fsrmm
fondation suisse de recherche sur les maladies musculaires
fondazione svizzera per la ricerca sulle malattie muscolari
schweiz. stiftung für die erforschung der muskelkrankheiten

13th Swiss Meeting on Muscle Research

Macolin / Magglingen

5th-7th December 2021

Program and Abstracts

The Swiss Meeting on Muscle Research was launched by the FSRMM in 1996 based on the initiative of its former Scientific Director, Prof. Denis Monard, as a biannual meeting to learn about the progress of funded projects.

Meanwhile, it has developed into a meeting for all researchers working in basic science and clinics who are devoted to the understanding and treatment of neuromuscular diseases in Switzerland. The purpose of the meeting is to discuss the latest results, initiate collaborations, exchange samples and ideas and to get to know each other better.

To foster scientific exchange and to promote young scientists, the FSRMM keeps the participation in this meeting free of charge.

Program

Sunday, December 5th

16:00-17:30 Arrival, Check-in

17:30-18:30 Welcome Apero

18:30-19:30 Dinner

19:30-19:35	Meeting opening (Markus A. Rüegg)
19:35-20:20	Keynote lecture: Muscles have feelings too – muscle spindles in healthy and diseased muscles Special guest: Stephan Kröger, Center for Neurosciences, Ludwig-Maximilians-University, Munich

Session 1: Targeting the extracellular matrix **Chair: Matthias Chiquet**

20:20-20:50	Therapeutic effect of linker protein-mediated gene therapy in a mouse model for *LAMA2*-related muscular dystrophy Judith Reinhard, University of Basel
20:50-21:20	New aspects of TGFβ signalling in muscle homeostasis and regeneration Michael Bachmann, University of Geneva

Monday, December 6th

Session 1: Targeting gene regulation — **Chair: Francesco Zorzato**

8:30-8:55	Targeted degradation of the DUX4 protein as a therapeutic strategy against FSHD Christian Klingler, University of Basel
8:55-9:20	An AAV/CRISPR tool for functional gene interrogation in adult skeletal muscle Marco Thürkauf, University of Basel
9:20-9:45	Identification of potential drugs to treat Myotonic Dystrophy Jochen Kinter, University of Basel
9:45-10:10	Identification of the critical regulators of protein synthesis in human muscle Lionel Tintignac, University of Basel
	10:10-10:40 Coffee break
10:40-11:05	Interaction of U1SNRNP with drugs and RNA binding proteins in splicing regulation and neuromuscular diseases Frédéric Allain, ETH Zürich
11:05-11:30	Pharmacological treatment of an animal model carrying recessive RYR1 mutations with inhibitors targeting epigenetic enzymes Alexis Ruiz, University of Basel

Session 2: Targeting NMJ and innervation — **Chair: Markus A. Rüegg**

11:30-11:55	CaMKII deregulation and changes in neuromuscular junctions in Type I Myotonic Dystrophy Perrine Castets, University of Geneva
11:55-12:20	Muscle contractions evoked using wide-pulse high-frequency neuromuscular electrical stimulation as a model to study muscle cramps Nicolas Place, University of Lausanne
	12:20-13:50 Lunch

Poster session 1:

13:50-15:30	POSTERS Nr. 1-14

Session 3: Diagnostic and Imaging **Chair: David Jacquier**

15:30-15:55 Automatic extraction of morphometry of peripheral nerves in magnetic resonance neurography
Olivier Scheidegger, University Hospital Bern

15:55-16:20 Muscular complications of COVID-19 infections
Werner Z'Graggen, University Hospital Bern

16:20-16:45 Recent advances in Muscle MRI
Francesco Santini, University of Basel

16:45-17:15 Coffee break

Session 4: T-cells involvement in disease **Chair: Michael Sinnreich**

17:15-17:40 Characterization of autoreactive T-cells in Guillain-Barré syndrome
Daniela Latorre, ETH Zürich

17:40-18:05 Single-cell profiling of myasthenia gravis identifies a pathogenic T cell signature
Bettina Schreiner, University Hospital Zürich

Session 5: Targeting sphingolipid metabolism **Chair: Leonardo Scapozza**

18:05-18:30 Mutations in the Serine-palmitoyltranferase (SPT) cause juvenile Amyotrophic Lateral Sclerosis (jALS)
Museer Lone, University of Zürich

18:30-18:55 Inhibition of sphingolipid de novo synthesis counteracts muscular dystrophy
Martin Wohlwend, EPFL Lausanne

19:00-20.30 Dinner

Evening program

20:30-22:30 free beer at poster site

Tuesday, December 7th

Session 1: Targeting metabolic pathways **Chair: Olivier Dorchies**

8:30-8:55 Involvement of metabolic remodelling in the development of dysferlinopathies
Regula Furrer, University of Basel

8:55-9:20 Distinct and additive effects of calorie restriction and rapamycin in aging skeletal muscle
Daniel Ham, University of Basel

9:20-9:45 Towards a tailored NADPH oxidases targeted therapy in models of Duchenne muscular dystrophy
Hesham Hamed, University of Geneva

9:45-10:10 Functional improvement of neuromuscular parameters with recombinant interleukin-6 myokine treatment in old mice
Christoph Handschin, University of Basel

10:10-10:35 Results of the Tamoxifen Trial
Dirk Fischer, University Hospital Basel

10:35-11:05 Coffee Break

Poster session 2:

11:05-12:30 POSTERS Nr. 15-32

12:30-14:00 Lunch

Meeting Poster prizes committee over lunch

Session 2: New model systems **Chair: Bernhard Wehrle-Haller**

14:00-14:25 Towards better models of Duchenne muscular dystrophy? Development and preliminary phenotyping of new murine lines
Laurence Neff, University of Geneva

14:25-14:50 A vascularized human muscle-on-a-chip to elucidate the contribution of vascular degeneration on the progression of muscular dystrophies
Simone Bersini, Ente Ospadaliero Cantonale, Lugano

Session 3: Targeting stem cells **Chair: Maud Frieden**

14:50-15:15 Integrative molecular roadmap for direct conversion of fibroblasts into myocytes and myogenic progenitor cells
Ori Bar-Nur, ETH Zürich

15:15-15:40 Metabolic regulation of adult muscle stem cells
Alexandre Prola, University of Geneva

15:40-16:05 Therapeutic potential of human myogenic reserve cells in Duchenne Muscular Dystrophy
Thomas Laumonier, University of Geneva

16:05-16:35 Coffee break

Session 3: Targeting mitochondrial function **Chair: Susan Treves**

16:35-17:00 Inter-organelle contact sites as novel therapeutic targets?
Francesca Amati, University of Lausanne

17:00-17:25 BCL2L13 at the hub of inter-organelle communication
Dogan Grepper, University of Lausanne

17:25: 17:50 A natural activator of mitochondrial calcium import increases muscle bioenergetics and performance during aging
Jérome Feige, Nestlé Institute of Health Sciences, Lausanne

17:50-18:10 Concluding remarks and poster prizes (Markus Rüegg)

-please remove posters now-

Departure

Abstracts

Contact: judith.reinhard@unibas.ch

Therapeutic effect of linker protein-mediated gene therapy in a mouse model for *LAMA2*-related muscular dystrophy

Judith R. Reinhard

Biozentrum, University of Basel, Basel, Switzerland

LAMA2-related muscular dystrophy (*LAMA2* MD or MDC1A) is the most frequent form of congenital muscular dystrophies. It is caused by mutations in *LAMA2*, the gene encoding laminin-α2, one chain of the heterotrimeric extracellular matrix protein laminin-211 (α2β1γ1). Most patients lack laminin-α2 due to biallelic loss-of-function mutations in *LAMA2*. The large size of the cDNA encoding laminin-α2 and the heterotrimeric structure of laminin-211 present a challenge for gene replacement or gene editing strategies. Here, we describe the development of an AAV-mediated gene therapy to functionally replace laminin-211 by two small linker proteins. Mini-agrin (mag) and αLNNd are predicted to be well tolerated as they are designed from domains that are found in proteins also expressed in *LAMA2* MD patients. Additionally, the linker proteins are secreted and act in the extracellular matrix, which allows for high targeting and expression efficiency by AAV. We have previously demonstrated the correction of the muscular dystrophy by the transgenic expression of mag and αLNNd in dyW/dyW (*Lama2* KO) mice. We now show high expression of both linkers by simultaneous, intravenous injections of two AAVs expressing either mag and αLNNd at postnatal day 1 and a significant improvement of the disease phenotype in *Lama2* KO mice. Starting at the age of 4 weeks, AAV-injected mice gained significantly more weight; at 8 weeks of age, the body and muscle weights were 2 times higher than in vehicle-injected *Lama2* KO mice. Further improvements included a significant increase in grip strength, a substantial increase in myofiber size and a strong reduction of fibrosis. In summary, our study serves as a proof-of-concept in mice and establishes systemic delivery of AAVs that express the two linkers as a promising gene therapy for treating *LAMA2* MD.

Contact : michael.bachmann@unige.ch

New aspects of TGFβ signaling in muscle homeostasis and regeneration

Michael Bachmann

University of Geneva

Cytokines of the transforming growth factor β (TGFβ) family control numerous aspects of muscle development and homeostasis. TGFβs are dimeric proteins and are anchored to the extracellular matrix in a latent complex, from which they are released by different mechanisms. Transcriptomic data indicate that mostly TGFβ2 and TGFβ3 are expressed in skeletal muscles under steady-state conditions. Interestingly, mutations in TGFβ2 or 3 have overlapping phenotypes affecting several organs including the musculo-skeletal system (e.g. Loeys-Dietz syndrome). Motivated by the highly overlapping phenotypes of TGFβ2/3-related syndromes, we asked, whether these TGFβs can heterodimerize. Indeed, we observed that TGFβ2 forms a heterodimer with TGFβ3 in different cell types including mouse myoblasts (C2C12). Heterodimeric forms of TGFβ have not been reported previously and their function and activation mechanism are therefore not described. Based on recent reports about inhibition of muscle fusion by TGFβ-signaling, we studied whether different TGFβs affect fusion of C2C12 cells and how integrins interact with different homo- or dimeric forms of TGFβs. Our results show that activated TGFβ2/3 inhibits muscle fusion similar to TGFβ1, a finding that correlates with enhanced TGFβ2 and 3 expression in skeletal muscle and in satellite cells. In order to activate signaling, TGFβ1 and TGFβ3, but not TGFβ2, rely on activation by integrins while being anchored to the extracellular matrix. We show that TGFβ2/3 binds to αVβ6 integrin expressing cells with high affinity, while homodimeric TGFβ2 fails to interact. Interestingly, we identified other integrins that bind TGFβ2, and are therefore additional potential candidates to activate heterodimeric TGFβ2/3 in vivo. To conclude, myoblast fusion is an essential part of muscle development and regeneration. TGFβ cytokines have not only been linked to muscle fusion, but also to proliferation and differentiation of muscle stem cells. Thus, understanding the different activation and signaling modes of TGFβs is crucial in order to help patients with myopathies. Our data describe a new heterodimeric TGFβ and demonstrate its impact on muscle fusion. Our future work will evaluate downstream signaling and activation of TGFβ2/3 and its importance for muscle homeostasis.

Contact: christian.klingler@unibas.ch

Targeted degradation of the DUX4 protein as a therapeutic strategy against FSHD

Christian Klingler

University Hospital Basel

Aberrant expression of the transcription factor double homeobox protein 4 (DUX4) can lead to a number of diseases including facio-scapulo-humeral muscular dystrophy (FSHD), acute lymphoblastic leukemia, and sarcomas. Inhibition of DUX4 may represent a therapeutic strategy for these diseases. By applying Systematic Evolution of Ligands by EXponential Enrichment (SELEX), we identified aptamers against DUX4 with specific secondary structural elements conveying high affinity to DUX4 as assessed by fluorescence resonance energy transfer and fluorescence polarization techniques. Sequences analysis of these aptamers revealed the presence of two consensus DUX4 motifs in a reverse complementary fashion forming hairpins interspersed with bulge loops at distinct positions that enlarged the binding surface with the DUX4 protein, as determined by crystal structure analysis. We demonstrate that insertion of specific structural elements into transcription factor binding oligonucleotides can enhance specificity and affinity. After circularization of DUX4 DNA-oligonucleotides to increase biological stability and attachment of several chemical modifications, we use these structures to establish experimental conditions and parameters in several biochemical assays like TR-FRET as well as cellular assays to generate proteolysis targeted chimeras (PROTACs) that bring the transcription factor DUX4 into proximity to an E3 Ubiquitin ligase, thereby initiating its ubiquitination as neo-substrate and subsequent degradation by the proteasome. Successful compounds will be an essential and valuable component in the strategy to counteract the hitherto untreatable disease FSHD and other DUX4-mediated diseases.

Contact: marco.thuerkauf@unibas.ch

An AAV/CRISPR tool for functional gene interrogation in adult skeletal muscle

Marco Thürkauf

Biozentrum, University of Basel

Traditional in vivo gene of interest (G.O.I) interrogation techniques are highly animal consuming and hardly multiplexable. This remains a huge bottleneck in the context of target gene screening in aging skeletal muscle. To overcome these limitations, we are establishing a method using AAV-CRISPR/Cas9 for functional testing of candidate genes in adult skeletal muscle. Here, we use a mouse model that expresses Cas9 specifically in skeletal muscle fibers and deliver single guide RNA via adeno-associated viruses (AAV) delivery. Using a novel and highly myotrophic AAV variant, AAVMYO (J. Weinmann, 2020) we achieve high infection rates that are sufficient to introduce permanent somatic mutations into the G.O.I., leading to the loss of protein. This method is highly efficient upon local or systemic delivery of the sgRNA-containing AAV. In summary, our data show that this method can be used to characterize the function of genes in adult skeletal muscle without the need to go through lengthy breedings. Moreover, this method may also allow to interrogate gene function of several genes at the same time.

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Identification of potential drugs to treat Myotonic Dystrophy

Jochen Kinter

University Hospital Basel

Myotonic Dystrophy type I (DM1) is the most common form of muscular dystrophy in adults with an estimated prevalence of 1:8'000. This autosomal dominantly inherited disease affects multiple organ systems, most visibly the skeletal muscle with wasting, weakness and inability to relax (myotonia). There is currently no causal treatment available for this disabling disease. In contrast to the majority of genetic diseases, DM1 is not caused by mutated protein(s) but by a toxic effect of an RNA molecule. A sequence of CTG triplet repeats, which is located in the 3'untranslated region of the DMPK gene, is abnormally elongated in patients. On the mRNA level the corresponding sequence of expanded CUG repeats (CUGexp) sequester the splicing factor muscleblind-like 1 (MBNL1). The resulting lack of available MBNL1, due to RNA binding, leads to misregulated alternative splicing of various genes, explaining the multisystemic involvement in DM1. Disease severity and age of disease onset correlate with the number of triplet repeat expansions. We have developed a screening and validation cascade for the identification of small molecular weight compounds for the treatment of myotonic dystrophy type 1 (DM1) and other diseases in which sequestration of the splice modulator MBLN1 by YGCY RNA motifs (Y=pyrimidine) is the pathogenic basis. The screening cascade includes biochemical assays to test for MBNL1–RNA complex inhibition, selectivity assays, RNA stabilization assays, cellular splice assays by multiplex PCR, as well as foci accumulation assays and isolated muscle fiber assays. In vivo assays include RNAseq analysis to monitor potential improvement in missplicing, as well as ex vivo myotonia measurements. With this screening cascade we have identified compounds that are active at biochemical and cellular levels, and which can improve a flight deficit in a transgenic drosophila model of (DM1), expressing 480 CUG repeats. We are currently designing the mouse model experiments in HSA-LR mice with the identified compounds.

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Identification of the critical regulators of protein synthesis in human muscle

Lionel Tintignac

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The imbalance between protein synthesis and protein degradation is a common pathogenic mechanism underlying the vast majority of muscle diseases. Therefore, identifying the molecular networks that specifically control the interplay between muscle protein synthesis and degradation has become a prerequisite to the development of therapeutic strategies counteracting muscle atrophy. Within the financial support of the FSRMM we established a cellular model for the identification of the endogenous protein networks and translational shift centered on 2 crucial and antagonistic regulators of protein metabolism in muscle, the f-subunit of the eukaryotic translation initiation factor 3 (EIF3F) and the muscle atrophy F-box protein (MAFbx). We genetically engineered human myoblasts cell lines endogenously expressing BioID-tagged (EIF3F or MAFbx) chimeric proteins for in situ proximity-dependent labelling assay. For this purpose, we employed the CRISPR-Cas9 technology to insert the optimized bacterial BirA* biotin ligase (HA tagged mutant R118G) in frame with the C-terminus sequence of respectively the EIF3F locus (Chr.11, Exon10) or the MAFbx locus (Chr.11, Exon9). We used flow cytometry assisted cell sorting to isolate and expand single cell colony before their selection by genotyping and sequencing of the recombined targeted locus. With this approach we successfully generated human immortalized myoblasts heterozygote for EIF3F_HA-BirA* or MAFBX_HA-BirA*. Here we will present the results generated with the EIF3F_HA-BirA* cell line where we used the muscle differentiation paradigm to explore the EIF3F functional regulation of translation and discuss our ongoing model for EIF3F dependent regulation of translation in muscle cells.

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Interaction of U1SNRNP with drugs and RNA binding proteins in splicing regulation and neuromuscular diseases

Frédéric Allain

ETH Zürich

U1snRNP is the first element of the spliceosome to recognize the pre-mRNA via base-pairing with the intron 5' splice-site (5'SS). Suboptimal base-pairing between the U1snRNA and the 5'SS is at the origin of the alternative-splicing of a great number of exons. Trans-acting splicing factor like SR protein and hnRNP protein or even small molecule can influence positively or negatively these key steps in the splicing reaction. We will present structural evidence using NMR spectroscopy and other structural biology techniques on how small molecule drugs or alternative-splicing factors can bind U1snRNA and enhance U1snRNP-5'SS recognition and therefore explain their mechanism of action in splicing regulation and neuro-muscular disease like SMA and ALS.

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Pharmacological treatment of an animal model carrying recessive RYR1 mutations with inhibitors targeting epigenetic enzymes

Alexis Ruiz

University Hospital Basel

Although recessive RYR1 mutations are the underlying molecular signature of severe congenital myopathies leading to proximal muscle weakness and a poor quality of life of affected children, few studies have focused on the elucidation of their pathophysiological mechanisms and on the treatment of these disorders. Recessive RYR1 mutations cause reduction of RyR1 expression in skeletal muscle and are associated with fibre hypotrophy and general muscle weakness. In addition, muscles of patients with recessive RYR1 mutations exhibit increased DNA methylation and expression of class II histone de-acetylases (HDACs). We developed a mouse model knocked-in for compound heterozygous RYR1 mutations (Q1970fsX16+A4329D) isogenic to those identified in a severely affected patient with recessively inherited multi-minicore disease. The phenotype of this mouse model closely resembles that of patients carrying a hypomorphic RYR1 allele + a missense mutation in the other allele, making it an ideal model to study the effect of drugs aimed at improving muscle function. In the present investigation we treated wild type and compound heterozygous RYR1 mutant mice with inhibitors of DNA methyltransferases and HDACs. Such a treatment resulted in improved muscle strength in the mutant mice starting from 6 weeks of drug administration. We will present the results of our study, in particular pharmacokinetics, in vivo and ex vivo physiological results as well as biochemical analysis of muscles from treated mice. The results of this study are important as they pave the way for the development of pharmacological strategies to treat patients affected by ryanodinopathies accompanied by a decrease in RyR1 protein content.

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CaMKII deregulation and changes in neuromuscular junctions in Type I Myotonic Dystrophy

Perrine Castets

University of Geneva

Myotonic Dystrophy type I (DM1) is a multi-systemic neuromuscular disorder, which constitutes the most common form of muscular dystrophy in adults. DM1 is caused by a (CTG)n repeat expansion in the 3' UTR of the *DMPK* gene, which leads to toxic elongated transcripts, sequestration of multiple RNA-binding proteins, and ultimately defective splicing of numerous genes. Especially, we and others have shown that mis-splicing affects the expression of Ca2+/calmodulin-dependent protein kinases (CaMK) in DM1 muscle. In parallel, previous studies have reported changes in pre- and post-synaptic compartments of neuromuscular junctions (NMJs) in muscle from DM1 patients and mouse models. However, the underlying pathomechanisms and whether NMJ decline contributes to muscle dysfunction remain unknown. CaMKIIs are key players in muscle plasticity, by regulating activity-dependent gene expression and the recycling of synaptic proteins, such as acetylcholine receptors (AChR) at post-synaptic sites. Thus, we investigated whether CaMKII deregulation may contribute to NMJ decline in DM1. To this end, we explored changes in NMJ and activity-dependent signalling in HSALR and Mbnl1 knock-out mice, two well-known mouse models for DM1. Both models displayed increased fragmentation of motor endplate, associated with high turnover of AChRs and increased expression of synaptic genes. These changes correlated with the loss of the muscle-specific isoform of CaMKIIβ (CaMKIIβM) and nuclear enrichment of histone deacetylase 4 (HDAC4). Upon nerve injury, endplate fragmentation and AChR turnover further increased in DM1. Unexpectedly, this occurred despite defective up-regulation of synaptic genes in DM1 denervated muscle. This abnormal response to denervation was likely independent from CaMKII deficiency. Overall, our study suggests that NMJ decline involves CaMKII-dependent and -independent mechanisms and contributes to muscle dysfunction in DM1.

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Muscle contractions evoked using wide-pulse high-frequency neuromuscular electrical stimulation as a model to study muscle cramps

Nicolas Place

University of Lausanne

Muscle cramps consist of a sudden, involuntary and painful contraction of a muscle, self-extinguishing within a few seconds to minutes aided by passive stretching. Cramps can be part of clinical syndromes, but most cramps are idiopathic. Despite a prevalence of 30-50% in healthy individuals, the aetiology and best management strategies to prevent and stop muscle cramps remain unclear. The prevailing hypothesis of muscle cramp development is the so-called neuromuscular hypothesis, supported by the observation of self-sustained firing of motor units. This central origin for muscle cramp contractions resembles the generation of muscle contractions using wide-pulse, high-frequency neuromuscular electrical stimulation (WPHF NMES). The use of WPHF NMES (pulse duration: 1 ms, stimulation frequency: 100 Hz) increases the electrically-evoked sensory volley travelling to the central nervous system via large diameter afferents and subsequent reflex activation of motor units in the spinal cord. Responders to WPHF NMES show progressive increases in force ('extra force') during the stimulation, which can reach up to 70% maximal voluntary contraction (MVC) force with a low stimulation intensity targeting an initial force of 5-10% MVC force. Current evidence suggests that this extra force is due to reflexive recruitment of motoneurons and may be attributed to the activation of persistent inward currents (PICs) in spinal motoneurons, which increase the input/output gain of the motor pool. Thus, there may be similarities between the origin of muscle cramp contractions and the extra force observed during evoked muscle contractions using WPHF NMES. The aim of the present study was to investigate whether WPHF NMES could constitute a model to study the mechanisms underlying muscle cramps. Twenty-seven healthy individuals (26±6 years) were recruited for this study and all experiments were performed on the dominant plantar flexors. The susceptibility to develop cramp was assessed using electrically-induced cramp threshold frequency, and the extra-force evoked during 20s WPHF NMES trains at an initial level of 5% MVC force was quantified. High-density surface electromyography (soleus and gastrocnemius medialis muscles) recordings and decomposition analysis were used to estimate PICs in crampers vs. non- crampers. Data analysis is ongoing and results will be presented at the conference. If our investigations prove successful, it may provide a model in which it is possible to test therapeutic possibilities for the management of motoneuron hyperexcitability, a hallmark of several neuromuscular disorders.

Contact: olivier.scheidegger@insel.ch

Automatic extraction of morphometry of peripheral nerves in magnetic resonance neurography

Olivier Scheidegger

University Hospital Bern

Magnetic resonance neurography (MRN) has emerged as a complementary diagnostic tool for peripheral neuropathy to the state-of-the-art relying on neurological examination and electrodiagnostic studies (EDS). MRN provides advantages compared to EDS such that it enables physicians to assess deeply situated nerves and nerves close to the trunk including their surrounding tissues. However, MRN remains a qualitative approach despite that a quantification could assist in the diagnosis and monitoring of peripheral neuropathies in terms of cross-sectional and volume biomarkers calculated from segmented peripheral nerves. Therefore, we developed an automatic machine learning method for segmenting peripheral nerves from MRN images of extremities based on convolutional neural networks (CNNs). We propose a three-step segmentation method: First, an MRN image is processed by a CNN to obtain a coarse probability map of the location of the peripheral nerves. Second, the probability map is transformed into a point cloud representation. Third, the point cloud is processed by a second CNN that classifies point-wise into peripheral nerve or background yielding a segmented peripheral nerve. For evaluation, we used 52 turbo spin-echo T2-weighted MRN images of the thigh of healthy volunteers (n=10) and patients diagnosed with peripheral neuropathy (n=42). Sequence parameters were TR of 4690ms, TE of 82ms, FOV of 384×330mm2, FA of 134°, voxel size of 0.52×0.52×4.0mm3, and 60 axial-oriented slices with gap of 0.4mm. The sciatic nerve was manually segmented by three raters for inter-rater variability quantification and four-fold cross-validation was applied for evaluation. The segmentation yields Dice coefficients of 0.866±0.044, volumetric similarities of 0.946±0.041, and 95th percentile Hausdorff distances of 4.5±9.7mm. Inter-rater variabilities are 0.802±0.091, 0.905±0.082, and 9.2±17.6mm. Therefore, the approach achieved statistically significant better results for the Dice coefficient and the volumetric similarity and on-par results for the Hausdorff distance compared to the inter-rater variability. Qualitatively, 3-D renderings show a high agreement between the automatic and manual segmentations. The results suggest that peripheral nerve segmentation from MRN is feasible and achieves human inter-rater performance. Such automatic segmentation might allow quantifying peripheral neuropathies for disease progression and outcome or serve as volume of interest for further quantitative MRN techniques.

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Muscular complications of COVID-19 infections

Werner Z'Graggen

University Hospital Bern

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing COVID-19 primarily targets the respiratory system but can affect also other organ systems. Two muscular complications of COVID-19 infections have now become apparent: (i) Critical illness myopathy, and (ii) direct muscular SARS-CoV-2 virus affection and/or parainfectious muscular complication. Critical illness myopathy is a primary and acquired myopathy that can develop as a sequel of intensive care treatment, and is associated with higher mortality and morbidity rates, the need for longer rehabilitation and reduced re-integration into former life, and ultimately increased health-care costs. In a prospective cohort study, we found that the incidence of critical illness myopathy in COVID-19 patients is almost twice as high as it has been reported in non-COVID-19 populations. Using muscle excitability measurements, we could show that changes of muscle membrane properties are part or even an early factor in the evolution of critical illness myopathy, and that muscle excitability measurements can be used for early diagnosis and disease monitoring. Furthermore, the previously postulated association of critical illness myopathy with neuromuscular blocking agents could not be confirmed. Unexpectedly, we also found muscle membrane depolarization and a low myosin:actin ratio, as a sign of an underlying loss of myosin filaments, in a young patient who experienced prolonged fatigue and exercise intolerance after a mild COVID-19 infection. The observed muscle membrane potential change and the loss of myosin as a sign of concomitant structural alterations may be attributed to either a direct muscular SARS-CoV-2 virus affection or a parainfectious muscular complication. Muscular involvement in Covid-19 may therefore play an important role for development of long-Covid.

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Recent advances in Muscle MRI

Francesco Santini

University of Basel

In recent years, the usage of MR for the imaging of the muscle has increased in popularity, because of its possibility of objectively quantifying important parameters over the whole organ, such as muscle volume, degree of fat infiltration, and edema. The focus has been shifting from qualitative imaging towards quantitative approaches, that deliver a higher level of confidence and have the potential of becoming biomarkers for the follow-up of neurodegenerative diseases and the monitoring of novel therapies. Additionally, the flexibility of MRI allows the visualization of the dynamics of the muscle during its contraction and the derivation of additional quantitative parameters, such as muscle strain and contraction speed. In order to obtain useful clinical information from these emerging methods, however, the availability of solutions that are both patient- and operator-friendly is necessary. Since its foundation in 2015, thanks to the support of the FSRMM, our group “Basel Muscle MRI” (BaMM) at the University of Basel has been striving to translate these technological advances in the field of muscle MRI into a clinical reality. Specifically, we have perfecting dynamic muscle imaging during electrical muscle stimulation, obtaining promising results in volunteers and patient populations with facioscapulohumeral dystrophy and cerebral palsy. However, we have also acquired a comprehensive experience in imaging in general, devising modern algorithms for image postprocessing and analysis, based on efficient computation and advanced deep learning methods. In this presentation, I will give an overview of the current state of the art and of the emerging technologies in muscle MRI, both from the experience of our group in Basel and from the international community.

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Characterization of autoreactive T cells in Guillain-Barré syndrome

Daniela Latorre

ETH Zürich

Guillain-Barré syndrome (GBS) is considered an autoimmune disorder of the peripheral nervous system (PNS) in which the contribution of pathogenic autoreactive T lymphocytes targeting PNS antigens has been strongly supported by in vivo studies. However, the underlying immune-mediated mechanisms in humans are far from clear. The overall aim of this study is to gain insights into this issue by investigating the existence and providing an in-depth characterization of the autoreactive T cell response in GBS patients during the acute and recovery phases of the disease. Flow cytometry analysis of ex-vivo PBMCs revealed altered frequencies of immune cell populations in GBS patients, thus pointing to an involvement of an aberrant immunity in the disease. Notably, by using a recently established sensitive workflow based on ex vivo T cell screenings, generation of single T cell clones and TCR sequencing, here we reveal the existence of self-reactive T cells in GBS patients. Memory CD4+ T cells targeting self-antigens of the PNS were detected in all GBS patients analyzed so far, whereas they resulted almost absent in healthy controls. Moreover, by analyzing more than 400 autoreactive single T cell clones, we found that these cells show a polyclonal TCR repertoire, target multiple epitopes of the self-antigens with some immunodominant regions and are mostly HLA-DR restricted. Collectively, our data provide the first description of self-reactive T cells directed against PNS myelin proteins in GBS patients, thus opening new perspective for biomedical application.

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Single-cell profiling of myasthenia gravis identifies a pathogenic T cell signature

Bettina Schreiner

University Hospital Zürich

Myasthenia gravis (MG) is an autoimmune disease characterized by impaired neuromuscular signaling due to autoantibodies targeting the acetylcholine receptor. Although its auto-antigens and effector mechanisms are well defined, the cellular and molecular drivers underpinning MG remain elusive. Here, we employed high-dimensional single-cell mass and spectral cytometry of blood and thymus samples from MG patients in combination with supervised and unsupervised machine-learning tools to gain insight into the immune dysregulation underlying MG. By creating a comprehensive immune map, we identified two dysregulated subsets of inflammatory circulating memory T helper (Th) cells. These signature ThCD103 and ThGM cells populated the diseased thymus, were reduced in the blood of MG patients, and were inversely correlated with disease severity. Both signature Th subsets rebounded in the blood of MG patients after surgical thymus removal, indicative of their role as cellular markers of disease activity. Together, this in-depth analysis of the immune landscape of MG provides valuable insight into disease pathogenesis, suggests novel biomarkers and identifies new potential therapeutic targets for treatment.

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Mutations in the Serine-palmitoyltranferase (SPT) cause juvenile Amyotrophic Lateral Sclerosis (jALS)

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of the lower and upper motor neurons characterized by severe muscle wasting, leading eventually to paralysis and death. Majority of ALS cases occur sporadically, however a small yet increasing number of cases are being related to pathogenic genetic variants with a Mendelian inheritance. Mutations in SPTLC1 subunit of the enzyme Serine-Palmitoyltransferase (SPT) were identified and associated with childhood-onset ALS. SPT catalyzes the first and the rate-limiting step in the de-novo sphingolipid synthesis. The activity of the endoplasmic reticulum (ER) localized SPT is regulated by a feedback inhibitory mechanism. This involves interactions of the only transmembrane domain of SPTLC1 with a family of sphingolipid sensory proteins, Ormdl1-3. The nine non-related families with six novel dominant mono-allelic pathogenic variants in SPTLC1 were associated with this condition. All ALS mutations map to exon-2, encoding the SPTLC1 transmembrane domain. Several SPT mutations are linked to HSAN1, a predominantly sensory neuropathy that is caused by pathological levels of neurotoxic 1-deoxysphingolipids. Analysis of sphingolipid profiles in SPT-ALS patient plasma revealed distinct sphingolipid profile, that was associated with a significant increase in certain sphingolipid species but the absence of 1-deoxysphingolipids. This project aims to understand how these observed perturbances in the SL homeostasis lead to ALS and whether the observed metabolic changes can be corrected therapeutically using either a pharmacological or genetic approach.

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Inhibition of sphingolipid *de novo* synthesis counteracts muscular dystrophy

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Duchenne muscular dystrophy (DMD), the most common muscular dystrophy, is a severe muscle disorder, causing muscle weakness, loss of independence, and premature death. Here, we establish the link between sphingolipids and muscular dystrophy. Transcripts of sphingolipid *de novo* biosynthesis pathway are upregulated in skeletal muscle of patients with DMD and other muscular dystrophies, which is accompanied by accumulation of metabolites of the sphingolipid pathway in skeletal muscle and plasma. Pharmacological inhibition of sphingolipid synthesis by myriocin in the *mdx* mouse model of DMD ameliorated the loss in muscle function while reducing inflammation, improving Ca2+ homeostasis, preventing skeletal muscle, heart, and diaphragmatic fibrosis, and restoring the balance between M1 and M2 macrophages. Combination therapy of myriocin with glucocorticoid used in standard care of DMD alleviated DMD phenotype more than glucocorticoid monotherapy. Our study identifies inhibition of sphingolipid synthesis, targeting multiple pathogenetic pathways simultaneously, as strong candidate for treatment of muscular dystrophies.

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Involvement of metabolic remodeling in the development of dysferlinopathies

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Dysferlinopathies are muscular dystrophies that are caused by a mutation in the dysferlin gene. Dysferlin plays a prominent role in membrane repair and dysferlin-deficient muscles develop a progressive muscular dystrophy. Currently, effective treatments for dysferlinopathies are lacking. Unexpectedly, even though experimental approaches can successfully restore membrane repair, muscles remain dystrophic, suggesting that additional, so-far unknown dysferlin-dependent functions might be involved in the development of the pathological phenotype. Therefore, alternative or complementary therapeutic interventions are needed. In many muscle diseases including Duchenne muscular dystrophy, overexpression of PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) in skeletal muscle ameliorates muscle fiber atrophy, integrity and function. Therefore, we were interested in the potential therapeutic effect of muscle PGC-1α on dysferlinopathy. Surprisingly however, the pathology of dysferlin-deficient mice was exacerbated by elevated levels of PGC-1α. Our findings furthermore reveal novel and intriguing aspects of dysferlinopathies that could provide important insights into the patho-etiology and –mechanism of the disease. We demonstrate that dysferlinopathy is not only a disease involving impaired membrane repair but also exhibiting a substantial metabolic dysregulation. The better understanding of processes involved in disease progression can have meaningful implications in the treatment of patients with dysferlinopathy.

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Distinct and additive effects of calorie restriction and rapamycin in aging skeletal muscle

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As global life expectancy continues to climb, maintaining skeletal muscle function is increasingly essential to ensure a good life quality for aging populations. Calorie restriction (CR) is the most potent and reproducible intervention to extend health and lifespan, but is largely unachievable in humans. Therefore, identification of "CR mimetics" has received much attention. CR, along with many of the interventions known to prolong lifespan, dampen activity of nutrient-sensing pathways, which center around the mammalian target of rapamycin complex 1 (mTORC1), thereby alleviating protein synthetic burden and promoting intrinsic quality control processes, like autophagy. The mTORC1 inhibitor, rapamycin (RM), has been proposed as a potential CR mimetic and is proven to counteract age-related muscle loss. Therefore, we tested whether RM acts via similar mechanisms as CR to slow muscle aging. Long-term CR and RM treatment both promoted a fast-to-slow muscle fiber type transition and improved multiple measures of relative muscle function in aging skeletal muscle. However, contrary to our expectation, CR and RM-treated geriatric mice displayed almost entirely distinct skeletal muscle gene expression profiles, suggesting different mechanisms of action. Moreover, CR improved muscle integrity and markedly reduced P62 accumulation in a mouse model where nutrient-insensitive, sustained muscle mTORC1 activity drives a premature muscle aging phenotype. Furthermore, and most conclusively, combined RM and CR treatment induced additive effects in aging mouse muscles, including improved relative grip strength, isolated muscle force and muscle mass as well as a fast-to-slow fiber type switch. These data demonstrate that RM and CR exert distinct, compounding effects in aging skeletal muscle and they open the possibility of parallel interventions to counteract muscle aging.

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Towards a tailored NADPH oxidases targeted therapy in models of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscular disease that causes premature death and for which no cure exists. There is a general consensus that a state of oxidative stress is one of the main pathophysiological features both in DMD and in animal models of this pathology. We have previously shown evidence that NADPH oxidases (NOXes) contribute to excessive production of reactive oxygen species in dystrophic muscles which subsequently results in enhanced calcium entry. We have also shown that targeting NOXes with pharmacological inhibitors improves various pathological features of this disorder both in vitro and in vivo. To further validate and explore NOXes as novel therapeutic targets in DMD, we have embarked on a multi-axis approach ranging from in silico transcriptomics and drug design to in depth in vivo analyses. Novel tools for monitoring NOX activity in muscle fibres have been evaluated. New NOX2 and NOX4 inhibitors have been tested in dystrophic cultures and isolated muscles. Profiling NOXes expression in different stages of the disease have also been done. In vivo testing of diapocynin in a curative rather than preventive setting was performed. Finally, the selective genetic invalidation of NOX4 from dystrophic muscles was done and followed over different stages of the disease. The findings of this project reinforced our understanding of the pathogenesis of DMD and revealed the role played by NOXes in aggravating muscle damage. It also consolidated the hypothesis that targeting NOXes can become a potential biochemical disease modifier.

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Functional improvement of neuromuscular parameters with recombinant interleukin-6 myokine treatment in old mice

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In elderly individuals, but also in many neuromuscular dystrophy patients, preservation of functional capacities is of uttermost importance. Unfortunately, training interventions in such populations are difficult due to issues with tolerance, acceptance and compliance. We therefore aimed at leveraging the effect of myokines, mediators of plasticity evoked by exercise, to modulate muscle functionality, using sarcopenia as experimental paradigm. Indeed, mice exposed to pulsatile injection of the myokine interleukin-6 showed an improved response to training interventions, without observable adverse effects and toxicity. Administration of recombinant IL-6 might therefore constitute a safe and well tolerated treatment modality to boost functional capacity in various pathological settings.

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Results of the Tamoxifen Trial

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Towards better models of Duchenne muscular dystrophy?

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Development and preliminary phenotyping of new murine lines Although the *mdx*/*mdx*5Cv mice and *dko* mice have been widely used for decades, they do not properly replicate the disease severity and progression experienced by Duchenne muscular dystrophy (DMD) patients. The D2-*mdx* mouse (DBA/2J background) that recently attracted a lot of interest also has pros and cons. The need for mouse models that would be better phenocopies of DMD is largely acknowledged in the Duchenne community. We have generated several new mouse lines designed to match more closely the pathogenic features, disease progression, and overall severity of DMD than do existing lines of dystrophic mice. After a preliminary phenotyping, we selected for further development: 5Cv/Uflox MC (for controlled down-regulation of utrophin from skeletal muscles) and 5Cv/L (bearing the pro-fibrotic Ltbp4DEL variant). A CRISPR-Cas9 strategy was used to obtain C57BL/6J founders in which exon 7 of utrophin (Utrn) was either floxed or deleted. After breeding with *mdx*5Cv dystrophic mice bearing a Muscle-Cre recombinase transgene controlled by rtTA, a reverse trans activator responsive to doxycycline (Dox), 2 lines were obtained: 5Cv/Uflox MC, dystrophic mice in which Utrn alleles can be deleted from skeletal muscles via Dox administration, and 5Cv/UKO MC, dystrophic mice constitutively null for Utrn (similar to *dko* mice). Wildtype, dystrophic, 5Cv/Uflox MC and 5Cv/UKO MC mice were exposed to Dox for 1 week at weaning (P28) before longitudinal monitoring for 12 months. In dystrophic mice, postnatal invalidation of Utrn caused a reduction in body weight, impaired motor function and kyphosis. Most muscles showed marked atrophy and enhanced fibrosis compared to regular dystrophic mice. Because of the DBA/2J background in which it was created, the D2-*mdx* mouse bears Ltbp4DEL, believed to promote fibrosis. However, it also bears Abcc6MUT, which causes ectopic calcification, not relevant for DMD and likely confusing for D2-*mdx* phenotype. By crosses, we generated dystrophic lines (C57BL/6J background) bearing Ltbp4DEL (5Cv/L), Abcc6MUT (5Cv/A), both mutant alleles (5Cv/LA) or none of them (5Cv/0, similar to *mdx*5Cv mice). Intriguingly, in the C57BL/6J context, Ltbp4DEL and Abcc6MUT failed to recapitulate the worsened clinical presentation observed in the DBA/2J background. These new lines are being investigated further with respect to fibrosis, regeneration and muscle function.

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A vascularized human muscle-on-a-chip to elucidate the contribution of vascular degeneration on the progression of muscular dystrophies

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None of currently available treatments can effectively stop the progression of muscular dystrophies. A major problem is the challenging delivery of drugs through the fibrotic microenvironment which characterizes these muscle diseases. Surprisingly, it is generally underestimated that damage to endothelial cells (e.g. cell-cell junction breaks, loss of cell identity) severely impacts the progression of fibrosis in muscle dystrophies. Currently there are no available human models which allow a comprehensive analysis of the functional consequences of vascular damage on the progression of muscle diseases, including its contribution to the reduced muscle contractility observed in dystrophic patients. To properly mimic the dystrophic muscle microenvironment, I am biofabricating a perfusable, contractile 3D human muscle-on-a-chip which allows to analyze and dissect the consequences of vascular degeneration in the progression of Duchenne Muscular Dystrophy (DMD). Using a combination of computer aided design, computational simulations and 3D printing I have designed and developed a prototype of the muscle-on-a-chip which is able to host a 2 cm long, suspended human healthy muscle fiber that can be maintained in culture for 2 weeks. Thin electrodes have been introduced in the system to provide electrical stimulation and promote the maturation of the fiber. Preliminary experiments have been performed to embed mature muscle fibers into collagen matrices containing human muscle-specific fibroblasts and endothelial cells. Once optimized, cells from DMD patients will be employed to biofabricate muscle fibers. Overall, this unconventional in vitro system will allow to simultaneously quantify key aspects of vascular damage and muscle fibrosis, including abnormal remodeling of the 3D extracellular matrix and changes in muscle contractility. Significantly, it is expected that impairing the progression of fibrosis through vascular normalization could improve the delivery of therapies targeting dystrophic muscles.

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Integrative molecular roadmap for direct conversion of fibroblasts into myocytes and myogenic progenitor cells

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Transient MyoD overexpression in concert with small molecules treatment reprograms mouse fibroblasts into induced myogenic progenitor cells (iMPCs). However, the molecular landscape and mechanisms orchestrating this cellular conversion remain unknown. Here, we undertook an integrative multi-omics approach to delineate the process of iMPC reprogramming in comparison to myogenic transdifferentiation mediated solely by MyoD. Utilizing transcriptomics, proteomics and genome-wide chromatin accessibility assays, we unravel distinct molecular trajectories which govern these two processes. Notably, reprogramming is uniquely characterized by gradual upregulation of stem cell and differentiation genes as well as signaling pathways, which manifest via rewiring of the chromatin in core myogenic gene promoters. By employing single cell RNA-Seq we demonstrate that stable iMPC clones are composed of activated satellite-like cells, committed myogenic progenitors, myocytes and myofibers, thus encapsulating a near complete myogenic differentiation program. Furthermore, we establish that FACS-purified Pax7+ iMPCs exceptionally express genes and signaling pathways which are associated with activated satellite cells in vivo in comparison to Pax7+ primary myoblasts, suggesting an augmented capture of a myogenic stem cell state in vitro. Lastly, we determine that only iMPC reprogramming is mediated by Notch pathway activation, which is indispensable for both iMPC formation and self-renewal. Collectively, our study charts divergent molecular blueprints for myogenic transdifferentiation or reprogramming and underpins the heightened capacity of iMPCs in modeling myogenesis *ex vivo*.

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Metabolic regulation of adult muscle stem cells

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Skeletal muscle shows robust regenerative capacity and can undergo several rounds of regeneration in response to multiple injuries. This capacity relies on adult muscle stem cells (MuSCs), which are quiescent cells that get activated upon muscle damage and then proliferate, differentiate and fuse to form new myofibres. For unclear reasons, MuSCs are dysfunctional in numerous pathological conditions, which contributes to muscle alteration. Control of MuSC quiescence and activation is key for efficient MuSC function but mechanisms at play are not clearly understood. Recent reports suggest that MuSCs have distinct metabolic signatures, dependent on their myogenic state (i.e. quiescent, proliferating or differentiating). Metabolic properties of MuSCs may hence contribute to fate determination and targeting metabolic pathways may offer new possibilities to control MuSC for therapeutic applications. As MuSC metabolism has never been explored in vivo, we developed an innovative procedure to profile their metabolic state at the single-cell level, in native conditions, by combining colorimetric enzymatic reactions with immunostaining against MuSC markers on muscle cryosections. Unexpectedly, our results revealed two distinct MuSC populations ranging from oxidative to glycolytic cells. Further, MuSCs with distinct levels of mitochondrial function behave differently, providing novel insights on the discriminative role of mitochondrial function in the accomplishment of early steps of myogenesis. Interestingly, there was a highly significant correlation between the metabolism of MuSCs and the metabolism of the neighboring muscle fiber. Thus, muscle fiber metabolism may govern MuSC metabolism and consequently, metabolic perturbations of muscle fibers may be responsible for MuSC dysfunction in diseases. In this context, we evidenced changes in MuSC metabolism in a myopathic mouse model for Collagen VI-related myopathies before the onset of muscle weakness. Hence, changes in MuSC metabolism may constitute primary pathogenic events, by affecting the myogenic function and capacity of the cells. Hence, our procedure provides a unique way to characterize MuSC metabolism in situ, and to identify thereby primary pathogenic changes, which may affect myogenic function in diseased muscle.

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Therapeutic potential of human myogenic reserve cells in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a severe and progressive neuromuscular muscle-wasting disorder caused by the lack of dystrophin. Currently there is no cure to stop muscle degeneration in DMD. Muscle stem cell (MuSC)-based cell therapy has long been explored as a promising approach for treatment of muscular dystrophies because it would allow genetic complementation and restore the regenerative capacity of muscles. However, the strategy still needs to be improved as the culture conditions alter the regenerative capacities of amplified MuSC. We demonstrated that human myogenic reserve cells (RC) generated in vitro, are quiescent Pax7+ MuSC with properties required for their use in cell therapy. Moreover, as compared to other MuSC, human RC hold the advantage to be generated in vitro in number compatible with therapeutic applications. Recently, we showed that human myogenic reserve cells (RC) are heterogenous for Pax7, with a $Pax7^{High}$ and a $Pax7^{Low}$ subpopulation. We performed bulk RNA-Seq on human RC subpopulations and our results suggest that the $Pax7^{High}$ RC are less primed to myogenic differentiation and adopt a more stem-like state. We also observed a distinct metabolic gene signature between RC subpopulations with a reduced glycolysis toward an increased fatty acid oxidation. Thus, $Pax7^{High}$ subpopulation may constitute an appropriate stem cell source for potential therapeutic applications in DMD. For this purpose, we will assess the regenerative capacity of human RC subpopulations ($Pax7^{High}$ and a $Pax7^{Low}$) after transplantation in immunodeficient dystrophic mice. We first developed strategies to isolate viable human RC subpopulations as intracellular staining for Pax7 is not compatible with in vivo injections. Among various methods tested, cellular autofluorescence appear as a promising biomarker for isolating viable human RC subpopulations. We will then evaluate their survival using non-invasive bioluminescent techniques and assess their function in vivo upon engraftment. These data will bring new information on the regenerative capacity of human RC subpopulations in pathological DMD conditions and will open major perspectives for the possible clinical use of human RC.

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Inter-organelle contact sites as novel therapeutic targets?

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Inter-organelle contact sites play an important role in maintaining competent organelles and modulating their activities. Organelles communicate through intermembrane exchanges of lipids, ions and other small molecules. Proteins that populate membrane contact sites can be structural proteins, containing lipid- and protein-interacting motifs that tether the two membranes, or functional proteins. While the role of endoplasmic reticulum-mitochondria contact sites is well established, the interaction between mitochondria and the endo-lysosomal machinery is poorly understood. Our recent FSRMM funded project allowed us to unveil a novel pathway connecting mitochondria to RAB7 positive endosomal vesicles. The concomitant recruitment of the retromer system at this contact site suggests the recycling of selective mitochondria components through the Golgi. The key player of this inter-organelle contact is a spliced isoform of a protein known to coat lipid droplets and modulate lipolysis through chaperone mediated autophagy. The newly observed pathway bypasses mitophagy, i.e. the complete organelle degradation through macroautophagy, and represents an alternative mitochondrial quality control machinery. Exploring the function and regulation of this specific organelle contact site, we are questioning its implications in neuromuscular disorders. First, we evidenced a higher global expression of our protein of interest in motor neurons derived from patients with amyotrophic lateral sclerosis. Further, we created chimeric peptides able to tether mitochondria to late endosomes or lysosomes. Our preliminary data show promising increases in mitochondrial function in cells from healthy donors as well as from patients. Currently we are running toxicity assays, dose-response paradigms and exploring the mechanistic and phenotypic effects in disease models. Taken together, we uncovered a novel specific and minute mitochondrial quality control mechanism independent from autophagy. Our discovery allows to hypothesize that boosting tethering between mitochondria and the endo-lysosomal system, in a controlled manner, may favor the maintenance of an efficient mitochondrial network. Chimeric peptides, which enable the modulation of these specific organelle contact sites, could pave the way for novel treatment strategies for neuromuscular disorders.

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BCL2L13 at the hub of inter-organelle communication

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The vital role of inter-organelle communication can be highlighted by the many processes taking place at mitochondria contact sites. These include ion transport, lipid exchange and utilization, mitochondrial bioenergetics, dynamics, and mitochondrial quality control. Dysfunctions of proteins located at organelle contact sites are associated with neuromuscular disorders such as Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis. The identification of new molecular players, which regulate organelle communication and tethering, is essential to understand the role of contact sites in neuromuscular diseases. BCL2L13, a member of the BCL2 family of proteins, was shown to be implicated in various cellular processes, such as apoptosis, mitochondrial fragmentation, mitophagy and energy metabolism. In chronic endurance exercise, a specific role of BCL2L13 for maintenance of mitochondrial quality was suggested. The goal of this study is to identify the role of BCL2L13 in inter-organelle communication and its physiological implications. HeLa cells overexpressing BCL2L13 present increased mitochondria-lysosome contacts and mitochondrial fragmentation. Specific domains allow BCL2L13 to interact with mitochondria and the endoplasmic reticulum. BCL2L13 is partially located at mitochondria-associated ER membranes. BCL2L13 knockout zebrafish are smaller, display decreased locomotion, and have lower maximum oxygen consumption rates compared to wild type fish. Our results suggest that BCL2L13 is necessary for inter-organelle communication and organelle dynamics. We believe that better understanding of membrane contact sites and inter-organelle communication could provide insight to the pathogenesis of neuromuscular disorders.

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A natural activator of mitochondrial calcium import increases muscle bioenergetics and performance during aging

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Physiological calcium import in mitochondria via the Mitochondrial Calcium Uniporter (MCU) couples contraction to energy production by regulating the activity of TCA dehydrogenases such as PDH. MCU is a multimeric complex composed of a Ca2+ channel across the outer mitochondrial membrane and regulatory subunits that tightly regulate calcium import to modulate ATP production. MCU mutations in humans cause a rare genetic myopathy and preclinical gain and loss of function experiments have demonstrated that MCU acutely regulates TCA activity and mitochondrial respiration in skeletal muscle, and controls chronic adaptations that promote muscle hypertrophy and prevent atrophy. In a human observational study of sarcopenia, we have identified a strong association between altered mitochondrial bioenergetics in muscle and loss of muscle mass and strength. From the mitochondrial signatures of sarcopenia, we discovered that the expression of the regulatory subunit MCUR1 is downregulated in sarcopenia. Mitochondrial calcium import is also reduced during aging in preclinical models and leads to lower activity of PDH and lower mitochondrial respiration, while knocking-down MCUR1 is sufficient to alter mitochondrial calcium import and bioenergetics and recapitulate muscle aging phenotypes. Finally, we performed a screen for natural activators that specifically increase mitochondrial calcium and identified the olive leaf derived polyphenol Oleuropein as a potent and direct activator of the MCU complex by binding to MICU1. Oleuropein acutely activates PDH, ATP production and mitochondrial respiration in muscle both in vitro and in vivo. Oleuropein treatment limits physical fatigue and increases treadmill performance in young and aged mice, while these effects are lost in MCU KO mice. Altogether, our work highlights a central role of mitochondrial calcium in the regulation of muscle performance and sarcopenia, and identifies a new nutritional solution available for human use to stimulate mitochondrial calcium import, boost muscle bioenergetics and improve performance for applications in healthy individuals and muscle wasting conditions.

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Linker proteins ameliorate *LAMA2*-related muscular dystrophy in dy3K/dy3K mice

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The heterotrimeric laminin-211, consisting of the α2, the β1 and the γ1 chain, is a major component of the myomatrix (basement membrane surrounding muscle fibers) and is required for the integrity of muscle structure and function. Deficiency of the laminin-α2 chain, caused by mutations of LAMA2, leads to congenital muscular dystrophy (*LAMA2* MD). In the dystrophic muscles, laminin-411 (α4,β1,γ1) is overexpressed, forming an unstable myomatrix, because laminin-411 cannot self-polymerize and binds only weakly to the muscle fiber membrane. We have designed linker proteins (mini-agrin and αLNNd) that anchors laminin-411 to the muscle membrane and allow laminin-411 self-polymerization. Transgenic expression of linker proteins in dyW/dyW mice, the most widely used mouse model for *LAMA2* MD, ameliorates the dystrophic phenotye. To test whether the linkers would also ameliorate the disease in another *LAMA2* MD mouse model and to see whether ubiquitous expression of the linkers would add addition benefit, we transgenically expressed both linkers under the CAG promoter in dy3K/dy3K mice. Mice that express both linker proteins, called dy3K/DL mice, show a much improved phenotype. Starting from the age of 4 weeks, dy3K/DL mice gained weight, while dy3K/dy3K mice remained small. The increase of body mass in dy3K/DL mice included both fat and lean mass. Muscle size and histology was strongly improved in dy3K/DL mice compared to dy3K/dy3K mice. Functional measurement of muscle, such as grip strength and ex vivo muscle force were strongly ameliorated in dy3K/DL mice. Finally, current studies measuring survival show that dy3K/DL mice reach a median lifespan of 258 days whereas dy3K/dy3K mice reach a median survival of 55 days. The oldest dy3K/DL mice has now reached an age of 46 weeks. We also find that the linkers, which are also expressed in the basement membrane of the endoneurium, prevent the severe hindlimb paralysis of the dy3K/dy3K mice. Consequently, many of the dy3K/DL are as active as their wild-type littermates. In summary, our data show that ubiquitous expression of the linker proteins ameliorates the muscular dystrophy and the peripheral neuropathy in dy3K/dy3K mice. Thus, the restoration of function is independent of the mutation in the *LAMA2* gene, suggesting that the linker approach is likely to improve the disease in all *LAMA2* MD patients.

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Deciphering DUX4 protein biogenesis

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscle dystrophy. In FSHD, the progressive wasting of muscle leads to a significant morbidity, with about 20% of patients becoming wheelchair-bound. FSHD results from the aberrant expression of the pioneer transcription factor Double Homeobox 4 (DUX4) caused by the loss of epigenetic repression of DUX4 locus on chromosome 4. To date, the knowledge on DUX4 biogenesis is sparse and we thought essential to decipher the physiological mechanisms involved. To this end, we employed tetracycline inducible DUX4 cells (in HEK293 and LHCN muscle cells) to establish that DUX4 protein is a short-lived nuclear transcription factor (1.5 to 2hr). Moreover, the inhibition of the UPS leads to a 2-fold accumulation of DUX4 protein in these cells. We further generated a series of DUX4 mutants either resistant to ubiquitination (Lysine mutant) or C-terminally deleted (putative destabilisation sequence/degron). Functionally, DUX4 lysine mutation reduces its activity as measured by a dual luciferase reporter assay, whereas deletion in the C-ter, where the transactivation domain (TAD) is located, fully inhibits DUX4 activity. To identify the endogenous UPS component responsible for DUX4 degradation we employed the BioID technology where the WT or mutated DUX4 protein were fused to a biotin ligase BirA*. The resulting chimera displays in cellulo biotin ligase activity allowing upon streptavidin pull-down the MS/MS identification of its proximity dependent biotinylated interactors. Such approach led us to identify known DUX4 binding protein involved in mRNA binding and splicing, histone binding and transcription regulation as well as several E3 ligase candidates. We are currently addressing the putative involvement of those UPS components in the degradation of DUX4 *in vivo*.

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The role of Klf5 in exercise-mediated skeletal muscle remodeling

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Regular physical exercise is a cornerstone of good health and longevity. Skeletal muscle is a highly plastic tissue and copious health benefits of exercise are due to the exercise-induced remodeling of skeletal muscle. Muscular exercise adaption consists of a plethora of physiological changes including increased vascularization, force generation, improved fatty acid utilization or hypertrophy, depending on the type of exercise. While these adaptions are well characterized on a physiological level, the molecular processes underlying these changes remain elusive. Exercise-induced remodeling of muscle happens on the bases of single bouts of exercise, to which the muscle reacts with a complex transcriptional network, unfolding in the hours post-exercise. These acute transcriptional changes are likely to provide the bases for long-term adaptions. In order to understand what constitutes a healthy muscle it is crucial to understand these processes during and after exercise. Krüppel-like factor transcription factors have previously been shown to regulate a wide variety of functions in skeletal muscle, including cell proliferation, amino acid metabolism or stress response. Transcriptomic analysis of skeletal muscle after an acute bout of exercise reveals an increase in expression and motif activity of Krüppel-like factor 5 (Klf5) but its role and function in an exercise-context is unknown. In this study, we investigate the role of Klf5 in an acute and chronic exercise setting via AAV-mediated gain- and loss-of function in mice. We show that knockdown of Klf5 in an acute exercise setting affects expression of known exercise responsive genes while in a chronic exercise setting, Klf5 depletion affects lipid metabolism by altering levels of multiple proteins involved in fatty acid synthesis. These findings point towards a pivotal role of Klf5 in response to exercise-induced muscle remodeling.

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Chronic exercise-induced multi-cellular adaptation the muscle

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Exercise-induced skeletal muscle adaptation has been proven to have a fundamental impact on systemic health, including the prevention of many of the deadliest diseases. Despite the evidence, the underlying cellular and molecular mechanisms of muscle adaptation remain elusive. Skeletal muscle is a highly plastic and complex tissue, consisting of many different cell types which orchestrate microenvironment remodeling upon individual biological cues. Traditional approaches using bulk muscle analysis oftentimes failed to see the intricate communication and response of individual cells in the muscle. In a holistic approach, using single-cell transcriptomics on trained and untrained mouse muscles, cell-type-specific adaptations and inter-cellular communication patterns will be revealed. With this approach, we aim to identify novel and essential drivers of exercise adaptation, which could be used as cell type-specific therapeutic targets in exercise-prevented diseases.

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Effect of intermittent high frequency stimulation on muscle velocity recovery cycle recordings

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Background: The technique of multi-fiber muscle velocity recovery cycle recordings (MVRCs) was developed as a diagnostic tool to assess muscle membrane potential changes and ion channel function *in vivo*.
Aim: This study was undertaken to assess the impact of intermittent 37 Hz stimulation on the different MVRC parameters and to study whether the changes can be modified by endurance training.
Methods: MVRCs with 1 and 2 conditioning stimuli were recorded in the left tibialis anterior muscle in 15 healthy subjects before (baseline), during and after intermittent 37 Hz stimulation for 6 minutes and its effects on early supernormality (ESN), late supernormality (LSN) and extra-late supernormality following two conditioning stimuli (XLSN) was analyzed. The recordings were conducted before and after two weeks endurance training. Training effect was assessed by measuring the difference in endurance time, peak force and limb circumference.
Results: Intermittent high frequency stimulation was successfully recorded in 12 subjects and showed a significant effect on MVRC parameters. While ESN showed a maximal reduction at the beginning of intermittent stimulation and recovered during stimulation, LSN showed a delayed decrease to its minimal value and stayed significantly reduced after the end of the intermittent high frequency stimulation. Training induced changes in peak force correlated positively with baseline changes of ESN.
Conclusion: Our results support the hypothesis that ESN represents membrane potential, which depolarizes in the beginning of high frequency stimulation. LSN probably reflects transverse tubular function and shows progressive changes during high frequency stimulation with delayed normalization.

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Leg pain and weakness in postural tachycardia syndrome are associated with altered muscle excitability

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Background: In neuropathic postural tachycardia syndrome peripheral sympathetic dysfunction leads to excessive venous blood pooling during orthostasis. Up to 84% of patients report leg pain and weakness in the upright position. Despite these symptoms being so frequent, their aetiology is still unclear and accordingly, therapy options are limited.
Aim: To explore possible pathophysiological processes underlying these symptoms the present study examined muscle excitability in patients with neuropathic postural tachycardia syndrome and healthy subjects depending on body position.
Methods: In ten patients with neuropathic postural tachycardia syndrome and ten healthy subjects multiple muscle excitability measurements were performed successively: One in the supine position, three during ten minutes of head-up tilt and two during six minutes thereafter. Additionally, circumferences of the lower leg were measured and subjective leg pain levels were assessed.
Results: In patients with neuropathic postural tachycardia syndrome muscle excitability was increased in the supine position, decreased progressively during tilt, continued to decrease after being returned to the supine position, and did not completely recover to baseline values after six minutes of supine rest. The reduction of muscle excitability during tilt was paralleled by an increase of circumference of the lower leg as well as of leg pain levels. No such changes were observed in healthy subjects.
Conclusions: The results indicate that in patients with neuropathic postural tachycardia syndrome muscle fibres are hyperpolarized in the supine position and depolarize progressively during tilt, with delayed recovery after being returned to the supine position. Depolarization of muscle fibres during tilt were associated with an increase of the lower leg circumference and pain levels. Insufficient perfusion as a consequence of blood stasis may cause a misery perfusion of the muscles, which could explain the occurrence of orthostatic leg pain and weakness in neuropathic postural tachycardia syndrome.

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Exploring the neuromuscular junction using snRNA-seq

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Voluntary contraction of skeletal muscle depends on a nerve-muscle interaction site named the neuromuscular junction (NMJ). The proteins necessary for initial contact between motor neurons and muscle fibers during embryonic development are well described. However, what happens downstream of this ligand-receptor interaction allowing full differentiation of NMJs is not well understood. Until recently it has been particularly challenging to study the NMJ as it covers only ~0.1% of muscle fiber area and only ~1-2% of nuclei in muscle fibers express NMJ-related genes. Using single nuclei RNA-seq we can overcome this challenge and can explore the transcriptome of sub-NMJ myonuclei. We identify a large number of new transcripts including T-Box Transcription Factor 21 (Tbx21). Using knockout mice, we are now characterizing the function of Tbx21 in the nerve-muscle interaction.

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Molecular mechanisms controlling the expression and dynamics of synaptic proteins in muscle

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Neuromuscular junctions (NMJs) are the synapses connecting motor neurons to skeletal muscle enabling contraction and conditioning muscle well-being. Synaptic proteins are selectively expressed in the sub-synaptic region of muscle fibres, but they get re-expressed all along the fibres upon nerve injury [1,2]. The mechanisms regulating these events to ensure NMJ maintenance and remodelling remain largely unknown. Here, we focus on the histone acetyltransferase p300 and the transcriptional repressor CtBP1, which may promote and repress synaptic gene expression in sub- and extra-synaptic regions, respectively [2,3]. p300 and CtBP1 were detected in both sub- and extra-synaptic nuclei in muscle fibres. Moreover, their transcript and protein levels remained unchanged in muscle after nerve injury. However, while the localization of p300 was unchanged upon denervation, the nuclear accumulation of CtBP1 transiently increased shortly after denervation. Interestingly, the regulation of both p300 and CtBP1 was altered in muscle from TSCmKO mice, characterized by constant activation of mTORC1 pathway and inhibition of PKB/Akt. Indeed, we observed that the transcriptional expression of p300 was increased and that the nuclear accumulation of CtBP1 was altered in mutant muscle. This suggested a role of the PKB/Akt - mTORC1 signalling in the regulation of both effectors. Ongoing experiments aim at characterizing the complexes formed by CtBP1 and p300, and the genes targeted, in both sub- and extra-synaptic muscle regions of innervated and denervated muscles, in order to get further insights on the role(s) of CtBP1 and p300 in NMJ maintenance. Understanding these processes will bring insights on the pathomechanisms compromising muscle innervation and on potential targets to limit muscle dysfunction in various diseases.

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Unraveling the function of novel subsynaptic NMJ transcripts

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The neuromuscular junction (NMJ) is the synapse formed between motor neurons and muscle fibers, that converts nerve action potentials into muscle contraction. In the postsynaptic muscle fiber, proteins required at the NMJ apparatus are locally and selectively encoded in a small number of subsynaptically enriched myonuclei, that are transcriptionally distinct from the neighbouring nuclei of the syncytial muscle fiber. Although the NMJ is one of the longest studied synapses, little is known about the transcriptional regulation of NMJ myonuclei, particularly in muscles of different fiber types – knowledge of which is paramount in tackling neuromuscular diseases. Using laser-capture microdissection combined with RNA-sequencing, we successfully generated NMJ gene expression profiles in prototypical fast- and slow twitch muscles and identified many novel NMJ genes. We further aim to characterise the role and function of selected NMJ-specific transcripts in health and diseases models.

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Quantification of fat fraction and water T1 in neuromuscular diseases by magnetic resonance fingerprinting with machine learning-based reconstruction

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Quantitative magnetic resonance imaging provides objective and sensitive biomarkers such as fat fraction (FF) and water T2 (T2H2O) for neuromuscular diseases (NMDs). The FF quantifies chronic degenerative changes of muscle, or more generally disease severity. The T2H2O quantifies the presence of edema or inflammation, or more generally disease activity. Recently, also water T1 (T1H2O), quantified with magnetic resonance fingerprinting (MRF), is also being considered as a possible biomarker of disease activity. While the principle of MRF provides very fast MR acquisitions (< 1 minute), it requires a computationally extensive image reconstruction based on dictionary matching resulting in hours of processing time, which makes T1H2O quantification unfeasible for clinical use. Therefore, we aim performing MRF reconstruction using machine learning for an accurate and fast quantification of T1H2O in NMDs. We used MRF T1-FF, an MRF sequence for quantification of FF and T1H2O in skeletal muscle. MRF T1-FF relies on a 1400 radial spokes FLASH echo train with variable echo time, repetition time, and flip angle schedules after an inversion pulse. Dictionary matching was used to reconstruct FF, T1H2O, off-resonance frequency (B0), and flip angle efficacy (B1) maps. To replace the dictionary matching, we propose a convolutional neural network (CNN), which operates both in the temporal as well as spatial domain reconstruct the four parametric maps. A highly heterogeneous clinical dataset consisting of 164 patients with various NMDs was used to evaluate our method. Visually, the four parametric maps show good agreement to the reference parametric maps reconstructed by dictionary matching. Region of interest analysis of the major muscles showed excellent agreement between CNN and dictionary matching reconstructions with coefficient of determinations of 0.99, 0.89, 0.99, 0.99 for FF, T1H2O, B0, and B1. Bland-Altman analysis showed little to no bias and 95% limits of agreement below the dictionary sampling step size for FF, B0, and B1. For T1H2O, the 95% limits of agreement were ±60ms. The CNN required one minute for reconstruction, whereas the dictionary matching required five hours. The results suggest that machine learning-based reconstruction of MRF is feasible with good accuracy and in clinically feasible time. Using our method, the quantification of FF and T1H2O for diagnosis and monitoring of NMDs is possible.

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Dynamic magnetic resonance imaging of muscle contraction in facioscapulohumeral muscular dystrophy

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Objectives: Quantitative muscle MRI (water-T2 and fat mapping) is being increasingly used to assess disease involvement in muscle disorders, while imaging techniques for assessment of the dynamic and elastic muscle properties have not been translated yet into clinics.
Methods: Here, we quantitatively characterized muscle deformation (strain) in patients affected by facioscapulohumeral muscular dystrophy (FSHD), a prevalent muscular dystrophy, by applying dynamic MRI synchronized with neuromuscular electrical stimulation (NMES). We evaluated the quadriceps muscles in 34 ambulatory patients and 12 healthy controls, at 6 month intervals.
Results: We found that while a subgroup of patients behaved similarly to controls, for another subgroup the strain significantly decreased over time (50% over 1.5 years). Dynamic MRI parameters did not correlate with quantitative MRI.
Conclusions: In conclusion, our results suggest that the evaluation of muscle ability to contract by NMES-MRI could be used to explore the elastic properties and monitor muscle involvement in FSHD and other neuromuscular disorders.

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Establishing a resistance training model in mice for better understanding the impact of resistance exercise on muscle growth, strength and health

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The loss of muscle mass and strength is a hallmark of many neuro-muscular diseases and aging. So far, the most effective intervention to induce muscle growth and strength gains, or to prevent their decline, is resistance training. The molecular mechanisms underlying the adaptation to resistance training are, however, still poorly understood. In particular, it is unclear how the repeated application of resistance exercise bouts translates into the phenotypic adaptations observed following training. To facilitate the study of resistance training adaptation, we aimed at establishing a mouse model that induces muscle hypertrophy and strength gains. To that end, we used running wheels with adjustable resistance and compared different protocols on their potential to increase muscle mass, muscle fiber cross-sectional areas and grip strength.

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Characterization of a novel mouse model carrying the homozygous p.F4976L RyR1 mutation, identified in a severely affected child

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Mutations in RYR1 are associated with a range of neuromuscular disorders including core myopathies, congenital fiber type disproportion and centronuclear myopathy. Here we present a new mouse model we created using the CRISPR/CAS9 technique, knocked-in for a homozygous *RYR1* mutation identified in a severely affected child. The child was born pre-term, presented feeding difficulties and very limited limb movement. Genotypic investigation revealed that he carried the homozygous c.14928C>G RYR1 mutation in exon 104, resulting in the substitution of p.F4976L. Both parents were heterozygous carriers for the same mutation. The presence of the heterozygous p.F4976L mutation in the new mouse model was verified by sequencing genomic DNA from founder mice. Heterozygous mice were mated to obtain homozygous Ryr1F4976L mice. The correct mendelian transmission of the mutation was confirmed, with a homozygous frequency below the expected frequency. The presence of the mutation was not associated with increased post-natal lethality, nor with obvious developmental changes and homozygous mice were not visibly different from their WT and Het littermates. Analysis of the growth curves of both male and female mice over a period of 15 weeks, did not show any difference in body weight between WTs, Het and homozygous mice. We will present the in vivo and ex vivo physiological characteristics of muscle function of the new mouse model, as well as changes occurring in the expression and content of the main proteins involved in excitation contraction coupling and force development. The results of this study are important as they will help understand recessive ryanodinopathies pathomechanism and will help identify targets and biomarkers useful for the development of therapeutic approaches aimed at improving muscle function in affected patients.

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STIM1L KO mice: is there a skeletal muscle phenotype?

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Store Operated Calcium Entry (SOCE) is activated to refill the endoplasmic/sarcoplasmic reticulum (ER/SR) with calcium and relies on the activity of the calcium sensors from the STIM family (STIM1, 2) and the plasma membrane Orai calcium channel family (Orai1, 2, 3). Since skeletal muscle contraction mobilizes calcium from the SR, the mechanisms for SR calcium refilling are essential to maintain optimal contraction capacity. Calcium extrusion occurs during the prolonged contraction, thus SOCE could be decisive in helping to maintain contractions. Our laboratory discovered that skeletal muscles highly express alternative splicing of STIM1, called STIM1L. Because the expression of STIM1L allows for a faster SOCE activation, we hypothesized that skeletal muscles need the muscle-specific expression of STIM1L to maintain prolonged contractions. To test this hypothesis, we generated constitutive STIM1L KO mice without affecting the expression of the ubiquitous STIM1 isoform. The study used males and females according to the ARRIVE guidelines. The STIM1L KO mice develop as well as the wild-type mice (wt). A spontaneous activity test using running wheels showed that while the females STIM1L KO run as much as the females wt, the males STIM1L KO run more and faster than the wt males. We will perform *ex vivo* experiments to determine the capacity of muscle laking STIM1L to sustain contractions. Preliminary data show that STIM1L deletion induces a mild shift from slow to fast fibers in EDL/TA muscles. This should be now confirmed on different muscles both in males and females, but that could explain the phenotype we observed on the running performance of the STIM1L KO mice.

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Seeding and spreading of dipeptide repeat proteins in C9ORF72 linked ALS

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The deposition of aggregated proteins and ubiquitin into intracellular inclusion is a common pathological hallmark for most neurodegenerative disease including AD, PD, HD and ALS. Each disorder is characterized by the misfolding and aggregation of disease specific proteins. Cell to cell transmission of such misfolded protein has been described recently for different neurodegenerative disorders. Transmission of such misfolded proteins acts as a nucleation seed resulting in sequestration and aggregation in the receiving cells. Hence, seeding and spreading of misfolded proteins has been emerging as a new theme in neurodegenerative disease. Although hexanucleotide repeat expansion (GGGGCC), the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is present in non-coding intron is able to translate into dipeptide repeat proteins (DPRs) through repeat-associated non-ATG (RAN) translation. Five different DPRs are currently known to be formed from both sense and antisense strands and are found to form neuronal inclusion throughout central nervous system in patients with the C9 repeat expansion. Thus, we are investigating whether individual DPR species are transmitted between neurons and astrocytes and how their uptake affects the receiving cells.

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Interleukin-6 potentiates endurance-training adaptation and improves functional capacity in old mice

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Interventions to preserve functional capacities at advanced age are becoming increasingly important. So far, exercise provides the only mean to counteract age-related decrements in physical performance and muscle function. Unfortunately, the effectiveness of exercise interventions in elderly populations is hampered by reduced acceptance and compliance as well as disuse complications. We therefore studied whether application of interleukin-6 (IL-6), a pleiotropic myokine that is induced by skeletal muscle activity and exerts broad systemic effects in response to exercise, affects physical performance and muscle function alone or in combination with training in aged mice. Sedentary old mice were compared to animals that either received recombinant IL-6 (rIL-6) in an exercise-mimicking pulsatile manner, were trained with a moderate-intensity, low-volume endurance exercise regimen or were exposed to a combination of these two interventions for 12 weeks. Before and at the end of the intervention, mice underwent a battery of tests to quantify endurance performance, muscle contractility *in situ*, motor-coordination, gait and metabolic parameters. Mice exposed to enhanced levels of IL-6 during endurance exercise bouts showed superior improvements in endurance performance, fatigue resistance *in situ*, motor-coordination and gait following training. Pulsatile rIL-6 treatment in sedentary mice had only marginal effects on glucose tolerance and some gait parameters. No increase in adverse events or mortality related to rIL-6 treatment were observed. In conclusion, rIL-6 administration paired with treadmill running bouts potentiates the adaptive response to a moderate-intensity low-volume endurance exercise regimen in old mice, while being safe and well tolerated.

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Integrated cross talk between cellular clearance machinery in C9orf72 ALS

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Recently, one of the most common genetic mutations associated with familial amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) has been identified as the hexanucleotide repeat expansion of GGGGCC (G4C2) in the intron of the gene chromosome 9 open reading frame 72 (C9orf72). Five different dipeptide repeat proteins (DPRs) with no known physiological function are generated via a non-ATG-mediated translation of G4C2 repeats. These DPRs accumulate within C9orf72 ALS neurons, causing pathogenenic responses. Although, the underlying mechanisms of ALS onset are unknown, new insights show impaired proteome homeostasis as a fundamental process underlying ALS pathogenesis. Motor neurons, which degenerate in ALS, are intrinsically vulnerable to endoplasmic reticulum stress (ER) as well as proteome stress. Misfolded or aggregated proteins are cleared by the cell's protein degrading machinery involving Ubiquitin proteasome system (UPS), Autophagy, and ER associated protein degradation (ERAD). However, in the context of neurodegenerative diseases such as ALS, these systems are either overwhelmed or defective due to protein aggregation and their progressive accumulation, eventually leading to cell death. Our aim is to examine two key questions using patient iPSC-derived motor neurons; the cross talk between the ER and other cell clearing systems such as autophagy and UPS in DPRs degradation during ER stress and to identify key molecules that would facilitate the degradation of distinct DPRs.

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Role of STIM2.1 on differentiation and function of human myotubes

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Store-operated Ca2+-entry (SOCE), a fundamental mechanism in myogenesis, involves the Stromal Interaction Molecule (STIM), a sarcoplasmic reticulum (SR)-membrane calcium sensor which detects SR calcium depletion and translocates to the plasma membrane. By gating the calcium channel ORAI1, it induces a Ca2+ influx into the cytosol. The SERCA pump allows then the re-filling of the SR calcium store. Skeletal muscle cells express both STIM1 and STIM2 proteins, and recently, a novel member of the STIM proteins family, STIM2.1 also named STIM2b, acting as a negative regulator of SOCE has been discovered. We confirmed by qPCR the presence of this new splicing variant STIM2.1 in human skeletal muscle cells, more specifically expressed in myotubes. The aim of our study was to understand the implication of STIM2.1 and STIM2.2 (classical form) on SOCE in human skeletal muscle cells, their possible impact on cell proliferation, differentiation and human myotube functions. The downregulation of STIM2.1 or STIM2.2 by siRNA did not prevent neither myoblast proliferation nor myotube differentiation. But downregulation of STIM2.2 led to the formation of larger human myotubes. Surprisingly, SOCE was reduced by about 25% both in siSTIM2.1 and siSTIM2.2 conditions. Then, using the ER-targeted Cameleon probe (D1ER), we observed a decrease of the total SR-Ca2+ content when reducing STIM2.1 or STIM2.2 expression. We then depolarized the myotube membrane with a 70mM KCl, mimicking an electrical stimulation, to assess EC coupling. In line with the reduced SR Ca2+ content, the Ca2+ released was reduced by 20%. Interestingly, compared to the full SR Ca2+ content, the amount of Ca2+ depletion upon KCl stimulation was reduced in case of STIM2.1 invalidation, suggesting a defect in the EC coupling. Indeed, both DHPR and RyR1 expression were decreased in siSTIM2.1 condition, while being unaffected by STIM2.2 downregulation. Moreover, SERCA2 level was also reduced, both at the mRNA and protein levels. Overall, these results indicate that the novel splicing variant STIM2.1, is playing a functional role in EC coupling of human myotubes, whereas STIM2.2, appears more implicated at the differentiation level, regulating the myotube size.

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Targeting NAPDH oxidases in Duchenne muscular dystrophy: diapocynin therapeutic effect on adult mdx mice

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Duchenne muscular dystrophy (DMD) is the most common muscular disease affecting children. It affects nearly 1 male birth over 3500. There is a general consensus that oxidative stress is a pervasive feature in the pathogenesis of DMD. Recent work on NADPH oxidase (NOX) showed that they can be a target of interest in diseases involving oxidative stress like DMD. Previously, we have shown that the putative NOX inhibitor, diapocynin, demonstrated high efficacy in inhibiting reactive oxygen species (ROS) production in dystrophic myotubes and preventing eccentric contraction induced damage in isolated dystrophic mice in situ. Diapocynin *in vivo* treatment also showed a restoration of spontaneous locomotor activity, enhanced wheel running capabilities, improvement in fatigue and diaphragm structure in young mice treated from 14 days post-natal to 12 weeks of age. In light of the encouraging results obtained with diapocynin in young *mdx*5Cv mice, we decided to test it further on the chronic phase of the disease. We report here a comprehensive analysis of diapocynin in adult mice treated between 6 and 9 months of age. Read-outs previously done in young mice were done in older mice. Classical methods such as grid tests, locomotor activity, resistance to fatigue and eccentric contractions among others were performed as well as histological evaluation and biochemical analyses. The treatment of dystrophic mice in advanced stage of the disease in a curative setting revealed the potential of NOX targeted therapy. Our findings showed that different functional and histological parameters were improved compared to control. These observations correlate with our findings on quantifying NOXes expression at different stages of the disease.

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Expression pattern of the Vma21 gene brings new insights on specific muscle phenotype in XMEA patients

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Over the last decades, several pathological conditions, including aging, have been associated with lysosomal dysfunction. In particular, defective lysosomal acidification impinges proteolytic capacity and triggers intracellular accumulation of undigested content. Consistently, mutations in genes encoding subunits of the vacuolar ATPase proton pump (v-ATPase) or associated assembly chaperones, cause several lysosomal disorders. Especially, mutations in the *VMA21* gene, encoding an essential v-ATPase chaperone, lead to a rare disorder called X-linked myopathy with excessive autophagy (XMEA). The disease is characterized by progressive atrophy of skeletal muscle, associated with the vacuolation of muscle cells. XMEA patients do not exhibit non-muscular clinical signs, raising questions about the specific dysfunction of skeletal muscle. Although previous reports suggested that *VMA21* deficiency impairs autophagy, the pathomechanisms underlying the specific muscle dysfunction in XMEA remain to be further investigated. To gain insights on XMEA pathogenesis, we examined the expression pattern of VMA21 in different cell types. Four isoforms are predicted in mouse: VMA21a (101 amino acids), b (120aa), c (134aa) and d (161aa). Transcript and protein levels of the main isoform, VMA21a, were detected in both muscle and non-muscle tissues. In contrast, VMA21c showed low expression levels detected only in liver and muscle, and *VMA21d* transcripts were undetected. Interestingly, VMA21b was expressed specifically in skeletal muscle, at both transcript and protein levels. Moreover, the expression of VMA21b strongly increased upon differentiation of C2C12 cells, while levels of VMA21a/c decreased. This suggests that VMA21 isoforms play different functions in muscle and non-muscle tissues, as well as during myogenesis. Ongoing experiments aim at determining the role of each isoform by assessing the consequences of their modulation *both in vitro* and *in vivo*. Unveiling yet-unknown function of VMA21 will help to understand the pathogenesis of XMEA and to identify potential therapeutic targets to delay the disease.

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Inactivation of sphingolipid *de novo* synthesis counteracts age-associated muscle dysfunction

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Age-related muscle dysfunction is a major cause of physical incapacitation in elderly, and currently lacks viable treatment-strategies. Here we report the involvement of sphingolipids in age-related muscle dysfunction and sarcopenia. Sphingolipids accumulate in skeletal muscle upon aging, and pharmacological inhibition of sphingolipid synthesis prevents age-related decline in muscle mass while enhancing strength and exercise capacity. In aged muscle, inhibition of sphingolipid synthesis confers increased myogenic potential, an effect verified in muscle progenitor cells. The relevance of sphingolipid pathways in human aging is demonstrated in two cohorts, the UK Biobank and Helsinki Birth Cohort Study in which genetic variants of SPTLC1 are associated with improved fitness of aged individual. These findings identify sphingolipid synthesis inhibition as an attractive therapeutic strategy for age-related sarcopenia and co-occurring pathologies.

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Role of Ca2+ signals in the activation mechanisms of human muscle reserve cells

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The molecular pathways, especially the calcium signaling required for muscle stem cell reactivation, are still unclear. Our work aims to study the reactivation of the muscle stem cells obtained in vitro, called reserve cells (RCs). We first tested the ability of fetal calf serum (FCS) to induce the proliferation of the RCs in the presence of the myotubes. Around 30% of the RCs were positive for EdU, proving that FSC can trigger their proliferation. Then, we used the Ca2+ dye Fura-2 AM to measure the Ca2+ signals evoked by FCS stimulation to establish whether the activation of the RCs by FCS is correlated with a specific Ca2+ response. Upon stimulation, we differentiate several patterns of responses (a transient Ca2+ response, an oscillating Ca2+ pattern, a sustained Ca2+ response, a delayed response and a minority of RC which do not respond at all). Most of the Ca2+ responses were IP3 dependent, while inhibitors of the store-operated Ca2+ entry pathway (SOCE; an ubiquitous Ca2+ influx mechanism) only partially inhibited the Ca2+ oscillations. However, the Ca2+ oscillations or sustained Ca2+ responses were abrogated after removing extracellular Ca2+. We observed that neither IP3 production blockers nor Ca2+ influx blockers prevented the activation of RC. Further, we assessed the activation potential of RC in low Ca2+ medium or with BAPTA-AM (intracellular Ca2+ chelator). In both cases, activation of RC was not modified by the Ca2+ chelators, which leads us to the unexpected conclusion that RC activation is independent of Ca2+ signals. Finally, we evaluated the migration potential of RC after stimulation. By tracking each RC individually, we determine that the cells displaying a Ca2+ response upon FCS were migrating more and faster than the one that did not elicit a Ca2+ response. In line, we showed that inhibitor of the IP3 pathway together with Ca2+ influx inhibitor strongly decreased the displacement of RC in the 48h following FCS stimulation. Our results highlight the independence of Ca2+ signals for RC activation while having a strong effect on their migration potential. However, the exact pathway linking the Ca2+ response to their displacement remains to be deciphered.

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Human myogenic reserve cells are heterogenous for Pax7 with distinct metabolic gene signatures.

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The capacity of the skeletal muscle to regenerate depends on Pax7+ satellite cells, the muscle stem cells (MuSC). We have demonstrated that human reserve cells (RC) are quiescent Pax7+ MuSC, arrested in a G0 reversible cell cycle state with properties required for their use in vivo. Recently, we observed that human RC are heterogenous for Pax7 and we demonstrated that the $Pax7^{High}$ RC subpopulation is less primed to myogenic commitment. In the present study, we characterize human RC metabolism in vitro and we expect to identify mechanisms governing the metabolic status of human RC subpopulation $Pax7^{High}$ and $Pax7^{Low}$.

We first evaluated the bioenergetic profile of human RC using Seahorse technology. Glycolysis, maximal glycolytic capacity, and ATP production were significantly reduced in RC as compared to proliferating myoblasts. By flow cytometry, we also observed in human RC, a significant decrease of glucose uptake (2-NBDG) that correlate with a significant increase in fatty acid uptake (fluorescent palmitate) as compared to myoblasts. We also showed by western blot a significant increase in AMPKα1 expression in RC that correlate with an increased in phospho-Acetyl-CoA Carboxylase expression (ratio pACC/ACC). Finally, we performed RNA sequencing on RC $Pax7^{High}$ and $Pax7^{Low}$ subpopulations. Our preliminary analyses identify a distinct metabolic gene signature between $Pax7^{High}$ and $Pax7^{Low}$ subpopulations.

Overall, these data demonstrate that quiescent human RC shift their metabolism from glycolysis to fatty acid oxidation. Moreover, their transcriptional profile suggest that human RC are also metabolically heterogeneous with a $Pax7^{High}$ subpopulation being more dormant and less metabolically active. Altogether, our results indicate that $Pax7^{High}$ human RC subpopulation adopt a more stem-like state and may constitute an appropriate stem cell source for potential therapeutic applications in DMD.

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CRISPR/Cas9 editing of directly reprogrammed myogenic progenitors restores dystrophin expression in a mouse model of muscular dystrophy

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Genetic mutations in dystrophin manifest in Duchenne muscular dystrophy (DMD), the most prevalent form of a genetically inherited muscle disease. Dystrophin is expressed in myogenic stem cells and fibers, playing a critical role in maintaining skeletal muscle structure, regeneration and function. Here, we report on direct reprogramming of fibroblasts from the Dmdmdx mouse model into induced myogenic progenitor cells (Dmdmdx iMPCs) utilizing transient MyoD overexpression in concert with small molecule treatment. Dmdmdx iMPCs proliferate extensively in vitro and express canonical skeletal muscle stem and progenitor cell markers including Pax7, Sox8 and Myf5. Additionally, Dmdmdx iMPCs readily give rise to highly contractile and multinucleated myofibers that express a suite of mature skeletal muscle genes however lack dystrophin expression. Utilizing an exon-skipping based approach with CRISPR/Cas9, we report on a genetic correction of the dystrophin mutation in Dmdmdx iMPCs and subsequent restoration of dystrophin protein expression in vitro. Furthermore, engraftment of genetically corrected Dmdmdx iMPCs into dystrophic limb muscles of Dmdmdx mice restored dystrophin expression in vivo. Collectively, our findings report on a novel in vitro stem cell-based model for DMD and further establish a new approach to restore dystrophin expression in vivo via a combination of direct cellular reprogramming, genome engineering and stem cell transplantation methods.

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Investigating the regenerative potential of muscle stem cells in the dyW/dyW mouse model of LAMA2-related congenital muscular dystrophy

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LAMA2-related muscular dystrophy (*LAMA2* MD) is the most common form of congenital muscular dystrophy in Europe. It is caused by an absence of laminin-α2, a basement membrane protein that plays an essential role in the maintenance of muscles' structural integrity by tethering muscles fibers to their surrounding basal lamina. In *LAMA2* MD, the loss of laminin-α2 results in muscle atrophy and weakness as patients present highly-damaged muscle fibers and high levels of intramuscular fibrosis and inflammation. To study this disease, we use dyW/dyW mouse models as they accurately recapitulate the defects in muscle function and structure observed in patients. Interestingly, the muscles of dyW/dyW mice deteriorate over their short lifespan despite observations that they contain a higher relative number of muscle stem cells (MuSCs) and newly-regenerated fibers. It could therefore be hypothesized that the regenerative capacity of MuSCs is reduced or lost in dyW/dyW mice. Consequently, we decided to assess the proliferative and myogenic capacities of MuSCs isolated from wild-type and dyW/dyW mice in vitro, before evaluating their regenerative capacity in vivo following a cardiotoxin-induced injury. Since disparities between the MuSCs may be caused by cell-intrinsic (loss of laminin-α2 production by MuSCs) and/or cell-extrinsic mechanisms, we also started to characterize other cellular components of the healthy and diseased muscles (e.g., Fibro-Adipogenic Progenitor cells). Ultimately, we aim to identify potential cell-intrinsic and/or cell-extrinsic processes that influence the characteristics of MuSCs in dyW/dyW mice, as they could impact the effectiveness of treatments for *LAMA2* MD patients, particularly the double-linker approach developed by our group.

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Transgene-free direct reprogramming of mouse fibroblasts into myogenic progenitors utilizing modified mRNA and small molecules

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Satellite cells denote a subpopulation of muscle-resident stem cells which ensure regeneration of skeletal muscle tissue throughout life. This regeneration capacity is impaired in muscular dystrophies which manifest in progressive muscle loss and oftentimes untimely death. Reprogramming of patient-derived somatic cells into engraftable myogenic stem cells resembling functional satellite cells may provide a therapeutic approach to ameliorate muscular dystrophies. Previous work has established a reprogramming protocol that enables direct conversion of mouse fibroblasts into satellite-like induced myogenic progenitor cells (iMPCs) by lentiviral vectors encoding for the myogenic factor MyoD in conjunction with a triad of small molecules. However, use of viral vectors is associated with random genomic integration which is an impediment for therapeutic applications. Establishing a transgene-free reprogramming method is therefore highly desirable for circumventing viral vector delivery safety issues. Here we present a modified reprogramming protocol for the direct reprogramming of mouse fibroblasts into iMPCs utilizing transfection of in-vitro-transcribed modified mRNA encoding for MyoD in concert with small molecules treatment. Furthermore, by screening a selection of small molecules we identified a novel compound that significantly augments myogenic conversion via inhibition of the c-Jun N-terminal kinase (JNK) signaling axis. Transgene-free iMPCs proliferate extensively in vitro, express a suite of myogenic stem cell markers including Pax7 and Myf5 and can produce contractile multinucleated myofibers. Collectively, this work reports on the first direct conversion of somatic cells into myogenic progenitors utilizing mRNA delivery and further resolves a major hurdle in respect to using iMPCs for regenerative medicine purposes.

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Studying the role of PGC-1α in skeletal muscle aging using transcriptomic analysis

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Mitochondrial dysfunction is one of the hallmarks of aging, and a main contributor to the age-related loss of skeletal muscle mass and function that poses a major challenge to healthy aging. The impact of mitochondrial dysfunction on muscle aging is multifaceted, ranging from a reduction in metabolic function, dynamics, quality control and turnover. Mitochondrial function and biogenesis in muscle are both strongly associated with the expression of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). It is reported that the expression and transcriptional activity of PGC-1α is reduced in aged muscle, but how this affects the PGC-1α-controlled transcriptional network and whether this is linked to impaired muscle function remains elusive. To study the role of PGC-1α in muscle aging, we conducted a transcriptomic study in muscle tissue from muscle specific PGC-1α gain- and loss-of-function mouse models, at the ages of three and twenty four months. We found that a number of genes related to inflammation were upregulated with age, irrespective of the expression level of PGC-1α. However, expression patterns of genes involved in many mitochondrial metabolic pathways were affected by both age and PGC-1α expression. The age effect was most pronounced in wildtype mice, resulting in the general downregulation of these genes. Young PGC-1α knockout mice showed a 'premature aging' signature similar to old wildtype mice, whereas overexpression of PGC-1α led to the upregulation of these genes compared with wildtype in both young and old animals. Genes involved in mitochondrial translation were affected in a similar manner. We are now studying the functioning of this process in further detail, considering that it produces subunits of some of the complexes in the electron transport chain required for proper functioning of mitochondria. We postulate that impaired mitochondrial translation could contribute to mitochondrial dysfunction, and quality of the aging muscle.

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Using high fat diet in zebrafish: a novel imaging methodology to illustrate mitochondria and lipid droplets distribution

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Mitochondria are key in maintaining the balance between energy consumption and storage. Mitochondrial dysfunction is involved in multiple metabolic and neuromuscular disorders, such as lipid storage diseases. These are characterized by lipid accumulation in various tissues including skeletal muscles. The zebrafish is an emerging model to study neuromuscular diseases. Little is known on the effects of high fat diet in this model, particularly when looking at mitochondrial dynamics and lipid droplets distribution. The goal of this study was to establish the effects of high fat diet in zebrafish larvae and the appropriate methodology to observe its effects in these organelles. In a time and dose response paradigm, zebrafish larvae were fed with two different high fat diets or control diet. During the course of their development, their total body fat content was measured through oil red O staining. Targets of fat metabolism and storage pathways were analyzed with RT-qPCR. We developed a new technique to visualize organelles in larvae sections using gelatin embedding, cryosections and immunohistochemistry on sagittal cuts to illustrate the mitochondrial network as well as lipid droplets distribution dynamics. Out of our three planned cohorts, which will be completely analyzed and finished for this presentation, the first one shows promising preliminary results. Lipid droplets distribution and accumulation, as well as adipocytes formation, were increased in both high fat diet regiments compared to the normal control diet. This was observed in larvae of different ages, including 15, 18, 21, and 25 days post fertilization. Lipid droplet and mitochondria dynamics are impaired in multiple neuromuscular disorders. This can either be caused by the underlying defect or as a consequence to the dysfunctional muscle. The methodology developed in this study, particularly the outputs measuring organelle distribution, may be of use to unveil mechanistic hypotheses using zebrafish models of these diseases.

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LACTB regulates mitochondrial complex I in zebrafish skeletal muscle

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Mitochondria are key organelles for muscle function. Impaired mitochondria are less energetically efficient, produce more ROS and impede cellular quality control mechanisms. For these reasons, exploring the loss of mitochondria functional integrity is important for the understanding skeletal muscle disorders. Mitochondria evolved from alpha-proteobacteria through endosymbiosis. Several mitochondrial proteins are evolutionarily related to bacterial proteins although not always keeping similar functional properties. A good example of this is Lactamase B (LACTB). LACTB, derived from the penicillin-binding/beta-lactamase protein family involved in peptidoglycan synthesis of bacteria, is in eukaryotic cells localized at the mitochondrial intermembrane space. Since mitochondria do not synthesize peptidoglycan, our working hypothesis is that LACTB may have gained one or more novel function(s). Using the CRISPR-Cas9 technology, we generated a LACTB knock-out zebrafish. Mutants are smaller, have reduced locomotion and lower in-vivo respiration. At the molecular level, LACTB deletion alters proteins of the mitochondrial electron transport chain, particularly decreasing the abundance and function of complex I in skeletal muscle. In parallel, proteins of the endoplasmic reticulum involved in stress response and in glycerophopholipid synthesis are modified. We also evidence changes in skeletal muscle lipidomics, particularly of specific phospholipids. Our findings reveal that LACTB is important in regulating mitochondria complex I and phospholipids in skeletal muscle. As mitochondrial phospholipids have been demonstrated to be important for mitochondrial electron transport chain stability, function and mitochondrial shape, we are now investigating whether the observed complex I dysfunction is primary or secondary to the changes in phospholipids. This project is timely and in line with recent works pointing to unique modifications of mitochondrial lipidome in specific neuromuscular diseases. Understanding the subjacent mechanisms is important to hopefully unveil specific therapeutic targets.

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Maintaining ER- mitochondria contacts is neuroprotective in preclinical models of ALS

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron (MN) disease in adults with a prevalence of 6-7 per 100 000 people in Europe. Sporadic and familial cases of ALS share similar clinical-pathological hallmarks, involving muscle atrophy, paralysis, speech and swallowing disabilities, and cognitive dysfunction due to selective MN degeneration in the spinal cord, brainstem and motor cortex. In 2011, mutations in the *C9ORF72* gene characterized by a long hexanucleotide repeat expansion (G4C2) up to thousands of repeats, was found to be highly prevalent in familial ALS and fronto-temporal dementia (FTD). Several cellular processes have been connected to C9ORF72 ALS pathogenesis such as haploinsufficiency of the C9ORF72 gene, repeat RNA mediated toxicity, and dipeptide protein toxicity; nevertheless, it has been challenging to delineate causal from consequential pathogenic alterations. Using MNs differentiated from C9ORF72 patients induced pluripotent stem cells (iPSCs), we have identified a MAM linker molecule GRP75 that serves to not only physically connect the two cellular compartments, but whose transient enhanced expression has a neuroprotective effect during the progression of the pathology. Moreover, taking advantage of the C9orf72 pre-clinical rodent model, we performed pharmacological and viral mediated modulation of ER stress and provide evidence that the ER compartment is critical for optimal mitochondrial function and restoring the physiological levels of ER stress-mitochondria contacts is able to rescue mitochondrial deficits. Lastly, we validate the therapeutic potential of GRP75 via gene therapy using adeno-associated viruses (AAVs) thereby highlighting a promising therapeutic target for ALS.

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High-throughput screening of natural bioactives which promote energy metabolism in primary human myotubes by activating the Mitochondrial calcium uniporter (MCU)

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Accumulation of Ca2+ in energized mitochondria via the Mitochondrial Calcium Uniporter (MCU) is a crucial biological process for energy metabolism, Ca2+ signalling and cell survival. This process is especially relevant in high energy-demanding tissues, including skeletal muscle, where the MCU complex couples contraction to energy production by regulating the activity of mitochondrial TCA dehydrogenases. Despite the recent identification of pharmaceutical MCU inhibitors, nutritional intervention improving MCU activity is poorly investigated, including its effect on skeletal muscle energy metabolism and performance. Here we developed high-throughput assay of mitochondrial Ca2+ import to select MCU-specific natural bioactives present in food. We used mitochondrial-targeted and cytosolic targeted Ca2+-sensitive aequorins to quantify mitochondrial and cytosolic Ca2+ in stimulated cell lines and in primary human myotubes.

Out of more than 5000 natural food compounds, we identified the polyphenol oleuropein as a direct MCU activator. Oleuropein and its metabolites enhanced mitochondrial but not cytosolic Ca2+ rise in stimulated Hela cells. In semi-permeabilized cells stimulated with Ca2+, this phenolic compound increased the activity of MCU, demonstrating the direct effect of oleuropein on the uniporter. In intact primary human myotubes, oleuropein enhanced caffeine-stimulated mitochondrial Ca2+ elevation and boosted the ATP-synthase-dependent component of respiration. Conversely, the effect of oleuropein was prevented when MCU was ablated, demonstrating that the effects of oleuropein on energy metabolism are specifically mediated by MCU activation in myotubes. At the molecular level, we identified by Molecular Docking and Dynamics in silico a binding mode of oleuropein and its aglycone on a binding cleft of the human MICU 1 subunit of the MCU complex. Finally, the Micu1-dependency of oleuropein was validated by measuring the increased activity of MCU in control but not in MICU 1-ablated cells.

In summary we developed a high-throughput discovery strategy to identify natural modulators of the mitochondrial calcium uniporter. We find that the olive leaf polyphenol oleuropein is a selective and direct activator of the MCU channel, which enhances energy metabolism in skeletal muscle via MICU 1 activation.

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Acute RyR1 Ca2+ leak triggers muscle mitochondrial Ca2+ uptake, PDH dephosphorylation and enhances NADH-linked mitochondrial respiratory capacity

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Sustained ryanodine receptor (RyR) Ca2+ leak is associated with pathological conditions such as heart failure or skeletal muscle weakness. We report that a single session of sprint interval training (SIT), but not of moderate intensity continuous training (MICT), triggers RyR1 protein oxidation and nitrosylation leading to calstabin1 dissociation in healthy human muscle and in in vitro SIT models (simulated SIT or S-SIT). This is accompanied by decreased sarcoplasmic reticulum Ca2+ content, increased levels of mitochondrial oxidative phosphorylation proteins, supercomplex formation and enhanced NADH-linked mitochondrial respiratory capacity. Mechanistically, (S-)SIT increases mitochondrial Ca2+ uptake in mouse myotubes and muscle fibres, and decreases pyruvate dehydrogenase phosphorylation in human muscle and mouse myotubes. Countering Ca2+ leak or preventing mitochondrial Ca2+ uptake blunts S-SIT-induced adaptations, a result supported by proteomic analyses. Here we show that triggering acute transient Ca2+ leak via RyR1 in healthy muscle may contribute to the multiple health promoting benefits of exercise.

Notes

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