of the secondary pathologies, which are evolving over time in DMD patients, are not fully corrected by the restoration of dystrophin expression. We recently identified perturbations of the mevalonate pathway and of cholesterol metabolism in DMD patients. Taking advantage of the mdx model for DMD, we then demonstrated that some of these perturbations are improved by treatment with the cholesterol-lowering drug, simvastatin.[1] In the present investigation, we tested whether the combination of the restoration of dystrophin expression with simvastatin treatment, could have an additive beneficial effect in the mdx model. We confirmed the positive effects of minidystrophin, and of simvastatin, when administrated separately, but detected no additive effect by their combination. Thus, the present study does not support an additive beneficial effect by combining dystrophin restoration with a metabolic normalization by simvastatin.



	Gr 1 WT	Gr 2 mdx	Gr 3 mdx	Gr 4 mdx	Gr 5 mdx	Gr 6 mdx
Mini-dystrophin	-	-	-	Low titer	Low titer	High titer
Simvastatin	-	-	+	-	+	

Key words: Duchenne Muscular dystrophy; Simvastatin; Gene therapy; AAV; minidystrophin; microdystrophin combined therapy; lipid metabolism.

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2022PDM3 Abstract 77

Metabolic dysfunction and exercise preconditioning in disuse muscle atrophy

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Disuse induced loss of muscle mass is a frequent phenomenon and a very relevant clinical problem. It exposes subjects to falls and fractures and favour further deconditioning. It is related to metabolic alterations, such as insulin resistance, and to low grade chronic inflammation which are among the major risk factors of chronic diseases. It worsens the prognosis of many chronic diseases 1, which benefit from exercise training.
We studied structural, functional and proteomic adaptations of skeletal muscle and the underlying cellular and molecular mechanisms induced by spaceflight in humans and by simulated microgravity both in humans, bed rest (BR) and unilateral lower limb suspension (ULLS), and in mice, hindlimb unloading (HU). As expected, in both human and mice models we found muscle atrophy and a slow to fast myosin isoform shift. Moreover, a downregulation of most myofibrillar proteins was observed by 2D proteomic and of myosin by quantitative one-dimensional electrophoresis.
To address the mechanisms of such adaptations, we studied intracellular signalling pathways controlling muscle mass and metabolism. In human bed rest and mice HU, we observed: upregulation of NRF2, a transcription factor sensing reactive oxygen species (ROS) in the cell; adaptations of major ROS buffering systems (e.g., SOD, catalase, peroxiredoxins, Hsp); protein oxidation. Such findings were consistent with a potential role of redox imbalance in causing muscle wasting as suggested by several previous findings. To test such hypothesis, we administered trolox, a potent antioxidant, to HU mice. Importantly, NRF2 was not up-regulated and no protein oxidation was found, indicating that redox imbalance was prevented by trolox. However, expressions of MuRF-1 and atrogin-1, major ubiquitin ligases of the ubiquitin proteasome system, were still up-regulated and muscle atrophy was not prevented by trolox. Therefore, redox imbalance was not likely to cause muscle atrophy, at least in mice.
Importantly, in human BR and ULLS and in mice HU, proteomic analysis indicated downregulation of both aerobic and anaerobic metabolic enzymes, i.e., a general derangement of energy metabolism. In human BR and mice HU, PGC1α, an activator of transcription controlling mitochondrial dynamics and biogenesis and inhibiting the ubiquitin proteasome system, was downregulated and expression of proteins involved in mitochondrial dynamics was altered. On such ground, we hypothesized that a metabolic program of muscle atrophy could be activated in disuse. To test such hypothesis, we subjected transgenic mice selectively overexpressing PGC1α in muscle to HU. In both soleus and gastrocnemius muscles of such mice, no upregulation of atrogenes expression was observed following HU and muscle atrophy was almost completely prevented confirming a major role of a metabolic program in disuse muscle atrophy.
Exercise is an attractive tool to prevent muscle wasting as it is known to cause mitochondrial adaptations and to enhance PCG1α expression. Few, but encouraging evidences on the use of exercise as a tool to prevent disuse atrophy were published. The mechanism of exercise-induced protection on muscle atrophy appears not clearly defined yet. We will present novel data on the mechanisms underlying prevention of muscle atrophy by exercise preconditioning.
Acknowledgements: Funding by ASI, MARS-PRE Project, PRIN Project 2017CBF8NJ.

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2022PDM3 Abstract 78

Energy balance and skeletal muscle in microgravity

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Energy balance maintenance is crucial to preserve astronaut's wellness during longduration-spaceflight. Nutritional intervention can counteract the detrimental effect of microgravity on body composition and metabolism. Methods and procedures: NutrISS is an Italian Space Agency biomedical experiment. This proof-of-concept study aim to monitor the body composition of astronauts and to provide nutritional advice during long-term mission. The study has been executed on the ISS and sponsored by ESA. The payloads selected for both missions (i.e. "BEYOND" and "COSMIC KISS") result from a public-call funded and coordinated by ASI, in the frame of its national mission of promoting and fostering the culture of space across the Country and providing access to the ISS as a laboratory in space to the Italian research community. The Italian astronaut Luca Parmitano already completed the protocol during his 6-month spaceflight. While Mathias Maurer is now starting the firsts measurements. Each astronaut underwent to baseline data collections, i.e. body composition, anthropometric evaluation and energy and metabolic assessment carried out by the scientific team. Body composition has been assessed by a BioImpedance Analyser device (Akern) modified by Kayser Italia. To maintain the near-neutral energy balance, the NutISS team monitored monthly the astronaut body mass during flight. Results are now classified for privacy reasons. The study was extended since collected data improved physical performance and quality of life of the astronaut during spaceflight and optimize the astronaut recovery-phases on Earth after landing. Obtained experimental data could have an important impact both on astronaut quality of life and clinical management of malnourished and/or obese immobilized patients, therefore improving the quality of human bedridden patients on Earth.

Fig 23. *S…………. …………………. ………………. …………. ………….. ……………… ………. ……. …………. ………………… ……………… …………. …………..*

Acknowledgements: Funding by ASI, NUTRISS and MARS-PRE Projects

Key words: Space flight; energy balance; body composition; muscle atrophy.

References

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2022PDM3 Abstract 79

Peripheral impairments of oxidative metabolism during exercise following inactivity

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Oxidative metabolism is responsible for most of ATP re-synthesis during work, leisure or sport activities lasting longer than 1-2 minutes. A substantial impairment of oxidative metabolism during exercise is known to occur following inactivity. Whereas the "central" (mainly cardiovascular) factors contributing to this impairment have been thoroughly investigated, "peripheral" impairments (related to microvascular and endothelial functions, intramuscular O2 delivery-O2 uptake coupling, peripheral O2 diffusion, skeletal muscle oxidative metabolism, mitochondrial respiration) have received relatively less attention. We recently investigated several central and peripheral factors potentially responsible for an impairment of oxidative metabolism during exercise in 10 young males undergoing 10 days of horizontal bed rest (BR) (Zuccarelli et al. 2021). As expected, peak pulmonary O2 uptake ($\dot{V}O2peak$) and peak cardiac output were significantly lower after (POST) vs. before (PRE) BR (44.4 ± 7.2 mL kg-1 min-1 vs. 40.3 ± 6.1, P=0.001; 25.2 ± 5.5 L min-1 vs. 19.0 ± 3.2, P=0.003, respectively). Microvascular/endothelial function was evaluated by measuring the blood flow increase in the common femoral artery, by Eco-Doppler, during 1-min passive leg movements (PLM). A significant impairment was observed following BR: the area under the blood flow vs. time curve during PLM was smaller (P=0.03) in POST (274 ± 233 mL) vs. PRE (427 ± 291). The intramuscular matching between O2 delivery and O2 uptake was evaluated by measuring the muscle deoxygenation "overshoot", by near-infrared spectroscopy (NIRS), during a rest-to-exercise transition. Also for this variable a significant impairment was observed following BR, with a significantly greater overshoot in POST vs. PRE (P=0.02). Resting vastus lateralis muscle $\dot{V}O2$, estimated by calculating the slope of muscle deoxygenation (determined by NIRS) during a transient ischemia of the limb, was 27% lower (P=0.006) in POST vs. PRE. Other variables related to skeletal muscle oxidative metabolism were unaffected (or even slightly improved) following BR. The kinetics of recovery of vastus lateralis muscle $\dot{V}O2$ (determined by NIRS and the repeated-occlusions method [Zuccarelli et al. 2020]) were unaffected by BR: time-constants were 21.7 ± 5.7 s in PRE vs. 22.2 ± 5.9 in POST (P=0.79). Skeletal muscle citrate synthase activity, taken as an estimate of muscle mass, was not different (P=0.11) in POST (131 ± 16 nmol.min-1.mg-1) vs. PRE (138 ± 19). Mitochondrial respiration was evaluated ex vivo by high-resolution respirometry in isolated vastus lateralis muscle fibers obtained by biopsy. Maximal ADP-stimulated mitochondrial respiration (JO2max) (66 ± 18 pmol.s-1.mg-1 [POST] vs. 72 ± 14 [PRE], P=0.41) was not affected by BR. The sensitivity of mitochondrial respiration to submaximal [ADP] was evaluated by calculating the [ADP] corresponding to 50% of JO2max: values were significantly lower (P=0.046) in POST (409 ± 398 □M) vs. PRE (1270 ± 1015), demonstrating an increased sensitivity to submaximal [ADP]. In conclusion, peripheral limitations to oxidative metabolism after a 10-day BR were "upstream" of mitochondrial function, at the level of microvascular O2 delivery/endothelial function, and at the intramuscular matching between O2 delivery and O2 uptake. On the other hand, mitochondrial content and mitochondrial respiration were unaffected or even improved (enhanced mitochondrial sensitivity to submaximal [ADP]). The decreased resting muscle $\dot{V}O2$ following BR could represent an adaptive phenomenon in response to simulated microgravity–inactivity, attributable to the fact that muscle catabolic processes within muscles are less expensive, in terms of energy, than anabolic ones. These concepts, besides being of interest from a basic science point of view, may be relevant for patients with pathological conditions characterized by relatively short periods of profound inactivity, and could affect the definition of countermeasures or rehabilitative interventions.

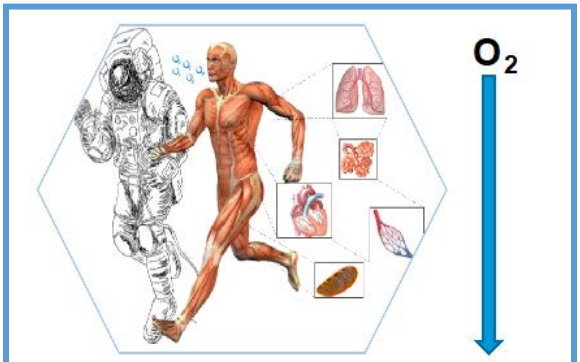

Fig 24. *Bed rest studies are widely utilized to evaluate the combined effects of microgravity and inactivity.*

Acknowledgements: Funding by ASI, MARS-PRE Project, DC-VUM-2017-006; PRIN Project 2017CBF8NJ; WP6, Project ALT FRAILTY, Active Ageing, UNIUD.

Key words: Microgravity; inactivity; skeletal muscle oxidative metabolism; microvascular/endothelial function; mitochondrial respiration.

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2022PDM3 Abstract 80

Alterations in the behaviour of individual motor units with inactivity

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A reduction in skeletal muscle activation (disuse), regardless the cause (i.e.: physical inactivity, disease and microgravity), can induce profound functional transformations affecting the metabolic, and neuromuscular properties of the the muscle tissue. It is also well known, both from animal and human studies, that although the main alteration caused by disuse is represented by muscle atrophy, there is evidence that similarly the quality of the muscle is altered, as demonstrated by the fact that the loss of muscle strength is considerably greater than that of muscle size.[1] The reduction of muscle quality can be ascribed to a complex interaction of various factors affecting neuromuscular transmission,[1] muscle architecture,[2] and contractile apparatus properties.[3] Short-term disuse is a common condition experienced by those individuals, that are bedridden because of an injury, surgery and/or chronic medical conditions. In the present study we focused our attention on the functional skeletal muscle alterations induced by short term disuse, consisting of 10 days of unilateral lower limb suspension (ULLS, walking with crutches), and the effect of a retraining period on the contractile properties of neuromuscular system investigated at the level of the single motor units (MUs). To this purpose, the electrical activity of the muscle vastus lateralis of 11 young males (22.1 ± 2.9 years) was recorded with high-density electromyography (HD-EMG), using a grid of 64 electrodes (5 columns × 13 rows; gold-coated; diameter of 1 mm; inter-electrode distance of 8 mm), during isometric trapezoidal contractions executed at 10, 25 and 50% of maximum voluntary contraction (MVC) and characterized by 3 phases: a linear increase (recruitment phase) in force at 5% MVC/s, a 20 s constant force at target (plateau phase) and a linear decrease (de-recruitment phase) in force at 5% MVC/s. These measures were performed at baseline (BLS0), and repeated immediately after the suspension period (LS10) and after 21 days of retraining (RT1). As expected, MVC decreased at LS10 (from 816.18 ± 97.22 to 573.20 ± 104.69 N; $p<0.001$) as did the ability of the participants to maintain the constancy (steadiness) of the isometric contraction, during the plateau phase, with a reduction observed at 25% MVC. After the HD-EMG decomposition and the removal of duplicated MUs, a total of 1422 unique MUs were identified. Moreover, we observed a reduction in absolute MUs recruitment and de-recruitment thresholds at all intensity of contractions. The MUs discharge rate decreased at 10 and 25% MVC, at all the points of evaluation (e.g., recruitment, de-recruitment and plateau), but not at 50%. The coefficient of variation of MUs interspike interval (COVisi), a marker of irregular firing pattern, increased during the steady state phase at 25% MVC. After the 21 days of retraining, MVC, recruitment threshold and discharge rate recovered to baseline levels while the COVisi and steadiness improved compared to baseline. In conclusion, these results highlight that 10 days of ULLS can induce significant alterations of the skeletal muscle function at the level of the single MUs and that 21 days of resistance training can restore these impairments.

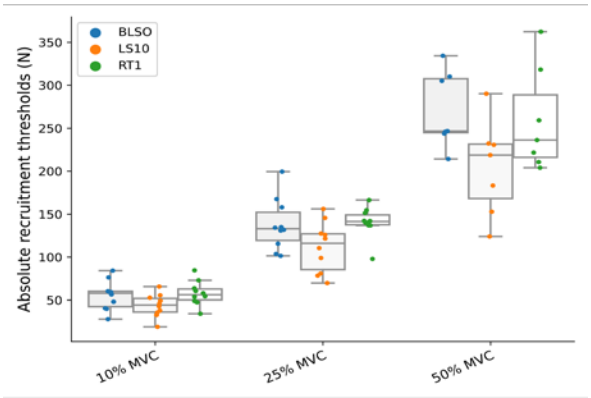

Fig 25. *Box and scatter plot of the absolute recruitment threshold expressed in newton (N) at 10, 25 and 50% of the maximum voluntary contraction (MVC). Every dot represents the average value of the observed motor units for each participant. Blue dots are for the baseline measurement (BLSO), orange for the post-suspension (LS10) and green for the post-retraining (RT1). *** p<0.001..*

Acknowledgements: Funding by ASI, MARS-PRE Project, DC-VUM-2017-006 is acknowledged

Key words: Motor unit; discharge rate; conduction velocity; disuse.

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2022PDM3 Abstract 81

Neuromuscular basis of disuse muscle atrophy and weakness

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Muscle atrophy is an inevitable consequence of chronic disuse. Until recently, in non-pathological conditions, the main cause of muscle wasting induced by inactivity was considered to be the lack of mechanical loading, resulting in a depression of protein synthesis and an increase in protein breakdown. However, there is now increasing evidence that neurodegenerative processes, triggered by inactivity, contribute to the loss of muscle mass and function. Indeed, instability of the neuromuscular junction (NMJ) and onset of denervation/reinnervation processes have been recently found after short-term and long-term inactivity in humans caused by bed rest or unilateral lower limb suspension (ULLS) (Salanova et al. 2011, Arentson-Lantz et al. 2016, Demangel et al. 2017, Monti et al. 2021, Sarto et al. 2022). The onset of these changes seems extremely fast as initial signs of fibre denervation/reinnervation, evidenced from the presence of neural cell adhesive molecule (NCAM) positive myofibres, can be observed after just 3-days (Demangel et al. 2017) and 10 days of bed rest (Monti et al. 2021) (Fig. 1). These changes are also accompanied by instability of the NMJ, as shown either by increased serum levels of c-terminal agrin fragment (CAF) after 10-day bed rest and after 10-day ULLS, or by a decreased expression of Homer proteins in muscle biopsies, after 60-day bed rest (Salanova et al 2011). These findings of NMJ instability and partial fibre denervation were associated with an impairment of sarcoplasmic reticulum (SR) calcium handling, since a significant depression in the caffeine-induced release of Ca2+ by the SR was found after 10-day bed rest (Monti et al. 2021), suggesting an impairment of excitation-contraction coupling. These findings support the view that the early onset of NMJ instability and impairment in SR function are eligible mechanisms contributing to the greater decline in muscle force than in muscle size observed in these bed rest studies and in most models of muscle disuse. *Acknowledgements*: Funding by ASI, MARS-PRE Project n. DC-VUM-2017-006, is acknowledged.

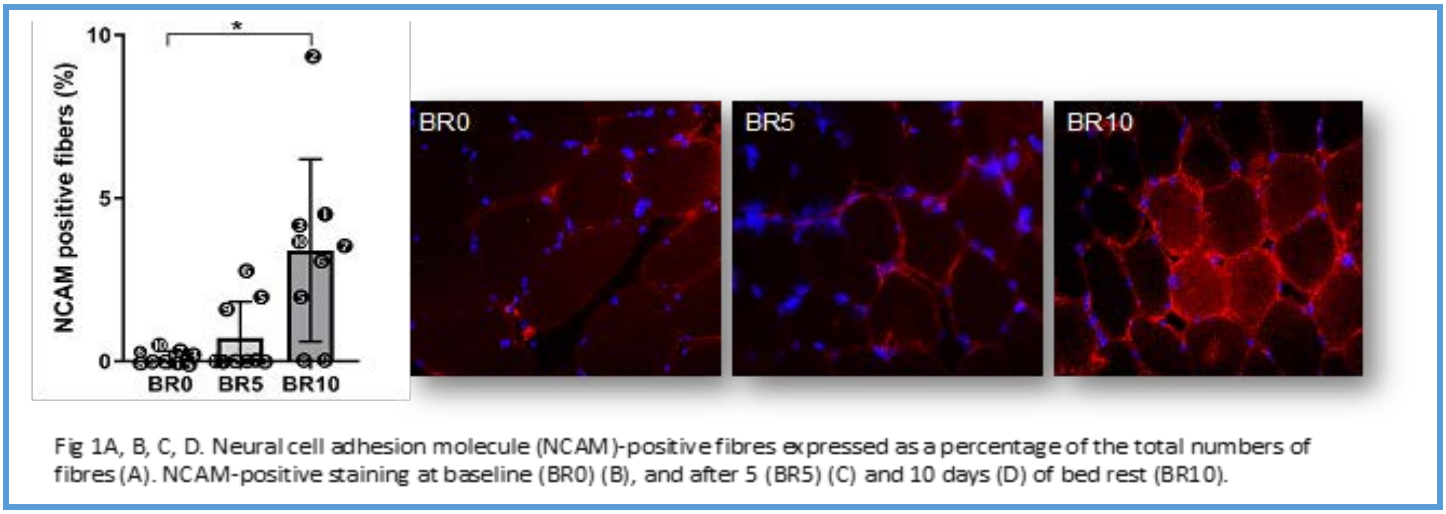

Fig 1A, B, C, D. Neural cell adhesion molecule (NCAM)-positive fibres expressed as a percentage of the total numbers of fibres (A). NCAM-positive staining at baseline (BR0) (B), and after 5 (BR5) (C) and 10 days (D) of bed rest (BR10).

Key words: …3 to 5…… …… …… …… …….

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2022PDM3 Abstract 82

On the role of proprioception in the sense of force

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Successful performance of motor tasks requires the adequate modulation of force output, which depends, in part, on the integration of information from various mechanoreceptors located in muscles, muscle-tendon interfaces, and joints. These signals form the basis for the perception of self-movement and body position – termed proprioception – which is defined as the conscious or unconscious perception of the position of different parts of the body in space. Along with the kinaesthetic sense (the senses of position and movement), the sense of force is another aspect of proprioception. The sense of force represents the ability to correctly perceive and reproduce a given level of force. However, it is still debated whether the sense of force really relies on proprioceptive inputs. This may depend on the experimental approach used for its assessment. Indeed, it can be evaluated by asking the participants to match with one limb the force produced by the contralateral limb (two-arm matching task), or by asking to reproduce a force with the same limb (force reproduction task). It has been suggested that the two-arm matching task does not require proprioceptive feedback but rather a centrally generated signal. In contrast, the force reproduction task is suggested to mainly rely on proprioceptive inputs, including signals from muscle spindles and Golgi tendon organs, in addition to sensory inputs from skin mechanoreceptors. By manipulating proprioceptive and visual inputs within different phases of the force reproduction task, we provide relevant elements supporting the role of proprioceptive signal from muscle and tendons sensors during the force reproduction task. Furthermore, we show a decrease in the sense of force with ageing, which could reflect age-related decline in sensorimotor integration of proprioceptive and somaesthetic inputs.

Fig 1. ……

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2022PDM3 Abstract 83

Two brothers with X-linked Charcot Marie Tooth disease and different lifestyle

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Aim of our study was to investigate CMT1-X and its different phenotypic expression in a family with an unreported frameshift mutation in GJB1 gene encoding connexin 32. In CMT1-X family two male siblings had a different clinical phenotype we investigated genetic and clinical features. Connexin 32 is a gap junction protein that is located in paranodal regions and Schmidt-Lanterman incisures. Genetic analysis and micro RNA-206 determination was performed on DNA from 2 cases on the following genes: BSCL2, EGR2, FGD4, FIG4, GARS, GDAP1, GJB1, HSPB1, IGHMBP2, LITAF, LMNA, MFN2, MPZ, MTMR2, NEFL, PMP22, PRX, SH3TC2. Muscle specific miRNAs was also investigated in serum. A muscle biopsy (1 case) and EMG with conduction velocities were performed in both patients. Clinical features and family history were collected. One brother of 55 years had diffuse hypotrophy in lower limbs since age 28 years, he had atrophic legs bilateral pes cavus bilateralis and ataxic gait, he had difficulty grasping object with hands his micro-RNA 206 level was elevated. The younger brother of 47 years had a distal weakness, tremor in upper extremities (right more than left) and stepping gait with thin legs and abnormal conduction velocity EMG (38 m/s). He had difficulty opposing thumb and a slight deficit (4/5 MRC score) of finger extension in upper limbs. His weakness was mostly in hands and slowly evolving in lower limbs. Muscle biopsy showed neurogenic changes, MRI of the spinal cord showed in the cervical region a slight disc protrusion on C5/C6 with a rectified cervical cord., a different level of micro RNA 206 was found In first genetic analysis, no duplication of PMP22 gene was found. A second genetic analysis of a panel of genes associated with inherited peripheral neuropathy showed a frameshift mutation of connexin 32 (GJB1 gene) c.281_287del in hemizigosis not previously reported that segregated with clinical phenotype in two male brothers. Circulating miR-206 was found up-regulated in both patients. While the older sibling had evident gait difficulty and presented a chronic invalidating weakness both in upper and lower extremities the second brother was a military pilot and had minimal weakness mostly in the right hand and tremor . There was evidence from the family history of an X-linked Hereditary Sensory Motor Neuropathy that was confirmed by the mutation in connexin 32 gene. We hypothesize that in CMT X-linked there is a different expression of the connexin-32 protein in relation to its markedly reduced abundance in gap region by a semi-dominant mechanism on other connexins and consequent demyelination. Such reduction might affect not only gap function formation but also myelin and resulted in differential micro-RNA 206 expressivity observed in this family. The level of these molecules reflects different muscle mass observed.

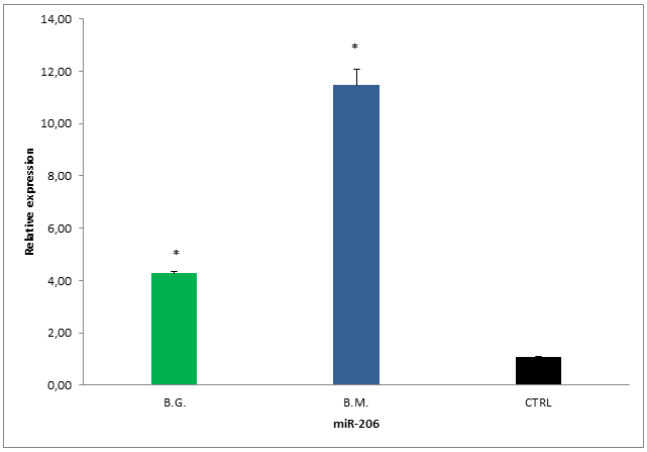

Fig 1. *Up-regulation of 4 times of miR-206 in serum of older (B.G.) 55 year old patient and over 10 fold in younger (B.M.) brother versus 7 controls (CTRL). The higher level of microRNA in less affected patient might be due to a relative better muscle regeneration or muscle trophism with less neurogenic atrophy.*

Key words: Charcot Marie Tooth; connexin-32; novel mutation in GJB1 gene; miR-206.

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2022PDM3 Abstract 84

Improvement of the expression of upper limb muscle strength and balance in a group of patients with multiple sclerosis, through a proprioceptive rehabilitation protocol combined with the application of photon emission devices

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In multiple sclerosis (MS) patients, symptoms such as: fatigue, lack of physical energy, spasticity, difficulty in performing movements, and motor coordination disorders, and with tremors, dizziness and postural instability are among the most common complications. Cattaneo et al. (2007) studied the effects of stability training on MS patients and described it as an effective intervention in reducing falls risk, improving stability, and strength. Therefore, the present study aimed to confirm early observations by the application of a proprioceptive rehabilitation program targeting balance and strength on MS patients. 13 subjects with MS, 5 male and 8 female, volunteered in the study. A MAP hand dynamometer was used to determine the handgrip strength, as the maximum isometric grip (MIG), recognized as an important health indicator for determining musculoskeletal function, as well as weakness and disability. Sensor Medica® systems allowed us to carry out baropodometric and stabilometric measurements using the associated program (Freestep® by Sensor Medica®). The device consists in a pressure platform with resistive sensors with conductive rubber. The rehabilitation protocol included: A) 10 minutes of Motomed; B) 10 minutes of Human Tecar proprioceptive path; C) 15 minutes in total of physical exercises; D) 15 minutes of massage therapy of the whole spine All patients wore the photon emission devices TAOPATCH by TAO technologies, according to a protocol used in a previous study (Amato et al., 2021). Testing procedures were carried out before and after the rehabilitation protocol. The paired sample t-test revealed statistically significant improvements for the baropodometric measures (p<0.05) associated with the intervention. In particular, plantar surface pressure, forefoot and hindfoot pressure improved significantly, as well as the maximal and mean foot pressures and the forefoot and hindfoot loads (figure 1a). In addition, the intervention induced a statistically significant improvement in the right (p = 0.023) and left (p = 0.021) handgrip strength (figure 1b). The combination of an adequate and specific rehabilitation protocol combined with the application of the photon emission devices Taopatch, emerged as a valid protocol to improve handgrip strength of the upper limbs and the expression of the body weight on the ground in contrast with the force of gravity. Thus, determining improvements in a cluster of parameters severely decompensated in MS patientsIn multiple sclerosis (MS) patients, symptoms such as: fatigue, lack of physical energy, spasticity, difficulty in performing movements, and motor coordination disorders, and with tremors, dizziness and postural instability are among the most common complications. Cattaneo et al. (2007) studied the effects of stability training on MS patients and described it as an effective intervention in reducing falls risk, improving stability, and strength. Therefore, the present study aimed to confirm early observations by the application of a In multiple sclerosis (MS) patients, symptoms such as: fatigue, lack of physical energy, spasticity, difficulty in performing movements, and motor coordination disorders, and with tremors, dizziness and postural instability are among the most common complications. Cattaneo et al. 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body weight on the ground in contrast with the force of gravity. Thus, determining improvements in a cluster of parameters severely decompensated in MS patientsproprioceptive rehabilitation program targeting balance and strength on MS patients. 13 subjects with MS, 5 male and 8 female, volunteered in the study. A MAP hand dynamometer was used to determine the handgrip strength, as the maximum isometric grip (MIG), recognized as an important health indicator for determining musculoskeletal function, as well as weakness and disability. Sensor Medica® systems allowed us to carry out baropodometric and stabilometric measurements using the associated program (Freestep® by Sensor Medica®). The device consists in a pressure platform with resistive sensors with conductive rubber. The rehabilitation protocol included: A) 10 minutes of Motomed; B) 10 minutes of Human Tecar proprioceptive path; C) 15 minutes in total of physical exercises; D) 15 minutes of massage therapy of the whole spine All patients wore the photon emission devices TAOPATCH by TAO technologies, according to a protocol used in a previous study (Amato et al., 2021). Testing procedures were carried out before and after the rehabilitation protocol. The paired sample t-test revealed statistically significant improvements for the baropodometric measures ($p<0.05$) associated with the intervention. In particular, plantar surface pressure, forefoot and hindfoot pressure improved significantly, as well as the maximal and mean foot pressures and the forefoot and hindfoot loads (figure 1a). In addition, the intervention induced a statistically significant improvement in the right ($p = 0.023$) and left ($p = 0.021$) handgrip strength (figure 1b). The combination of an adequate and specific rehabilitation protocol combined with the application of the photon emission devices Taopatch, emerged as a valid protocol to improve handgrip strength of the upper limbs and the expression of the body weight on the ground in contrast with the force of gravity. Thus, determining improvements in a cluster of parameters severely decompensated in MS patients.

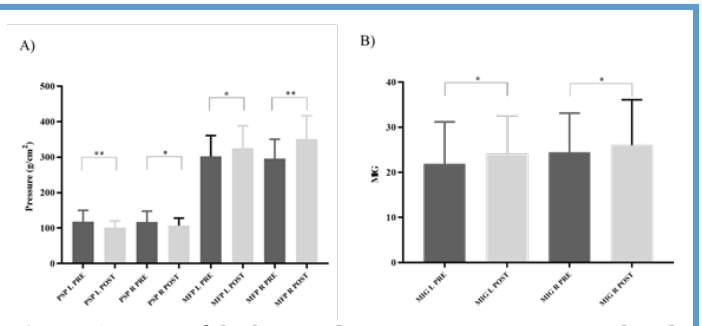

Fig 1. *A) Some of the baropodometric parameters considered in the study and their changes from pre to post intervention; B). Handgrip strength changes from pre to post intervention. Notes: *: p<0.05; **:p<0.01; PSP: plantar surface pressure, MFP: mean foot pressure; MIG: maximal isometric grip.*

Key words: Balance; multiple sclerosis; neuromuscular exercise; muscular strength.

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2022PDM3 Abstract 85

Glutathione and collagenase activity in skeletal muscle after 10 days of bed rest

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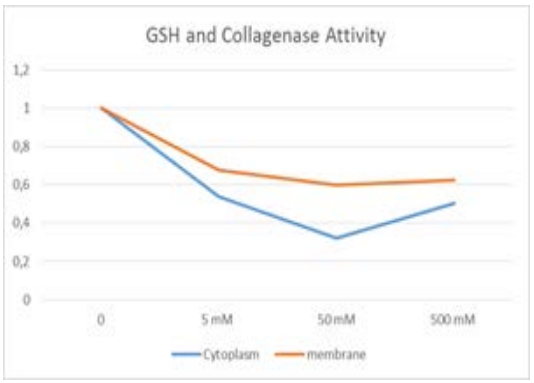
Management of muscle mass loss during microgravity (bed rest, sedentary lifestyle, space flights) is of foremost relevance in healthcare, but its pathophysiology is not completely understood. Tissue remodeling, a key step of muscle turnover, is at least in part ascribed to the balance between a class of intra and extracellular endoproteases named Matrix MetalloProteases (MMPs) and their inhibitors (Tissue Inhibitors of MMPs, TIMPs). One of these MMPs, a collagenase named MMP1 has received attention in muscle reactivation of sedentary women (1), but the mechanisms of its regulation, sites of activity and substrates are not investigated. Further, microgravity reduces muscle mass and modifies the glutathione levels as expression of the redox state (2, 3). Our hypothesis is that combination of GSH with proteins (transglutathionilation), and specifically of MMPs, might explain reduced muscle use and mass loss. The present study investigates the association of GSH/GSSG concentration measured in red blood cells, a recognized marker of whole body redox state (4), with collagenase activity observed in muscle biopsies (in-situ zymography) abd quadriceps volume changes in 10 volunteers before and after 10 days of bed rest. Bed rest induces a bimodal pattern in GSH/GSSG concentrations, with a reduction at 5 days, followed by an increase of GSSG at 10 days. At day 10, a consistent reduction of collagenase activity can be observed in cytoplasm of muscle fibers, but not on their membranes. On linear regression, GSSG at 10 days is the only variable predicting the amount of reduction of collagenase activity. Pretreatment of muscle biopsies with 0,5 micromolar GSH/GSSG reduces the collagenase activity. The amount of collagenase activity lost during bed rest is correlated with the variation of quadriceps volume.



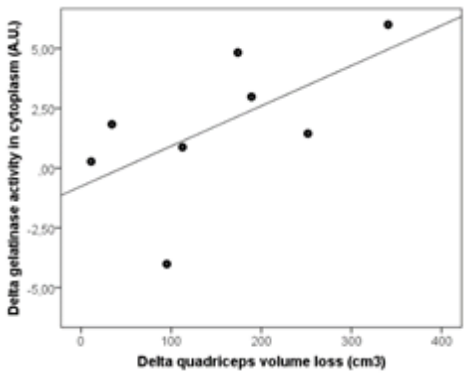


Fig 1. *Left panel: Effect of GSH on collagenase activity on skeletal muscle. At physiologic GSH concentrations it is possible to observe a reduction in collagenase activity. Right panel: a correlation exists between amount of muscle loss and loss of collagenase activity.*

Key words: Bed rest; Glutathione; skeletal muscle; matrix metalloproteinases; collagenases; in-situ zymography.

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2022PDM3 Abstract 86

A combined treatment protocol for postural instability in Pisa Syndrome

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Postural disorders such as Pisa Syndrome (PS) are frequently observed in Parkinson's Disease (PD). PS is characterized by postural instability that affects both balance and gait pattern, leading to progressive loss of autonomy, increase in fall risk and deterioration of quality of life. To date, no consensus has been reached on its pathogenesis, nor on the definition of a specific standard therapeutic protocol. Taking into consideration a central etiopathogenetic hypothesis, characterized by dystonic activation of trunk muscles, in particular the external oblique muscle (EOM), we evaluated the efficacy of an intensive rehabilitation training combined with botulinum toxin type A (BoNT-A) treatment. Patients with PD and PS, defined as a reversible lateral trunk flexion >10°, were selected. All patients were on chronic antiparkinsonian therapy with dopaminergic drugs, stable on their daily during the 4 weeks previous the study. Exclusion criteria were presence of other neurological diseases, clinically significant psychiatric disturbances, and orthopedic spine abnormalities. Before treatment (T0), all patients underwent the following evaluations:- 3D Gait Analysis (GA); - Needle EMG of the EOM: if hyperactivation was detected, concomitant BoNT-A inoculation was Performed - Berg Balance Scale (BBS) (Bronstein, 2013); - Clinical-Functional Scales: Walking Test (10mWT, TUG, 6MWT), Berg Balance Scale (BBS), Parkinson Disease Questionnaire (PDQ-8), Fall Risk scale (FRS), Falls Efficacy Scale (FES-I). Numerical Rating Scale (NRS); - Truncal flexion angle (TFA) measurement with "Image J" system. One week after T0, patients underwent a three-week specific high intensity rehabilitation program (trunk muscles stretching, balance and postural control) structured in 5 session per week. At the end of treatment (T1) initial evaluations were repeated. Nine patients were enrolled and completed the protocol (age 71± 4,1 y; M: F=5:4). The comparison of the T0 and T1 data displayed a significant reduction of TFA (T0= 11,45 ± 4,74; T1= 7,71 ± 6,21; p= 0,027), NRS (T0= 6 ± 1,8; T1= 1,77 ± 1,48; P= 0,007) and BBS (T0= 48,88 ±4,85; T1= 52,11 ± 3,29; p= 0,018). 3D Gait. Analysis showed an improvement in the time-space parameters after the treatment, specifically in stride duration (T0= 1,36 ± 0,21; T1 = 1,25 ± 0,20; p= 0,03) and cadence (T0= 90,18 ± 14,90; T1= 97,66 ± 15,15; p= 0,05). In conclusion, the results seem to confirm the importance of EOM in PS. Our protocol was well tolerated and showed to be successful in reducing the postural abnormality and posture related pain, as suggested by the significative variation of TFA and the major improvement seen in clinical-functional scales

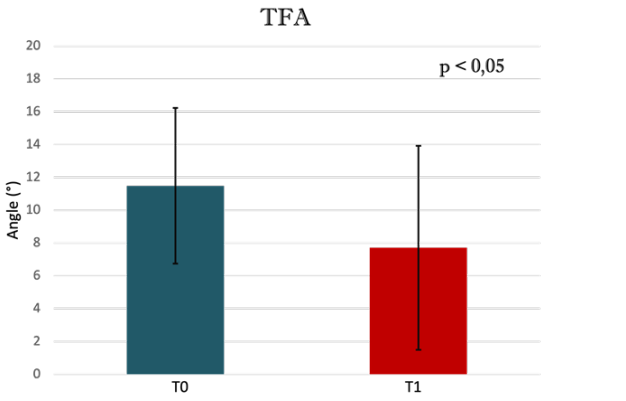

Fig 1. *The comparison of the T0 and T1 data displays a significant reduction of Truncal flexion angle (TFA).*

Key words: Parkinson's Disease; postural deviation; Pisa Syndrome; trunk dystonia.

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2022PDM3 Abstract 87

Focal Muscle Vibration and Action Observation: a novel approach for muscle strengthening

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Focal vibration (FV), when applied with specific intensities and frequencies on belly muscle, can improve muscle strengthening inducing peripheral and, in the long term, central modifications. Therefore, this approach is useful when subjects need a strengthening treatment after o during prolonged immobility or disabling diseases that prevent them from performing traditional protocols. Similarly, action observation (AO) and "motor imagery" techniques are used to intensify the effect of rehabilitation on muscle strength and to reduce recovery time. The synergy effect of the two approaches was not demonstrated. The aim of this study is to assess the effectiveness of the integration of a strengthening protocol based on FV associated with an AO paradigm. The study involved healthy subjects, who were randomly assigned to a specific treatment: a group underwent 100 Hz FV treatment on the right femoral quadriceps and a second group underwent the same protocol associated with an AO paradigm of the muscle training (AO+FV) for two weeks, five days/week. Before (T0), after the first week (T1), at the end of treatment (T2), and after a week from the end (T3) for the follow-up, we assessed the maximum voluntary contraction (MVC) expressed at knee extension bilaterally with dynamometer and at T0, T2 and T3 we assessed changes in cortical excitability by transcranial magnetic stimulation (Resting Motor Threshold - RMT; Silent Period - SP). Preliminary data seems to show that MVC increases bilaterally in both groups at T1 and T2. A major increase is evident in the AO+FV group, this increase is maintained even at T3. RTM is reduced at T3 in AO + FV. The SP is reduced only in group AO + FV at T2 and T3. In conclusion, AO seems to be able to enhance the effect of muscle reinforcement induced by FV on maximum strength. This effect seems to be mediated by peripheral and central modifications Further evidence is needed to support treatment effectiveness, which could be a valuable addition to existing protocols as well as an alternative treatment when the subject is not able to perform traditional reinforcement protocols.

Fig 1. *Subject undergoing the proposed integrated treatment of FV and AOT.*

Key words: Muscle-strengthening; focal vibration; action observation treatment.

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2022PDM3 Abstract 88

Form vs. function: strategies to deliver IGF-I for muscle therapeutics

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Skeletal muscle is a post-mitotic tissue, yet has the ability to regenerate through the activation of muscle satellite cells and the coordinated actions of multiple growth factors. A key player in the regeneration process is Insulinlike Growth Factor-I (IGF-I).[1] IGF-I helps to resolve damage by promotion of satellite cell proliferation and differentiation, suppression of proinflammatory cytokines, and fiber formation. A long-standing goal in our laboratory has been to optimize IGF-I forms to improve its actions in skeletal muscle. While the most well characterized form of IGF-I is the small mature 7kD protein, complexity of alternative splicing and posttranslational modification leads to several additional forms of this growth factor. These larger forms have been shown to have more efficient storage in muscle tissue as well as enhanced receptor activation,[2,3] and raise the possibility that improvements in therapeutic potential could occur through the use of a different IGF-I form. To understand the necessity of glycosylated and mature IGF-I forms, gene editing was employed to remove glycosylation sites in the C-terminal portion of IGF-IA, and to eliminate the ability for cleavage of the C-terminal extensions of all IGF-I forms (Figure 1).[4] Mice were viable, with normal force generation. To determine the effects on regeneration after acute injury, cardiotoxin injections were performed in male and female mice from all genotypes, and monitored for changes in fiber size. In both 2ND and 3RA genotypes, there was an enhanced recovery of fiber size following injury. This suggests that the pro-IGF-I forms, common to both genotypes may contribute to accelerating the resolution of damage in muscle. Future studies will take advantage of these forms to develop more effective therapeutics for muscle.

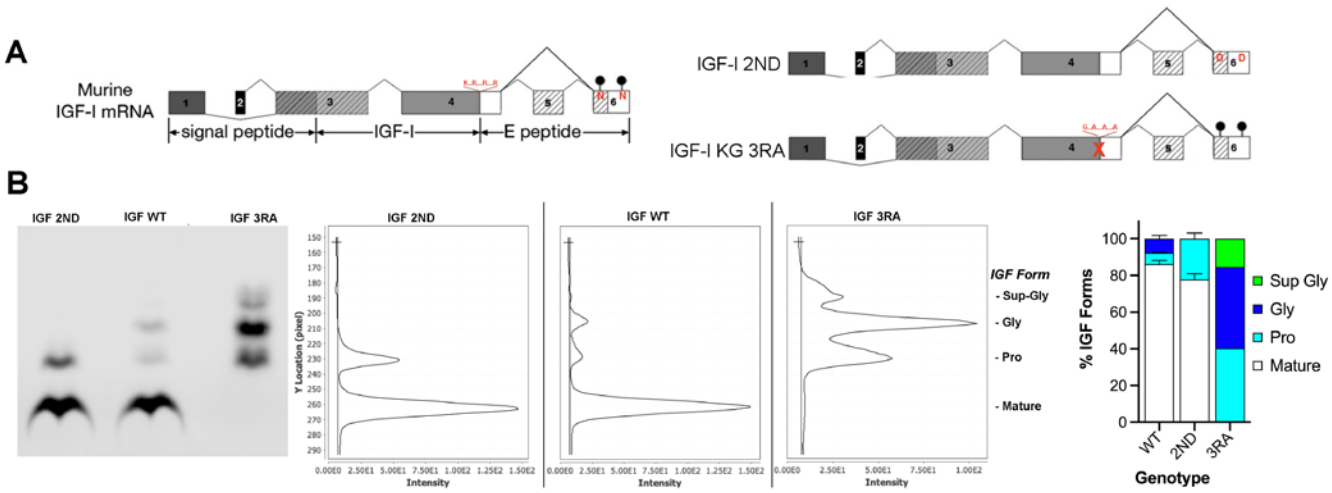

Fig 1. *A. Alternative splicing and glycosylation sites in Igf1a, with gene edited mutations of N92D/N100D (2ND) and K68G/R71A/R74A/R77A (KG3RA). B. Serum shows elimination of glycosylated IGF-I in 2ND mice, and elimination of mature IGF-I in KG 3RA mice.*

Key words: Muscle regeneration; IGF-I forms.

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2022PDM3 Abstract 89

Metabolic link between mitochondrial and contractile abnormalities

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Impaired mitochondrial function and abnormal muscle bioenergetics are evident in several diseases and aging and have been associated with loss of muscle contractile function. However, a direct link between metabolic dysfunction and loss of muscle force or power has not been established. Mitochondrial respiration impairments lead to accumulation of metabolites of the tricarboxylic acid cycle that are upstream of the electron transport system such as succinate and succinyl-derivatives.[1] High fat diet, diabetes, hypoxia/ischemia, cancer, and even exercise disrupt the metabolism succinate and succinyl-derivatives resulting in several fold increase in succinate.[1-5] We have found that postmenopausal heart failure with preserved ejection fraction (HFpEF) causes muscle mitochondrial dysfunction and weakness,[6] which are accompanied by elevated plasma [succinate] that reflects increased cellular concentration and export from the most metabolically active tissues: adipose, liver, and skeletal muscle. This presentation will focus on preliminary findings showing that: 1) HFpEF disrupts the abundance of proteins involved in mitochondrial succinate transport and lysine succinylation, resulting in increased myofibrillar protein succinylation; 2) acute exposure of intact skeletal muscle and permeabilized single fiber to succinate, which causes succinylation of myofibrillar proteins, impairs isometric and isotonic contractile function; and 3) the effects of succinate in vitro are independent of ROS from mitochondrial complex II. Overall, our findings suggest that succinate and lysine succinylation are a novel link between metabolic and contractile dysfunction in health and disease.

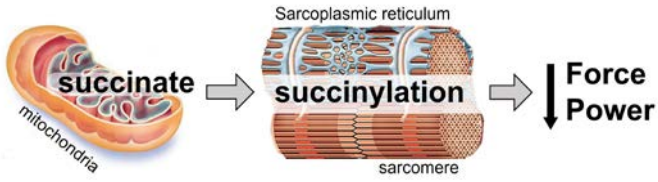

Fig 1. *Schematic representation of the working hypothesis.*

Key words: Heart failure; succinate; weakness; peak power.

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2022PDM3 Abstract 90

Topical aspects of neuro-rehabilitation

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The goal of neuro-rehabilitation is to create optimal conditions for the patient's active engagement in domestic and social life, a return to active social and domestic activities and, ultimately, to improve the quality of life of both the patient himself and his/her relatives. The main tasks of neuro-rehabilitation are the impact on the restoration of the patient's vital functions using physical and mental methods, the impact on his/her body with the help of drug therapy to improve and normalize metabolic processes, as well as the development of an adaptation system for the changes caused by the pathological process. It is important to take into account theoretical aspects of the organization of physiological and pathological movements, as well as the formation of short- and long-term compensatory processes of damaged structures and the impaired functions of the body systems in the course of neuro-rehabilitation. The formation of compensatory mechanisms is greatly influenced by signaling from the external and internal environment of the body, i.e. feedback.[1] A reliable brain function is ensured by dynamic inter-neuronal activity, resulting in morphologically redundant connections. Neuro-rehabilitation is also based on the systemic organization of the brain functions, the reserve capacity of the brain in reorganizing its structures under the conditions of the pathological process. This reorganization causes the patient's motor retraining, which leads to the restoration or compensation of the impaired functions. When conducting neurorehabilitation, it is necessary to strive for a true restoration of the disturbed functions of the patient's body. This level of HP seems to be the highest and lies in the fact that the functions of the body completely return or are as close as possible to the original state. The main mechanism ensuring a true functional recovery is the disinhibition of inactivated nerve elements by means of techniques aimed at stimulating them.[2] With brain damage, recovery occurs due to intact functional systems. This process is ensured by the plasticity of the nervous system and the anatomical connections between its departments. The plasticity of the brain lies in the ability of the nervous tissue to change its structural and functional organization under the influence of external and internal factors. It is due to the following mechanisms: the functioning of the previously inactive connections; sprouting of the fibers of the remaining neurons (renewed of growth of the affected axon, changes in fiber branching, the area and density of the dendritic spines); formation of new synapses; synaptic remodeling (changes in the configuration and properties of synapses); reorganization of neuronal circuits; extrasynaptic transmission of excitation; a change in the structure of astrocytes, an increase in the number of contacts between synapses and astrocytes. During the medical and social rehabilitation, one must bear in mind that severe disabling neurological diseases cause permanent disability and social maladjustment. Thus, regarding the main aspects and principles of neuro-rehabilitation, it is important to understand the complexity, aims and problem orientation of this process, which is primarily aimed at increasing the degree of recovery of neurological functions of patients after a stroke, their level of social adaptation and, ultimately, improving the quality of life of patients and their relatives. [3]

Key words: Neuro-rehabilitation; stroke; neuroplasticity.

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2022PDM3 Abstract 91

Early rehabilitation of ischaemic stroke with medicinal acupuncture: A clinical study

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Purpose of this study was to assess the effect of a course of homeopathic preparations using acupuncture points on the severity of motor impairment against the background of early rehabilitation of the ischaemic stroke (IS). More than 60 patients with different localisation of ischaemic stroke (vertebro-basilar and carotid territories) were treated. All the patients were treated according to the standards of medical care for ischaemic stroke. The degree of impairment was assessed using National Institutes of Health Stroke Scale (NIHSS), which included impairment of consciousness, degree of paresis (0 - no paresis, 4 - no active movements), sensory disturbance and speech disorders manifested in the form of aphasia and dysarthria. The control group consisted of 10 patients with ischaemic stroke. All the patients underwent a computer-assisted tomography of the brain to rule out the hemorrhagic nature of the stroke. Treatment was given during the acute period of IS within 1 to 3 days after onset. Exclusion criteria: hemorrhagic and mixed strokes, thrombolysis, history of cancer. The basic therapy of IS was carried out according to the standards. HEEL homeopathic medicines with anti-inflammatory, metabolic and antioxidant effects were used, which were administered on the common action points of the Yang meridians of the paretic limbs. The control group received acupuncture according to the same scheme on the paretic limbs. The treatment duration was 10 sessions; the observation lasted for 21 days, according to the standards of hospitalization for stroke patients. The degree of functional impairment of patients treated with medicinal acupuncture and the patients of the control group assessed by the National Institutes of Health Stroke Scale (NIHSS), (Fig. 1,2). The outcomes of the medicinal acupuncture treatment: positive effect in the form of a reduction in the degree of paresis (Fig. 3,4) were observed in patients, who received medicinal acupuncture. The lowest treatment outcome was observed in patients with IS in the vertebro-basilar system. Medicinal acupuncture can be used in early rehabilitation for patients with mild and moderate IS, since its possible mechanism is the enhancement of neuroplasticity in the conditions of brain damage. The use of homeopathic medicines can increase the positive effect compared with acupuncture. The most promising "candidates" for this treatment are the patients with IS in the carotid territory.

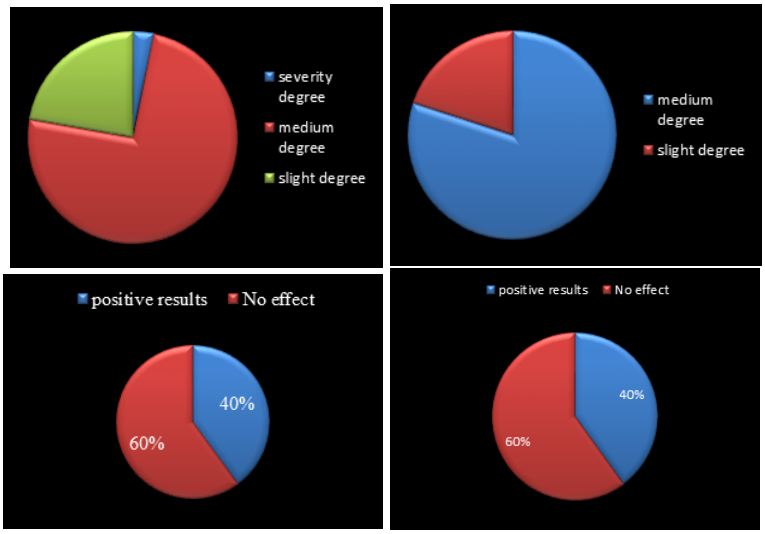

Fig 1. *Top left panel: Baseline degree of functional impairment in ischaemic stroke patients treated with medicinal acupuncture; Top right panel: Baseline degree of functional impairment in ischaemic stroke patients in the control group; Bottom left panel: Positive outcome in the group of patients, who received medicinal acupuncture; Bottom right panel: Positive outcome in the control group of patients.*

Key words: ischaemic stroke; medicinal acupuncture.

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2022PDM3 Abstracts 92

Molecular mechanisms of cancer-induced muscle wasting

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Purpose of this study was to assess the effect of a course of homeopathic preparations using acupuncture points on the severity of Cancer cachexia is a multifactorial condition characterized by skeletal muscle loss that impairs longevity and quality of life of the vast majority of cancer patients (1, 2). However, the ability to develop therapeutic strategies to counter cachexia is impeded by the limited understanding of the underlying mechanisms of cachexia in human cancer patients. The purpose of this study was therefore to identify the proteomic signature of skeletal muscle obtained from cachectic pancreatic ductal adenocarcinoma (PDAC) patients, who exhibit one of the highest rates of cachexia (3, 4). Muscle biopsies of the rectus abdominis were obtained from cachectic PDAC patients (n=8) undergoing tumor resection surgery as well as age and sex-matched non-cancer controls (n=6). Total proteome profiling of skeletal muscle protein isolate was performed using 10-plex tandem mass tag labeling and liquid chromatography tandem mass spectrometry (LC-MS/MS). MS/MS spectra were evaluated using SEQUEST and differentially expressed proteins were identified through the UniProt Homo Sapiens database [adjusted P value (FDR) ≤0.01; -1.25≥fold change≥1.25]. The total number of identified peptides and proteins are shown in Figure 1, which further shows that 383 proteins were identified to be differentially expressed in the skeletal muscle of PDAC patients compared to controls. To identify altered biological processes within the skeletal muscle of cachectic PDAC patients, differentially expressed proteins (DEP) were analyzed using several bioinformatic platforms. The results of these bioinformatic analyses will be presented, along with immunohistochemical analyses to further support changes in select proteins of interest.

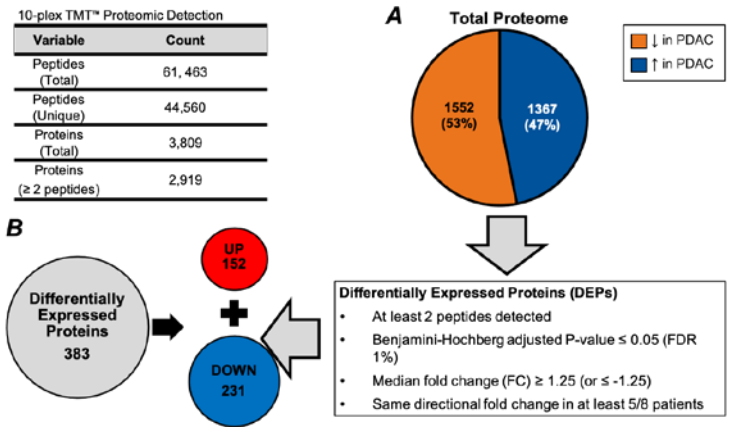

Fig 1. *Following LC-MS/MS analysis, 61,463 peptides were detected among all samples, of wich 44,560 peptides were uniquely identified. In total, 3,809 proteins were identified (1% FDR), and 2,919 proteins identified annotated to at least 2 distintctly detected peptides (A). Following differential expression analysis, 383 proteins were identified (B), of wich 152 (39.7%) were upregulated and 231 (60.3%) were downregulated in the skeletal muscle of PDAC versus controls.*

Key words: Cachexia; pancreatic cancer; proteomics.

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2022PDM3 Abstracts 93

Therapeutic potential of combined cycling and isometric strength training in patients with DMD: preliminary findings

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Duchenne muscular dystrophy (DMD) is a devastating, rapidly progressive neuromuscular disease caused by mutation of the dystrophin gene and is characterized by severe muscle weakness and fatigability, early loss of ambulation and premature death. The search for effective therapies is ongoing; understanding the potential of exercise alone, or as an adjuvant to developing therapies, is likely to be beneficial but is limited by (i) a lack of understanding of exercise prescription parameters (type, intensity, target muscle groups) that are safe and effective for patients with DMD; and (ii) a lack of accessibility to exercise equipment suited to the limitations of dystrophic muscle. High intensity, eccentric actions are known to be damaging, however our group recently reported moderate-intensity isometric exercise to be safe and increase muscle strength1 in boys with DMD. Dynamic cycling was shown to benefit boys and delay loss of muscle function2,3, however improvements in muscle strength and endurance were not detected likely relating to the low intensity of the cycling exercise which as motor-assisted. Our objective was to engineer a therapeutic exercise device for patients with DMD (Fig. 1), allowing for safe and appropriate overload of muscle (using both isometric strengthening and aerobic cycling with assistive and resistive capabilities) to induce remodeling and attenuate disease progression. The device is used in-home with remote video supervision by the study team to allow high-resolution real-time quantification of dynamic and isometric force profiles and capture physiological measures (i.e. heart rate and perceived exertion). Our goal is to develop guidelines and advance exercise as therapy for DMD. Outcome measures to assess intervention safety and efficacy include standard clinical function tests, magnetic resonance imaging of leg muscle (cross sectional area, fat fraction and T2 quantification), muscle strength and aerobic capacity. In this ongoing study (ClinicalTrials.gov NCT04322357), one boy with DMD on daily steroids (age 8-yrs) completed the 6-month training program (34 sessions of cycling and 20 sessions of strength training). Preliminary findings are shown in Figure 1.

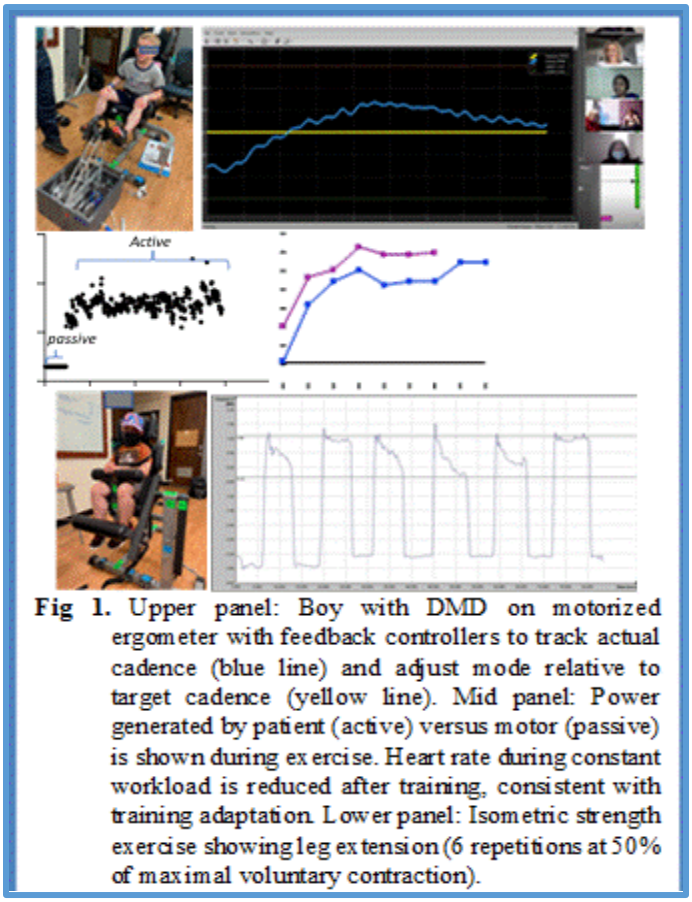

Fig 1. Upper panel: Boy with DMD on motorized ergometer with feedback controllers to track actual cadence (blue line) and adjust mode relative to target cadence (yellow line). Mid panel: Power generated by patient (active) versus motor (passive) is shown during exercise. Heart rate during constant workload is reduced after training, consistent with training adaptation. Lower panel: Isometric strength exercise showing leg extension (6 repetitions at 50% of maximal voluntary contraction).

Supported by Department of Defense Grant number: W81XWH1910330.

Keywords: Duchenne muscular dystrophy; aerobic exercise; strength training; physiological adaptation; combination therapy.

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2022PDM3 Abstracts 94

Development of an experimental setup for exact measurement of time in event chain of patellar reflex test, transcutaneus spinal cord stimulation (tSCS) and H-reflex analysis in healthy, spinal cord injured and brain insulted individuals

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2022PDM3 Abstracts 95

Network neuroscience evaluation of the relation between electroencephalography (EEG) signals during electrophysiological and movement tests

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2022PDM3 Abstracts 96

Brain activity and event-related potential analysis in healthy, spinal cord injured, and brain insulted individuals

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2022PDM3 Abstracts 97

Transcutaneus spinal cord stimulation (tSCS) review and recent progress

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2022PDM3 Abstracts 98 to 122

To be submitted by 26 Senior Speakers, who sent expression of interest to join or Title, (highlighted in YELLOW in the 2022 PDM3 On-site PROGRAM), but not their Abstracts.