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

15th Swiss Meeting on Muscle Research

Macolin / Magglingen

16th-18th November 2025

Programme 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.

Programme

Sunday, November 16th

16:30-17:15 Arrival, Check-in

17:15-18:30 Welcome Apero

18:30-19:30 Dinner

19:30-19:35 Meeting opening - Alain Pfulg and Markus Rüegg

19:35-20:20 **Keynote lecture:**

Unraveling the pathogenesis of Duchenne muscular dystrophy to advance therapy development
Maaike van Putten, Leiden University Medical Center, Leiden, The Netherlands

Session 1: Fibrosis in neuromuscular disorders **Chair: Markus Rüegg**

20:20-20:40 Persistent tissue regeneration and transforming growth factor-β induced fibrosis in the masseter muscle of mdx5Cv mice
Aouatef Djeraba Ait-Lounis, University of Geneva

20:40-21:00 Biofabrication of a 3D microphysiological platform for the study of the human muscle microenvironment in aging and disease
Simone Bersini, Ente ospedaliero cantonale, Lugano

Monday, November 17th

Session 1: New diagnostic approaches **Chair: Werner Z'Graggen**

8:30-8:50	Towards an effective multimodal biomarker panel for ALS/FTD spectrum diagnosis Nils Briel, Universitätsspital Zürich
8:50-9:10	ALS Registry Switzerland Jan Loosli, Universitätsspital Zürich
9:10-9:30	Open and reproducible pipeline for the acquisition and analysis of muscle MRI data in Facioscapulohumeral Muscular Dystrophy Claudia Weidensteiner, Universität Basel
9:30-9:50	Open MRI Pipeline for muscle strain calculation Introduction Marta Brigid Maggioni, Universität Basel
9:50-10:00	Téléthon

10:00-10:30 Coffee break

Session 2: Mitocondrial involvement in health and disease **Chair: Antoine Clery**

10:30-10:55	The silent isoform: alternative splicing of PLIN3 reveals mitochondrial targeting with elusive protein expression Axel Aguettaz, University of Lausanne
10:55-11:20	How does nutrition impact skeletal muscle metabolic health? Novel connections involving lipids Cassandra Tabasso, University of Lausanne
11:20-11:45	An updated inventory of genes essential for oxidative phosphorylation identifies a mitochondrial origin in familial Ménière's disease Marcell Harhai, University of Lausanne
11:45-12:10	New aspects of TGFβ signalling in muscle regeneration Jérémy Kessler, University of Geneva

12:10-13:30 Lunch

Poster session

13:30-15:30 ALL POSTERS, coffee and tea

Session 3: Muscle structure and performance — Chair: Susan Treves

15:30-15:55 Characterization of skeletal muscles from STIM1L KO mice
Stéphane Koenig, University of Geneva

15:55-16:20 The novel roles of STIM1S and STIM1L in skeletal muscle architecture
Tahir Idris, University of Geneva

16:20-16:45 A subsynaptic kinase regulates muscle fiber identity and its restoration ameliorates cancer cachexia
Volkan Adak, University of Basel

16:45-17:10 Zebrafish as a model for human skeletal muscle quality: identifying critical windows for intervention
Mauricio Castro Sepulveda, University of Lausanne

17:10-17:35 Muscle spindle function and its impact on the musculoskeletal system
Alexis Ruiz, University of Basel

17:35-17:45 Break

Session 4: Satellite cells and regeneration — Chair: Francesca Amati

17:45-18:10 Chemical reprogramming of committed mouse myoblasts into myogenic stem cells with satellite cell characteristics
Ori Bar-Nur, ETH Zürich

18:10-18 :35 Human muscle reserve cells represent a quiescent and heterogeneous stem cell population characterized by distinct Pax7 expression and stem cell states
Thomas Laumonier, University of Geneva

18:35-19:00 Orai3 and AHNAK2 regulate the activation of human skeletal muscle stem cells in vitro
Mélanie Forgeaud, University of Geneva

19:00-20:30 Dinner
Poster prize committee meets over dinner

Evening program

20:30-23:00 free beer at poster site

Tuesday, November 18th

Session 1: New therapeutic perspectives **Chair: Christoph Handschin**

9:00-9:25	Wif1 modulates the inflammatory microenvironment in LAMA2 Muscular Dystrophy Eleonora Maino, University of Basel
9:25-9:50	Dual AAV gene therapy using laminin-linking proteins ameliorates muscle and nerve defects in LAMA2-related muscular dystrophy Judith Reinhard, University of Basel
9:50-10:15	Pharmacological inhibition of LIN28 reverses dysregulated ion channel and miRNA expression in myotonic dystrophy Alok Behera, University of Geneva / ETH Zürich
10:15-10:40	Lysosomal signalling meets glucose homeostasis: a novel perspective in the fight of Pompe disease Andrea Armani, University of Zürich
10:40-11:05	High pressure melts toxic fibrils and uncovers hidden biomolecular states driving neuromuscular disease Mihajlo Novakovic, ETH Zürich

11:05-11:15 Concluding remarks and poster prizes -Markus Rüegg

11:15-11:45 Coffee Break and Poster removal

Departure

Abstracts

Contact: aouatef.ait-lounis@unige.ch

Persistent tissue regeneration and transforming growth factor-β induced fibrosis in the masseter muscle of *mdx5Cv* mice

Aouatef Djeraba Ait-Lounis

Division of orthodontics, University clinics of dental medicine, Faculty of Medicine, University of Geneva

Clinical observational studies have shown that patients with Duchenne Muscular Dystrophy (DMD) often develop orofacial dysfunction. However, most DMD mouse model studies have focused on limb and respiratory muscles, showing an extensive wave of muscle necrosis-regeneration in juvenile followed by a low-intensity chronic disease in adults. In the present study, we investigated the impact of DMD on the masseter muscles in mice and observed persistence and progression of the conditions at least until 12 months of age. Masseter and limb muscles from mdx5Cv mice aged 3, 6, and 12 months were compared with those from control mice (C57BL/6J background), measuring levels of necrosis, regeneration, inflammation, and fibrosis. In addition, mRNA expression of markers associated with fibrosis and transforming growth factor-beta (TGF-β) signalling were examined.

Our findings revealed that regeneration and inflammation were increased in dystrophic masseter muscles at 3 months of age and persisted to older ages. Fibrosis was more pronounced in the dystrophic masseter muscles at 6 months of age and revealed an increase of the fibro-adipogenic progenitors cell population. Notably, we found elevated deposition of fibronectin and TGF-β in fibrotic foci of the dystrophic masseter muscles. Increased TGF-β signalling was confirmed by a significant increase in nuclear localization of phosphorylated SMAD2 (pSMAD2) in dystrophic masseter and limb muscles. Our results suggest that masseter muscles may exhibit more sustained dystrophic damage than locomotor muscles. We speculate that any therapeutic developed for DMD may benefit to orofacial issues, although their efficacy remain to be established.

Contact : simone.bersini@eoc.ch

Biofabrication of a 3D microphysiological platform for the study of the human muscle microenvironment in aging and disease

Simone Bersini

Laboratories for Translational Research, Ente Ospedaliero Cantonale AND Euler Institute, Faculty of Biomedical Sciences, Università della Svizzera Italiana

Tissue fibrosis is a hallmark of organ degeneration during aging and disease. However, our knowledge of the biological mechanisms regulating the progression of fibrosis is still limited and a few therapeutic treatments are available[1]. Skeletal muscle fibrosis characterizes physiological aging and incurable diseases, including dystrophies. Beyond myofibroblast involvement, recent findings highlighted a complex cellular cross-talk within the muscle microenvironment, finally resulting in vascular and tissue damage. This complexity is not captured by current simplistic in vitro models, hence compromising the development of effective therapies[2]. Here, we biofabricated a human fibrotic skeletal muscle where a suspended myobundle is surrounded by a vascularized microenvironment. Based on our previous model[3], a miniaturized platform was microfabricated using high-resolution 3D-printing. This platform embedded flexible pillars and a custom-made electrical stimulation for triggering contraction in 36 independent tissues. A 1-cm long, free-standing myobundle (diameter 500µm) was biofabricated and cultured for three weeks. Combination of imaging-based and RNAseq analyses demonstrated myobundle maturation over time, with upregulated genes connected with sarcomere organization/muscle contractility. Electrical stimulation triggered contraction with a force of 350µN/mm2. To test the model in fibrotic conditions, a 3D matrix containing muscle-specific fibroblasts (from Duchenne patients or healthy donors), endothelial cells (ECs) and macrophages was cast around the muscle. Dystrophic fibroblasts impaired contraction, while analysis of the stromal compartment revealed activation of endothelial-mesenchymal transition. Indeed, retrieved ECs analyzed through flow cytometry, qRT-PCR and immunofluorescence showed upregulation of mesenchymal markers associated with vascular damage (e.g. PAI1,CDH2). Similar results were achieved within an inflammatory microenvironment embedding M1-macrophages. The compromised muscle contractility was partially reverted with Nintedanib. Indeed, biochemical assays and mass-spec demonstrated reduced collagen deposition and improved mitochondrial functionality upon treatment, resulting in restored contractility. This miniaturized platform combined contractile myobundles with a complex microenvironment, demonstrating its potential for testing/repurposing anti-fibrotic drugs and for analyzing the interactions among different cell populations in the broad context of muscle diseases and aging.

[1]Rockey,2015,N.Engl.J.Med. [2]Bersini,2018,Adv.Drug.Deliv.Rev. [3]Bersini,2018,Cell Reports
ACKNOWLEDGEMENTS: Fondation Suisse de Recherche sur les Maladies Musculaires; Novartis Foundation for medical-biological research..

Contact: nils.briel@usz.ch

Towards an effective multimodal biomarker panel for ALS/FTD spectrum diagnosis

Nils Briel

Department of Neurology, University Hospital and University of Zurich

The diagnosis and prognosis of the amyotrophic lateral sclerosis and frontotemporal dementia spectrum (ALS/FTD) is still largely determined by clinical assessments due to a lack of established fluid biomarkers. We aimed to identify a multimodal biomarker panel readily available in tertiary care centers, that accurately differentiates ALS/FTD from other neurodegenerative diseases. We conducted a retrospective, cross-sectional study analyzing routine clinical, neuroimaging, cerebrospinal fluid (CSF) and serum data from 229 samples, including 45 from patients with ALS, 26 with FTD, 158 with other neurodegenerative diseases, and 29 from healthy controls. Receiver operator curve (ROC) analysis was used to evaluate the predictive ability of individual and combined markers integrated in multivariable machine learning models. The CSF phospho-Tau181:total-Tau ratio (pTau:tTau) was confirmed as a robust individual predictor of ALS/FTD (AUC = 0.65) and ALS (0.77), performing poorly in FTD (0.57). A ten-items CSF + clinical parameters panel (including pTau:tTau, CSF amyloid-β, CSF and serum immunoglobulin levels, global cognition, and temporal measures of disease onset and duration) significantly improved performance in diagnosing ALS/FTD (AUC = 0.79), ALS (AUC = 0.93) and FTD (AUC = 0.68). Incorporating information from neuroimaging (i.e., mesiotemporal and gobal cortical atrophy) added to an increased predictability of FTD (AUC = 0.7).

These findings support that a concise multimodal biomarker panel centered on pTau:tTau, immunological and atrophy measures as well as temporal disease dynamics offers high diagnostic performance for ALS/FTD in this real-world cohort including typical disease-mimicking conditions. Further validation of these models is warranted to estimate their utility for earlier and more reliable biomarker-supported diagnoses.

Contact: jan.loosli@usz.ch

ALS Registry Switzerland

Jan Loosli

Department of Neurology, University Hospital Zurich

The amyotrophic lateral sclerosis (ALS) registry Switzerland aims to explore environmental and genetic risk factors as well as biomarkers to facilitate diagnosis. The registry consists of a case-control study and a biobank for serum, EDTA blood and cerebrospinal fluid (CSF). Using an extensive baseline questionnaire and yearly follow-up questionnaires that are filled out by patients as well as clinical data from regular visits, we aim to determine environmental, behavioral, and genetic risk factors to generate preventive strategies, delay the onset of the disease and slow disease progression. The questionnaires inquire about lifestyle factors, medication, depression, family history, comorbidities, and head traumas. Clinical data contain information on ALSFRS-R, neurological and nutritional status and lung function. The establishment of a biobank containing blood and liquor samples aims to investigate biomarkers that can help in the monitoring of disease progression and in the diagnosis, which could result in earlier treatment and care. Laboratory analyses will include markers for glucose and fat metabolism, neurofilament light chains, TDP-43, amongst others. For the project, a total of 300 cases and 600 controls will be included. Inclusion criteria are age >18 and the diagnosis of an at least possible ALS assessed via El Escorial criteria or a positive diagnosis according to Gold Coast Criteria. Patients and controls will be matched for sex and age. As of today, four Swiss clinics (University Hospital Zurich, Ente Ospedaliero Cantonale, Hôpitaux Universitaires de Genève, Cantonal Hospital Lucerne) are participating in the registry, which thus far have recruited 88 patients.

Contact: claudia.weidensteiner@unibas.ch

Open and reproducible pipeline for the acquisition and analysis of muscle MRI data in Facioscapulohumeral Muscular Dystrophy

Claudia Weidensteiner

Department of Radiology, University Hospital Basel and Department of Biomedical Engineering, University of Basel

Facioscapulohumeral muscular dystrophy (FSHD) is a rare neuromuscular disease characterized by muscle degeneration and fat infiltration leading to progressive muscle weakness. The main quantitative MR imaging measures for FSHD and other neuromuscular diseases are water T2 (wT2), which indicates edema and acute disease activity, and the fat fraction, which assesses muscle degeneration by fat infiltration. Only recently, effective drug candidates are entering clinical development. For upcoming clinical multicenter studies, standardized, easy-to-use data acquisition and post-processing tools have to be available that are capable of acquiring and processing MRI data on/from a range of scanners.

The aim of this project was to create a fully reproducible, vendor-independent solution for the acquisition of MR biomarkers of FSHD, from image formation to postprocessing, by refining existing open-source tools and combining them with new solutions. For data acquisition, we developed a scanner-independent acquisition sequence based on multi-echo spin-echo (MESE) sequences for the measurement of wT2. For data analysis, we adapted the existing open-source tool for wT2, and adapted an existing tool for the calculation of fat fraction based on the most commonly used 2-point-Dixon fat/water measurements. The developed sequence was tested on volunteers before and after a software upgrade of the MRI scanner. Testing of the post-processing tools was performed on existing data of clinical FSHD studies (patients and healthy volunteers). We published the MESE sequence and the postprocessing pipeline on GitHub, where it is openly available. The goal of our open source software solution is to advance independent, reproducible academic research especially in (but not limited to) the field of FSHD.

Contact: martabrigid.maggioni@unibas.ch

Open MRI Pipeline for muscle strain calculation Introduction

Marta Brigid Maggioni

University of Basel

In this work, we present a vendor-agnostic workflow for measuring forearm muscle strain induced by NMES, covering all steps from accelerated CINE 4D-flow data acquisition to reconstruction and analysis to addresses challenges in quantitatively evaluating rare neuromuscular diseases and standardizing flow data processing, which is often non-standardized and not openly available, limiting reproducibility and multi-center studies. Materials and Methods: A 4D-flow sequence was implemented in PyPulseq (v1.4.3) using a 3D cartesian GRE acquisition, with CINE acquisition triggered by NMES-induced forearm contractions. Elliptical k-space trimming and Poisson disc undersampling (factor 9, with a fully sampled center) were applied. Complex data were reconstructed using coil sensitivity maps from BART's ESPIRiT algorithm. The protocol was tested on eight healthy subjects (age 24–31, 5 females, 3 males) on a 3T scanner (Prisma, Siemens Healthineers) during NMES-evoked contractions, with an MRI-compatible force sensor recording force. Our analysis pipeline, which calculates strain tensors from velocity and displacement to assess muscle dynamics by computing the deformation gradient tensor, deriving the Eulerian strain tensor, and extracting its eigenvalues to represent principal strains was used. Strain rates are quantified by fitting time-varying strain curves with sigmoid functions. Flow sequence parameters included: 27 phases, 2 lines/segment, TR=6.7 ms, TE=4.5 ms, FA=10°, 1.5×1.5×1.5mm³ resolution, venc=0.2 s, and a 5-minute acquisition time. Sequence and analysis tools are available at https://github.com/BAMMri/Pulseq-4DFlow and https://github.com/BAMMri/3D-Dynamic-Velocity. Results: Undersampling and elliptical scanning reduced the acquisition time from 28 to 5 minutes. NMES-activated muscles are clearly distinguishable on the calculated displacement and strain eigenvalue maps. The build-up rate, determined via fitting, was 2.035 s^{-1} in the Flexor Digitalis Superficialis. Combining force sensor data with strain curves showed that strain peaks before force, due to elastic properties in the tendon-muscle complex. Discussion and Conclusion:

This work presents a fully open-source 4D-flow sequence and analysis pipeline for extracting velocity, displacement, and strain from NMES-stimulated skeletal muscles. Future improvements will focus on including automated registration of anatomical scans and adopting standardized data formats like musclebids..

Contact: axel.aguettaz@unil.ch

The silent isoform: alternative splicing of PLIN3 reveals mitochondrial targeting with elusive protein expression

Axel Aguettaz

The Aging and Muscle Metabolism Lab, University of Lausanne

Lipid homeostasis is essential for skeletal muscle function, as fatty acids provide a major source of energy for contraction. Disturbances in lipid metabolism and storage are a hallmark of several neuromuscular diseases, where lipid accumulation, mitochondrial dysfunction, and impaired energy supply contribute to muscle weakness and degeneration. Lipid droplets (LD), dynamic organelles that regulate lipid storage and consumption, are central players in this process. Perilipin3 (PLIN3) is a ubiquitous member of the Perilipins LD-coating protein family. PLIN3 contributes to LD growth, lipophagy, phosphatidylcholine synthesis and to the cellular protection from lipid toxicity. PLIN3 is highly expressed in skeletal muscle, where its levels have been correlated with fatty acid oxidation and exercise training.

We report on an uncharacterized PLIN3 transcript originating from an alternative splicing event, here named PLIN3B. PLIN3B overexpression in cells highlighted a specific mitochondrial targeting in multiple imaging and cell fractionation approaches. Electron micrographs revealed alterations of the mitochondrial suborganellar organization. In accordance with localization and expression phenotype, mass spectrometry analysis of PLIN3B interactors identified several mitochondrial partners. In cells, we confirmed the alternative splicing event with splicing-modulating antisense oligonucleotide experiments. Despite being able to tune PLIN3B mRNA expression and detect the alternative transcript in multiple human cell lines and tissues, we could not detect endogenous PLIN3B protein with biochemical approaches, proteomics and databases exploration. Despite confirming PLIN3B splicing variant at the transcriptional level, the translated protein appears to be undetectable in humans. This project opens new questions on the complexity of protein expression regulation and organelle targeting.

Contact: cassandra.tabasso@unil.ch

How does nutrition impact skeletal muscle metabolic health? Novel connections involving lipids

Cassandra Tabasso

Aging and Muscle Metabolism Lab, University of Lausanne

Inflammation and muscular function are tightly coupled with nutrition. Western diet (WD), consisting in increased total energy, saturated fat and fructose, is a modern cause behind insulin resistance (IR), obesity, neuromuscular dysfunction and neuroinflammation. While dietary fat composition is known to influence systemic lipid metabolism and insulin sensitivity, its impact on subcellular lipid classes distribution and fatty acid integration in muscle remains poorly characterized. Here, we tested two hypotheses: 1) That modulating dietary FA intake in mice would modify muscle mitochondria and lipid droplet (LD) lipid profiles, including typical lipotoxicity candidates like DAG. 2) That the changes in intracellular lipid profiles would be related to metabolic health parameters. Mice fed with a 12-week WD became obese and IR. LD and mitochondria were isolated from Soleus for organelle-specific lipidomics. DAG FA composition parallelled dietary FA supply with no subcellular compartment specific differences. Among phospholipids (PL), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were increased in WD LD. FA composition was altered, with changes being class-specific and unrelated to diet composition in all PL classes. FA composition in PL was compartment-specific, with mitochondria and LD being modulated together, highlighting the importance of subcellular lipidomics studies. Correlations between lipid classes abundance and metabolic parameters showed that substrate usage for energy production was strongly related to PL abundance in healthy mice, but not WD. LD sn1,3-DAG content appeared to be a strong predictor of healthy body morphotype, which correlated with ATGL abundance. PE and PG were related to obesity markers in all mice.

This study, combining in vivo phenotyping and organelle-specific lipidomics, points to a potential causal mechanism linking dietary FA composition to muscle metabolism. This nutritional linked mechanism may worsen muscle metabolism in specific muscular diseases. Dietary interventions may be of interest to alleviate muscle decay.

Contact: marcell.harhai@unil.ch

An updated inventory of genes essential for oxidative phosphorylation identifies a mitochondrial origin in familial Ménière's disease

Marcell Harhai

Department of Immunobiology, Faculty of Biology and Medicine, University of Lausanne

Mitochondrial disorders (MDs) are among the most common inborn errors of metabolism, and dysfunction in oxidative phosphorylation (OXPHOS) is a hallmark. Their complex mode of inheritance and diverse clinical presentations render the diagnosis of MDs challenging and, to date, most lack a cure. Here, we build on previous efforts to identify genes necessary for OXPHOS and report a highly complementary galactose-sensitized CRISPR-Cas9 "growth" screen, presenting an updated inventory of 481 OXPHOS genes, including 157 linked to MDs. We further focus on FAM136A, a gene associated with Ménière's disease, and demonstrate that it supports intermembrane space protein homeostasis and OXPHOS in cell lines, mice, and patients. Our study identifies a mitochondrial basis in familial Ménière's disease, provides a comprehensive resource of OXPHOS-related genes, and sheds light on the pathways involved in mitochondrial disorders, with the potential to guide future diagnostics and treatments for MDs.

Contact: jeremy.kessler@unige.ch

New aspects of TGFβ signalling in muscle regeneration

Jérémy Kessler

University of Geneva

Muscle regeneration is a multistep process that is tightly regulated by various factors including dimeric cytokines of the transforming-growth-factor-β (TGFβ) superfamily. Typically discussed and studied as homodimers, heterodimeric combinations between different family members also exist and increase the diversity of cytokine signalling. Another shared feature of all TGFβ-members is their co-expression of the cytokine with a prodomain which undergoes processing and proteolytic cleavage prior to secretion and activation in the extracellular space. Here, we present data for an undescribed heterodimerization of a prodomain/cytokine complex in the TGFβ-family. Genetic data and biological experiments indicate an important role of this heterodimeric cytokine in many developmental processes including skeletal muscle regeneration. Co-expression assays demonstrated that two TGFβ-family members preferentially form heterodimers, despite being previously described as homodimers. This preference is driven by a distinct cysteine pattern in their prodomains, and our data suggest that at least one homodimer aggregates. These folding issues imply that the heterodimer is likely the more abundant cytokine in vivo. Gene expression profiles indicate that this heterodimer is widely expressed across different tissues, including skeletal muscle. In particular, the respective protomers are expressed by myogenic-cells, tendon-cells, and fibro-adipogenic-progenitors, highlighting the potential of the heterodimeric-cytokine in muscle homeostasis and regeneration.

With support from the FSRMM, we further characterized this heterodimer structure and function in muscle. Activation assays revealed that, unlike homodimers, human myotubes activate the latent heterodimeric-cytokine, through αvβ6-integrin in a time- and space-dependent manner. We found that the active heterodimer in myogenic cells primarily triggers SMAD2/3 pathway, unlike a related homodimer linked to fibrosis, which activates SMAD1/5/9 alongside SMAD2/3. Based on structural modelling, we propose that the heterodimer possesses a unique receptor-binding-epitope that elicits a distinct signalling response. This signalling drives non-fibrotic muscle regeneration while preserving reserve-cells in a quiescent state, inhibiting premature differentiation, and preventing excessive myotube fusion, thereby avoiding muscle-hypertrophy. In summary, this new cytokine has strong effects on skeletal muscle regeneration characterizing it as a physiological regulator of tissue homeostasis. A comprehensive understanding of TGFβ-superfamily dimerization holds promise for targeted therapeutic interventions and the development of novel strategies to modulate cellular responses in various pathophysiological conditions.

Contact: stephane.konig@unige.ch

Characterization of skeletal muscles from STIM1L KO mice

Stéphane Koenig

Department of Cell Physiology and Metabolism, University of Geneva

The Store-Operated Calcium Entry is a common mechanism that helps replenish calcium in the endoplasmic or sarcoplasmic reticulum. It depends on the combined actions of calcium-sensing proteins from the STIM family (STIM1, 2) and plasma membrane calcium channels from the Orai family (Orai1, 2, 3). We discovered a unique, longer isoform of STIM1, called STIM1L, which is highly expressed in skeletal muscles and the brain. Interestingly, STIM1L is found only in tetrapods. We showed that this STIM1L variant accelerates the activation of SOCE in human myotubes in vitro. We developed genetically modified mice with a constitutive deletion of *STIM1L* while maintaining the expression of the common STIM1 form. The *STIM1L KO* mice exhibited normal development and displayed no obvious phenotypic traits. However, 18-week-old male and female *STIM1L KO* mice exhibited lower running capacity in running-wheel experiments, indicating potential muscle fatigue. Ex vivo force-frequency and fatigue tests on isolated EDL and soleus muscles from KO and wild-type (WT) mice showed, however, no defect. Additionally, fibers from KO mice display an α-actinin striation pattern identical to WT, again pointing to normal fiber formation Myotubes derived from cells of WT or KO mice show, as expected, that SOCE develops more slowly in KO myotubes. However, the SOCE has the same amplitude in KO and WT, indicating a compensatory mechanism, confirmed by the overexpression of STIM1S (the classical isoform) in some muscles like the quadriceps.

Our results indicate that the EDL and soleus muscles of *STIM1L KO* mice are equally resistant to fatigue compared to WT muscles. The lack of a muscle phenotype could be due to compensation of SOCE by the overexpression of STIM1S. The decreased running activity in these mice might relate to STIM1L expression in other tissues, such as the brain.

Contact: tahir.idris@unige.ch

The novel roles of STIM1S and STIM1L in skeletal muscle architecture

Tahir Idris

Department of Cell Physiology & Metabolism, University of Geneva

Ca^{2+} homeostasis is highly essential for normal cell function and is regulated by several key proteins including the Stromal Interaction Molecule 1 (STIM1) in the sarco/endoplasmic reticulum (SR/ER). Along with the plasma membrane channel Orai1, STIM1 triggers store-operated calcium entry (SOCE) upon Ca^{2+} depletion in the SR/ER, replenishing intracellular stores. A longer isoform of STIM1, STIM1 Long (STIM1L), was previously shown to be expressed specifically in skeletal muscle and brain cells. STIM1L contains an additional 106 amino acids with characteristics of an actin binding domain (ABD) thus suggesting roles beyond SOCE. As a result, this study hypothesizes that STIM1L contributes to the regulation of cytoskeletal architecture in skeletal muscles. AlphaFold and co-immunoprecipitation experiments revealed a specific interaction between the ABD of STIM1L and α-actinin.

To further explore the role of STIM1L on muscle architecture, we employed a lentivirus-based doxycycline-inducible system to downregulate either all STIM1 isoforms (the long and short) or only STIM1L during myotube differentiation. Disrupting STIM1L expression led to a disorganization and an increase in protein expression of α-actinin. Further experiments with the translation inhibitor cycloheximide suggested that the protein stability of α-actinin is also enhanced upon downregulation of either STIM1L or both isoforms of STIM1. We also investigated the role of STIM1L in regulating the extracellular matrix (ECM), an essential component of muscle architecture. STIM1L downregulation significantly reduced laminin expression in nascent myotubes, while fibronectin levels were unaffected, though its fiber organization was disrupted. These findings reveal a novel role for STIM1L in coordinating cytoskeletal and ECM components in skeletal muscles. Further research is ongoing to shed further light on the distinct functions of STIM1L and its shorter counterpart in muscle architecture.

Contact: volkan.adak@unibas.ch

A subsynaptic kinase regulates muscle fiber identity and its restoration ameliorates cancer cachexia

Volkan Adak

Biozentrum, University of Basel

The neuromuscular junction (NMJ), a specialized synapse connecting motor neurons and muscle fibers, is essential for muscle contraction and exhibits distinct functional properties across muscle fiber-types. Fast-twitch fibers, crucial for rapid and powerful movements, are disproportionately vulnerable to wasting in conditions like sarcopenia and cancer cachexia, yet the molecular drivers of this selective susceptibility are poorly understood. Using fiber-type specific transcriptomics of the NMJ, we identified a subsynaptic kinase as a key regulator of fast-twitch myofiber identity, necessary for maintaining neuromuscular integrity and motor performance. This kinase is consistently downregulated in muscle wasting models. Therapeutic restoration of its expression in a cancer cachexia mouse model reversed molecular cachexia signatures, preserved NMJ morphology and function, and mitigated muscle loss. Our findings reveal a mechanistic link between synaptic signalling and muscle fiber vulnerability, highlighting a potential target for cachexia intervention.

Contact: mauricio.castrosepulveda@unil.ch

Zebrafish as a model for human skeletal muscle quality: identifying critical windows for intervention

Mauricio Castro Sepulveda

Department of Biomedical Sciences, University of Lausanne

Multiple diseases, as well as aging, impact skeletal muscle (SM) quality, that encompasses muscle function, size and metabolism. This project aims to analyze SM quality during the aging in zebrafish to identify specific windows for preventive and therapeutic interventions. Zebrafish were divided into five age groups: 7-12 [Y], 19-24 [M], 30-36 [MO], 42-48 [O] and 54-55 [OO] months-old; 11 females /11 males per group. Morphometric and physiological assessments included spontaneous locomotion, in vivo subcutaneous adipocytes imaging and maximal oxygen consumption (MO2max). After euthanasia, SkM samples were analyzed via immunofluorescence to evaluate fibers cross-sectional area (CSA), electron microscopy to evaluate mitochondria and LD content and morphology, and Western blotting to evaluate target proteins. Two-way ANOVA was performed to evaluated age and sex effects. OO fish develop scoliosis and have larger adipocytes. Spontaneous swimming velocity and MO2max/surface-area declines with age, although males maintain higher velocity swimming and MO2max. Fast fiber CSA decreases in OO fish with sex-specific patterns. Mitochondrial density and morphology vary by fiber type and age; LD accumulate specifically in O fish slow fibers. Despite age-related declines in MO2max, key mitochondrial proteins (e.g., OXPHOS complexes, dynamics regulators) remain stable. SKM quality in zebrafish show similar decline compared to human aging studies. We identified two specific time-points where quality drops significantly, including loss of type II fibers CSA, reduction of mitochondrial content, increase in LD numbers and declines in exercise capacity. These could be used to investigate mechanistic queries related to aging or diseases involving loss of muscle quality.

Contact: alexis.ruiz@unibas.ch

Muscle spindle function and its impact on the musculoskeletal system

Alexis Ruiz

University Hospital Basel

Muscle spindles are stretch receptors lying deep within the muscle belly involved in detecting changes in muscle length and playing a fundamental role in motor control, posture and synchronized gait. Dysfunction of muscle spindles leads to abnormal proprioceptor function, which has been linked to aberrant bone and cartilage development, scoliosis, kyphosis and joint contractures. RYR1, the gene encoding the calcium release channel of the sarcoplasmic reticulum, is the most common target of mutations linked to human congenital myopathies, a condition often accompanied by skeleton alterations and joint contractures. So far, the link between RYR1 mutations, altered muscle spindles and skeletal defects has not been investigated. To this end, we investigated heterozygous mice carrying recessive *Ryr1* mutations isogenic to those present in a severely affected child. We show that a massive reduction of mutant RyR1 in intrafusal muscle fibers leads to altered expression of intrafusal fiber proteins, severe whole-body alterations, and gait coordination. These results support the hypothesis that RYR1 mutations not only affect the function of extrafusal muscles, but might also affect that of intrafusal muscles.

Contact: ori.bar-nur@hest.ethz.ch

Chemical reprogramming of committed mouse myoblasts into myogenic stem cells with satellite cell characteristics

Ori Bar-Nur

ETH Zürich

Quiescent muscle stem cells, commonly known as satellite cells, are essential for muscle repair and can convert into committed myoblasts capable of proliferation and differentiation in vitro. However, myoblasts frequently lose myogenic potential following prolonged propagation, limiting their utility in research and clinical applications. Here, we demonstrate that exposing committed mouse myoblasts to a small-molecule cocktail targeting the cAMP, TGF-β and WNT pathways, elicits their conversion into expandable satellite-like myogenic progenitor cells (MPCs). Using a novel dual-fluorescent reporter for Pax7 and MyoD, we demonstrate that the small molecules de-differentiate PAX7+/MYOD+ myoblasts into PAX7+/MYOD- satellite-like MPCs within days. This conversion is characterized by upregulation of signalling pathways associated with muscle stem cells in vivo including Notch, Calcitonin, P53 and EGFR. Accordingly, genetic ablation of Notch1-expressing cells abrogated MPC cultures but not parental myoblasts. Furthermore, single-cell transcriptomic analysis comparing MPCs to myoblasts and in vivo-derived freshly isolated and activated satellite cells revealed that the stem cell subset in MPCs shares common features with a subpopulation of self-renewing activated satellite cells. Collectively, our study presents a method to dedifferentiate committed myoblasts into MPCs that exhibit satellite cell attributes, offering a new avenue for studying myogenesis and advancing muscle disease therapeutics.

Contact: thomas.laumonier@unige.ch

Human muscle reserve cells represent a quiescent and heterogeneous stem cell population characterized by distinct Pax7 expression and stem cell states

Thomas Laumonier

Cell Therapy & Musculoskeletal Disorders Lab, University Hospital and Faculty of Medicine, Geneva

In recent decades, muscle stem cells (MuSCs) have emerged as a promising therapeutic target for muscle diseases, demonstrating significant potential for muscle cell therapy. However, optimising this strategy is crucial, as culture conditions can reduce the regenerative capacity of expanded MuSCs. Previous research has shown that human muscle reserve cells (MuRCs), which are generated in vitro, are quiescent Pax7+ MuSCs that are arrested in a reversible G0 cell cycle state. These cells have an enhanced regenerative potential compared to proliferating human myoblasts. Through transcriptomic analysis, we discovered that human MuRCs exhibit heterogeneity in Pax7 expression. A Pax7-High subpopulation was found to be in a deeper state of quiescence and less primed for myogenic differentiation, exhibiting lower metabolism. Furthermore, we demonstrated that human MuRCs are multipotent stem cells exhibiting strong immunosuppressive properties in vitro. Recently, we observed that intracellular autofluorescence (AF) can be used to isolate viable and functional human MuRC subpopulations without the need for labelling. AF enables the identification of human MuRC subsets; the AF-High subpopulation is associated with increased lipid content. AF-High MuRCs are enriched in Pax7-High cells and demonstrate delayed activation and slower proliferation, as well as comparable engraftment efficiency to the AF-Low subpopulation. We have also investigated cellular heterogeneity within the human MuRC population using single-cell RNA sequencing.

Our initial findings show that the human MuRC pool comprises eight distinct cell clusters in various states. Interestingly, our data revealed a key subpopulation in a deeper quiescent state characterised by high co-expression of Pax7, the mitotic inhibitor Cdkn1c and the transcription factor Hes1. Overall, this research opens up significant possibilities for the clinical application of human MuRCs in the treatment of skeletal muscle diseases.

Contact: melanie.fourgeaud@etu.unige.ch

Orai3 and AHNAK2 regulate the activation of human skeletal muscle stem cells in vitro

Mélanie Fourgeaud

University of Geneva

Skeletal muscle repair and maintenance rely primarily on the activation of precursor cells, known as muscle stem cells (MuSC). Upon activation, skeletal MuSC re-enter the cell cycle and proliferate as myoblasts, which subsequently either differentiate and fuse to form new fibers or return to quiescence to maintain long-term regenerative capacity. Understanding the molecular process that governs the activation and self-renewal of human MuSC is crucial, as any dysfunction can negatively affect muscle regeneration in both aging and muscle-related diseases.

Our work used an in vitro model of human primary muscle cells to study the serum-induced activation of stem cell-like cells, called reserve cells (RC). Our laboratory discovered that the calcium channel Orai3 promotes RC activation. Using a protein proximity assay (BioID), we have identified the large scaffold protein AHNAK2 as a new potential partner of Orai3, showing similar effects on RC activation. However, the downregulation of AHNAK2 and Orai3 has divergent effects on myoblast differentiation into myotubes. Furthermore, we found that AHNAK2 promotes RC activation via myotube-derived signals, suggesting that the two proteins utilize different regulatory pathways. Our study has potentially important implications for disease conditions. Indeed, we found that dystrophin downregulation increased the expression of AHNAK2 and Orai3. Preliminary proteomic analysis revealed that AHNAK2, like dystrophin, plays a role in connecting the extracellular matrix to the cytoskeleton. Further research into these proteins may provide new insights into the pathological mechanisms underlying Duchenne muscular dystrophy.

Contact: judith.reinhard@unibas.ch

Dual AAV gene therapy using laminin-linking proteins ameliorates muscle and nerve defects in LAMA2-related muscular dystrophy

Judith Reinhard

Biozentrum, University of Basel

LAMA2-related muscular dystrophy (LAMA2 MD) is a congenital muscular dystrophy caused by recessive loss-of-function mutations in the LAMA 2 gene, which encodes laminin-α2, a key structural component of the muscle fiber basement membrane. The disease manifests in two forms: a severe, early-onset congenital type, typically due to biallelic null mutations leading to complete loss of laminin-α2, and a milder, late-onset form associated with hypomorphic variants that permit residual expression. No therapy currently exists, highlighting a critical unmet medical need. Adeno-associated virus (AAV)-mediated gene replacement is a promising strategy for genetic disorders but is challenged in LAMA2 MD by two major obstacles: (i) the LAMA2 coding sequence exceeds AAV's packaging capacity, and (ii) severely affected patients lack endogenous laminin-α2, raising concerns about immune tolerance.

To overcome these limitations, we engineered two linker proteins derived from endogenous extracellular matrix components. These linker proteins reconstitute laminin receptor binding and polymerization, thereby restoring basement membrane assembly in the absence of laminin-α2. Dual AAV delivery of the linker proteins in a severe LAMA2 MD mouse model achieved robust expression, improved muscle histology, and significantly enhanced motor function. Use of myotropic AAV capsids further increased therapeutic efficacy at reduced vector doses. Notably, muscle-restricted expression unmasked an underlying LAMA2-related peripheral neuropathy. To address this, we implemented a dual-promoter approach: one linker was expressed under the muscle-specific Spc5-12 promoter, while the other was driven by the ubiquitous Cbh promoter. Delivery was achieved by using either AAV8 or AAV9. Both AAV variants have successfully been tested in human clinical trials in neuromuscular disease. Our dual-promoter approach yielded near-complete phenotypic restoration when administered neonatally and substantial functional benefit when applied at advanced disease stages.

These data show that our approach provides a mutation-independent, size-compatible, and potentially immune-tolerable treatment strategy for LAMA2 MD. These findings establish the basis for a clinically translatable therapy addressing both the muscular dystrophy and peripheral neuropathy manifestations of the disease.

Contact: eleonora.maino@unibas.ch

Wif1 modulates the inflammatory microenvironment in LAMA2 Muscular Dystrophy

Eleonora Maino

Biozentrum, University of Basel

Muscular dystrophies (MDs) are a group of genetic disorders characterized by progressive muscle degeneration, typically accompanied by inflammation and fibrosis. The phenotypic variability observed in MDs emerges from the complex interplay among distinct cell types within skeletal muscle. To investigate the molecular mechanisms underpinning muscle dysfunction in MDs, we performed comprehensive transcriptomic profiling on the muscles of the *dyW/dyW* mouse, a model of severe LAMA2-related muscular dystrophy (LAMA2 MD). By integrating bulk RNA-seq, single-nucleus RNA-seq, and spatial transcriptomics, we identified a pronounced downregulation of Wif1, an underexplored Wnt pathway inhibitor in the context of MDs, in the muscles of *dyW/dyW* mice.

Our analysis revealed that Wif1 is primarily expressed in a population of mature perimysial tenocytes, which are depleted in LAMA2 MD muscles along with reduced Wif1 expression. To assess the functional role of Wif1 in LAMA2 MD pathology, we overexpressed Wif1 in *dyW/dyW* muscles. This intervention mitigated dystrophic features by modulating the muscle pro-inflammatory environment, reducing the abundance of fibrotic macrophages, complement activation, and cytokine secretion. Together, these findings provide strong evidence for a pivotal role of Wif1 and Wnt signalling in MD progression, and underscore the influence of non-muscle cell populations in disease manifestations, suggesting promising therapeutic avenues for MD treatment.

Contact: alok.behera@pharma.ethz.ch

Pharmacological inhibition of LIN28 reverses dysregulated ion channel and miRNA expression in myotonic dystrophy

Alok Behera

University of Geneva / ETH Zürich

Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are autosomal dominant disease caused by expansion RNA repeats that affect multiple tissues. Myotonic dystrophy type 1 is caused by binding of CTG expansion repeats in the 3' untranslated region of the Dystrophia Myotonica Protein Kinase, DMPK gene. Myotonic dystrophy type 2 is caused by CCTG repeat expansion in the first intron in CNBP gene. The mutant transcripts containing toxic RNA repeats sequesters muscleblind- like (MBNL1) protein and causes functional loss of MBNL1 and gain of CUGBP Elav like family member 1(CELF1) RNA binding protein (RBP). The sequestration of these proteins by toxic repeats results mis-splicing of several ion channels and miRNA dysregulation which contribute to the pathology of the disease. Intriguingly, for the first time, we have shown an additional RBP and embryonic stem cell factor, LIN28 binding to CTG repeats and dysregulate several ion channels through its miRNA network. Cardiac defects and myotonia are prominent clinical features in DM patients and are directly associated with dysregulation of small number of ion channels that are targets of miRNAs, e.g. miR-1, miR-9, miR-30, miR-107 and miR-181. We have shown that precursors of these miRNAs bind to MBNL1 and LIN28 in in vitro assays, and furthermore are lowly expressed in myotubes from DM1 and DM2 patients compared to those from healthy volunteers. Treatment of cells with C1632, an inhibitor of LIN28, increased levels of several of these miRNAs in DM1 and DM2 myotubes, and reduced levels of several ion channels which includes CACNA1C, CACNA1S and KCNJ2.

Our combined data suggests small molecule therapeutic strategy for DM1 and DM2. We are currently screening other small molecules which has similar LIN28 inhibition activity as an RNA therapeutics strategy towards pathophysiology of myotonic dystrophies or cardiac defects.

Contact: andrea.armani@uzh.ch

Lysosomal signalling meets glucose homeostasis: a novel perspective in the fight of Pompe disease

Andrea Armani

Institute for Neuropathology, University of Zürich

Lysosomal signalling meets glucose homeostasis: a novel perspective in the fight of Pompe disease. Pompe disease (PD) is a rare genetic disorder stemming from mutations inactivating the gene encoding acid maltase (GAA), the enzyme responsible for glycogen breakdown within lysosomes. Due to the impairment of these organelles function, PD has been the first described lysosomal storage disease, affecting 1 in 40,000 live births. GAA deficiency leads to pathological glycogen accumulation in several tissues; in the infantile form, this triggers a severe cardiomyopathy that can be successfully reverted by enzyme replacing therapy (ERT). Moreover, both infantile and late onset (LOPD) forms exhibit a severe, ERT resistant and progressive skeletal muscle wasting and weakness that force patients to wheelchairs and mechanical ventilation; to date, respiratory failure is still the main cause of death in LOPD patients even if under treatment. Studies clarifying the pathogenic cascade guided the optimization of available therapies and the development of next-generation ERT; however, no systematic assessment of lysosomal signalling abnormalities during PD progression has been made due to major technical challenges in the isolation and characterization of functional organelles.

For this reason, we developed an innovative in vivo model crossing GAA KO mice with LysoTag carriers and exploited this system to optimize a robust and reliable protocol for rapid lysosomes immunopurification (LysoIP) starting from Pompe tissues. Thanks to this innovative methodology, we captured highly resolved lysosomal dynamics; remarkably, proteomics analysis revealed the enrichment of all ten glycolytic enzymes in the lysosomal fraction of healthy muscles, leading us to hypothesize the existance of glycolysis microdomains on lysosomal surface. To clarify the contribution of this phenomenon to PD pathogenesis, we investigated Pompe muscles and found that lysosomes deficient of glucosidase activity and lysosomal glucose production exhibit from an early time point a significant and progressive reduction in mature lytic enzymes content, indicating that the lack of lysosomal resident glycolysis leads to an early severe lysosomal failure.

Therefore, despite further studies are needed to clarify the mechanistic details of the phenomenon, our approach identified a neglected but fundamental cellular process occurring on lysosomal surface that could be a major breakthrough in reinterpreting the precocious cascade of events contributing to Pompe disease pathogenesis.

Contact: mihajlo.novakovic@bc.biol.ethz.ch

High pressure melts toxic fibrils and uncovers hidden biomolecular states driving neuromuscular disease

Mihajlo Novakovic

ETH Zürich

Liquid–liquid phase separation (LLPS) allows proteins and RNAs to form dynamic, membraneless assemblies known as biomolecular condensates. These condensates normally facilitate cellular organization and regulation, but when LLPS is perturbed, they can harden into solid-like aggregates and fibrils. Such aberrant phase transitions, especially of proteins like FUS and TDP-43 or repeat-expansion RNAs, are strongly linked to neuromuscular diseases including amyotrophic lateral sclerosis and myotonic dystrophy. We use nuclear magnetic resonance (NMR) to probe condensates under high hydrostatic pressure stress. Pressure favors low-volume, high-energy states, thereby acting as a lens to reveal hidden biomolecular conformations.

Our preliminary data show that FUS undergoes transition to an unexpected high-pressure LLPS state above ~2000 bar, with distinct condensate properties and protein dynamics. In a matured FUS sample, we detected that prolonged exposure to pressure dissolved preformed fibrils, which was accompanied by unfolding of the Zinc finger domain. This suggests that structural destabilization drives fibril melting and redistribution of protein populations. These findings establish high pressure as a unique perturbation to manipulate condensate equilibria and access molecular states invisible at ambient conditions that might be directly responsible for aberrant behavior. Beyond mechanistic insights, they raise the possibility that transient pressure changes in vivo, such as those during head trauma, may alter condensate stability and influence disease risk. Next, we aim to extend this approach to TDP-43 and repeat-expansion RNAs, and to define how pressure-dependent hydration, dynamics, and structural transitions contribute to aberrant phase separation in neuromuscular disease.

Posters

Contact: alikerem.kalkan@hest.ethz.ch

Engineering a 3D in vitro human skeletal muscle model for joint model integration

Ali Kerem Kalkan, Ori Bar-Nur

Department of Health Sciences and Technology, ETH Zürich

Musculoskeletal disorders are a major cause of mobility impairment worldwide, encompassing conditions such as osteoarthritis, rheumatoid arthritis, osteoporosis, and gout. While animal models are commonly used to study these diseases, they often fail to replicate human pathophysiology or predict clinical outcomes. Similarly, conventional two-dimensional human cell cultures lack sufficient maturation and complexity to model disease effectively. To address this gap, advanced in vitro systems incorporating multiple human tissue types in three-dimensional (3D) structures are needed. Here, we present a light-inducible 3D human skeletal muscle model designed for rapid integration into multi-tissue joint models. Under standard differentiation conditions, the construct generated contractile forces of up to 1 mN and maintained functional activity for at least three weeks in vitro, demonstrating robust maturation of human skeletal muscle. This platform provides a versatile foundation for building physiologically relevant musculoskeletal disease models.

Contact: parth.chansoria@hest.ethz.ch

Prolonged cell encapsulation and gravity-independent filamented light biofabrication of muscle constructs

Michael Winkelbauer[1], Jakub Janiak[1], Johannes Windisch[2], Hao Liu[1], Maria Bulatova[1], Max Von Witzleben[2], Hugo Oliveira[3], Sophie Dani[2], Richard Frank Richter[2], Nicolas L'Heureux[3], Ori Bar-Nur[4], Michael Gelinsky[2], Marcy Zenobi-Wong[1], Parth Chansoria[1]

[1] Institute for Biomechanics, Department of Health Sciences and Technology, ETH Zürich
[2] Center for Translational Bone, Joint and Soft Tissue Research, University Hospital and Faculty of Medicine, TU Dresden, Germany
[3] Univ. Bordeaux, Tissue Bioengineering INSERM U1026, France
[4] Laboratory of Regenerative and Muscle Biology, Department of Health Sciences and Technology, ETH Zurich, Schwerzenbach Institute of Biochemistry, Department of Biology, ETH Zurich

The prospects of fabricating human tissue grafts or models using cell-laden bioresins in space has garnered significant interest in recent years. While there has been tremendous progress in extrusion or light-based bioprinting in microgravity conditions, printing of aligned tissues (e.g., muscle, tendon, cardiac, etc.) remains a challenge. Furthermore, current photoresin formulations do not allow long-term cell encapsulation and are difficult to handle in microgravity conditions. In this study, we demonstrate a new gravity-independent filamented light (G-FLight) biofabrication system which can create viable muscle constructs within seconds. We also demonstrate new photoresin formulations based on gelatin methacrylate (GelMA) for encapsulation of primary cells (murine myoblasts) and storage in printing cuvettes for at least a week at 4°C or -80°C. The tissues printed in microgravity based on the new formulations exhibited higher cell viability, number of proliferating cells and higher numbers of myotubes and fusion index compared to control formulations (i.e., GelMA dissolved in phosphate buffered saline). Importantly, the microgravity-printed tissues also featured similar myotube density and fusion index to those printed using the same resins on-ground. The G-Flight printing concept, together with the new resins enabling refrigeration or cryopreservation with encapsulated cells, offers a promising solution for biofabrication in space.

Contact: sabine.raeuber@unibas.ch

Low-cost, open-source, MRI-compatible grip force sensor for NMES-synchronised dynamic muscle MRI

Sabine Melanie Räuber[1,2], Marta Brigid Maggioni[1,2], Francesco Santini[1,2]

[1] Department of Biomedical Engineering, Basel Muscle MRI (BAMM), University of Basel
l[2]Department of Radiology and Nuclear Medicine, University Hospital Basel Department of Clinical Chemistry, University Hospital Zürich

Synchronising dynamic MRI with force measurements and neuromuscular electrical stimulation (NMES) enables standardised assessment of muscle activity but requires MR-compatible force sensors. This study presents the design and validation of an open-source, low-cost, MR-compatible grip force sensor for dynamic forearm muscle MRI. Using a 3D-printed handle housing with two aluminium beam load cells, the device is connected to a microcontroller outside the scanner room via an Ethernet cable. A custom low-pass filter shields the microcontroller and further electronics from MRI interference. The sensor's MRI compatibility was evaluated in a 3T MRI scanner. We assessed its accuracy using calibrated weights and quantified its impact on image quality via phantom measurements of signal-to-noise ratio (SNR), B0 field homogeneity, and radiofrequency (RF) noise. Furthermore, the force sensor was tested in five healthy subjects during dynamic MRI with NMES-evoked muscle contractions. The sensor demonstrated excellent linearity both inside and outside the scanner ($R^2 > 0.99$). With the sensor active, phantom tests showed a minor 3.7% decrease in SNR ($p<10^{-4}$), while B_0 inhomogeneities and RF noise levels were minimally affected. The in-vivo study successfully recorded voluntary contraction force profiles, confirming the setup's reliability for dynamic MRI experiments.

We have demonstrated that a low-cost, open-source grip force sensor built from commercially available components can be safely and effectively integrated into a dynamic muscle MRI workflow. The device maintains high accuracy with an acceptable impact on image quality, providing a robust and accessible tool for characterising forearm muscle activity, with potential applications in the study of myotonic dystrophy.

Contact: matthias.exl@insel.ch

In-vivo recordings of single muscle fibers velocity recovery cycle

Matthias Thomas Exl[1,2], *Werner Z'Graggen*[1,3]

[1]*Department of Neurosurgery, Inselspital, Bern University Hospital, University of Bern*
[2]*Graduate School for Health Science, University of Bern*
[3]*Department of Neurology, Inselspital, Bern University Hospital, University of Bern*

Background: Muscle velocity recovery cycles (MVRCs) measure how the propagation velocity of a muscle action potential changes on the timing of a preceding action potential. MVRCs can be used to asses muscle fiber membrane properties in vivo. As a standard, MVRC recordings are made by stimulating multiple muscle fibers. Objective: This study aimed to compare the MVRC recordings of single muscle fibres with multi-fiber recordings, and to investigate whether muscle velocity recovery cycles could be used to identify different fibre types (type 1 and type 2).

Methods: We recorded MVRCs of single fibres in 20 healthy subjects. These recordings were made in the tibialis anterior muscle using a single conditioning stimulus. The recordings were categorised according to their recovery behaviour and the resulting data were compared with normative data of multi-fiber MVRCs.

Results: Two different behaviours of single muscle fibres could be observed. In general, single fiber recordings showed less supernormality than multi-fiber recordings with one type exhibiting blocking behaviour with shorter inter-stimulus intervals.

Conclusion: Using single muscle fiber MVRC recordings, two different types of MVRC patterns can be identified. Further research is needed to evaluate if the MVRC pattern corresponds to different muscle fiber types.

Contact: hadi.salem@unil.ch

Decoding lipotoxicity: Role of diacylglycerols in organelle specific mechanisms of muscle dysfunction

Hadi Salem[1], Cassandra Tabasso[1], Gary Domeniconi[2], Axel Aguettaz[1], Sylviane Lagarrigue[1], Milena Schuhmacher[2], Francesca Amati[1]

[1] Department of Biomedical Sciences, University of Lausanne
[2] Institute of Bioengineering, EPFL

Mitochondrial dysfunction in skeletal muscle is involved in multiple muscle disorders characterized by intramuscular lipid accumulation (myosteatosis) and local inflammation. Impaired mitochondria promote incomplete fatty acid oxidation, producing lipotoxic intermediates that further affect metabolism. Among these are diacylglycerols (DAG) which induce insulin resistance through phosphorylation of protein kinase C (PKC) and decrease glucose uptake following insulin stimulation. DAG-activated PKC also affect neurotransmitters release at the neuromuscular junction. The DAG family comprises chemically distinct species, which have different cellular spatial distributions. How localization affect metabolism is poorly understood.

Here, we characterize the effects of spatially and temporally controlled DAG release at mitochondria. Using UV-labile mitochondria-targeted probes, we released 3 different DAG species into the mitochondrial inner membrane. We selected one saturated, one monounsaturated and one polyunsaturated DAG based on their previously reported abundance in human skeletal muscle. Given that fibroblasts retain donor metabolic characteristics and are easy to use compared to myotubes, we used this cellular model for our in vitro experiments. First, we confirmed that the 3 DAG probes localize specifically to mitochondria. We evaluated fusion and fission at different timepoints post DAG release, revealing a time-dependent effect of DAG accumulation. Mitochondrial function, assessed by respirometry, illustrated DAG-related alterations. We will further assess DAG effects by measuring fatty acid oxidation and glycolysis, and evaluate individual respiratory complexes capacity.

The potential of this project is to unveil mitochondrial DAG-mediated mechanisms of action and uncover how specific lipid species are implicated in metabolic pathologies. Studying DAG function at the species-specific level, may explain the many different roles of DAG as messengers, toxic lipid metabolites, or structural components of membranes. This project has the potential to unveil the role of nutrition on mitochondrial DAG composition and leverage novel pharmacological interventions.

Contact: julien.duvernay@unil.ch

The role of LACTB in skeletal muscle maintenance and age-associated degeneration

Julien Duvernay, Sylviane Lagarrigue, Cassandra Tabasso, Mauricio Castro-Sepulveda, Francesca Amati

Aging and muscle metabolism lab, Department of Biomedical Sciences, University of Lausanne

Sarcopenia, the progressive loss of skeletal muscle mass and function with age, is a major contributor to frailty and reduced mobility in the elderly. This condition is closely linked with mitochondrial dysfunction, which impairs energy production and accelerates muscle atrophy. Identifying molecular factors that preserve mitochondrial integrity in aging muscle is essential to understanding age-related decline. LACTB, a mitochondrial serine protease known to regulate lipid metabolism in cancer cells, has an uncharacterized role in skeletal muscle aging.

We generated a zebrafish *LACTB KO* using CRISPR-Cas9 and studied a 23-month-old cohort. Muscle samples were embedded in OCT, cryosectioned transversely, and stained to distinguish fast and slow fibers. High-resolution imaging with a spinning disk microscope is being used to quantify cross-sectional area (CSA) of fast and slow fibers separately to assess fiber type-specific effects of *LACTB* deletion. In parallel, lipid droplet abundance is being quantified by immunohistochemistry to evaluate possible alterations in lipid storage. Electron microscopy of muscle sections is also being performed to analyze mitochondrial number, size, and morphology in both intermyofibrillar and subsarcolemmal compartments. These analyses will clarify whether LACTB contributes to sarcopenia by modulating lipid metabolism and mitochondrial structure in aged skeletal muscle. Based on preliminary results from our laboratory, we expect *LACTB KO* to exhibit reduced CSA in fast fibers and disrupted mitochondrial morphology. Such findings would support the hypothesis that LACTB protects muscle architecture and mitochondrial integrity during aging.

Contact: sylviane.lagarrigue@unil.ch

Effect of RYR1 mutations on muscle spindle function and their impact on the musculoskeletal system

Sylviane Lagarrigue, Mauricio Castro-Sepulveda, Adrien Martinotti, Jocelyn Fleurimont, Cassandra Tabasso, Francesca Amati

Aging and muscle metabolism lab, Department of Biomedical Sciences, University of Lausanne

Mitochondrial dysfunction is a hallmark of many muscular diseases, including myopathies and sarcopenia. Lactamase B (LACTB), a mitochondrial protein derived from the bacterial penicillin-binding/β-lactamase family, is an intriguing candidate regulator of muscle metabolism. Although its ancestral bacterial role was linked to peptidoglycan synthesis, mitochondria lack this pathway, suggesting LACTB has evolved novel functions in eukaryotic cells.

To investigate its role in muscle physiology, we generated a zebrafish *LACTB knockout* (KO) using CRISPR-Cas9. KO fish exhibited normal body weight and length but showed increased body surface area and altered locomotor activity, characterized by reduced low-speed swimming and longer pause times. During incremental swimming tests, KO fish consumed more oxygen than controls at the same speeds, consistent with impaired mitochondrial efficiency or altered energy substrate utilization. At the tissue level, fast-twitch fibers of KO fish displayed increased mitochondrial circularity and reduced respiratory capacity, whereas slow-twitch fibers maintained normal mitochondrial respiration but accumulated lipid droplets alongside elevated expression of lipid synthesis genes. These findings reveal that LACTB contributes to muscle homeostasis through fiber type–specific regulation of mitochondrial structure, respiratory function, and lipid metabolism. By linking altered mitochondrial dynamics and metabolic inefficiency to muscle performance, our study positions LACTB as a potential player in pathways underlying muscular pathologies.

Understanding how LACTB shapes muscle bioenergetics may provide new insights into mechanisms driving mitochondrial myopathies, lipid accumulation disorders, and age-related sarcopenia.

Contact: florent.chabry@unige.ch

Characterization of mitochondria remodeling following muscle denervation

Florent Chabry[1], Gaëtan Lopez[1], Olivia Cattaneo[1], Nicolas Liaudet[2] and Perrine Castets[1]

[1]Department of Cell Physiology and Metabolism, University of Geneva
[2]Bioimaging Core Facility, University of Geneva

Neuromuscular junctions (NMJs) are the synapses connecting motor neurons to skeletal muscle, enabling contraction and maintaining muscle homeostasis. Nerve injury disrupts muscle function and induces profound remodelling of muscle fibers. In particular, denervation causes severe muscle atrophy, a strong induction of synaptic and myogenic genes, and marked shifts in the contractile and metabolic properties of muscle fibers. In mice, denervation of fast muscle, such as tibialis anterior (TA), induces a transition toward slower oxidative fibers, which involves HDAC4-dependent regulation of genes encoding myosin heavy chains and glycolytic enzymes. Conversely, denervation drives a shift towards faster muscle fibers in slow muscles, such as soleus, although the underlying mechanisms remain poorly understood. Mitochondria remodelling accompanying these metabolic changes is largely unexplored.

Here, we analyzed changes in mitochondria function and organization following muscle denervation. Electron microscopy and immunostaining of isolated fibers, combined with 3D reconstruction and MATLAB-based analyses, revealed that the mitochondria network shifts from a transversal to a longitudinal orientation relative to the fiber axis after 2 and 3 weeks of denervation. Imaris 3D volumetric reconstitution further showed a reduction in mitochondria volume compared to innervated muscle. Notably, this remodelling occurred without any changes in the transcript levels of genes encoding mitochondria fusion/fission machinery (e.g., Mfn2 or Opa1) at 2 days or 2 weeks post nerve injury. These ultrastructural changes coincided with the increased proportion of fibers with high NADH activity in TA muscle, consistent with the oxidative shift induced by denervation. Although the expression of nuclear genes encoding respiratory chain sub-units was globally down-regulated 2 days after denervation, there was no major change in the protein levels of respiratory complexes I to V at 2 days and 2 weeks of denervation. Ongoing analyses aim to define the kinetics of mitochondria remodelling after denervation and to compare these responses in fast and slow muscles.

These studies will provide new insights into the muscle response to nerve injury and may help to understand the mechanisms underlying pathological conditions involving loss of neuromuscular integrity, such as sarcopenia.

Contact: ioannis.roussis@unibas.ch

Investigating the molecular basis and function of PGC-1α in skeletal muscle following exercise stimulation

Ioannis-Marios Roussis[1], Sandrine Quirim[1], Gesa B.S. Santos[1], Stefan Steurer[1], Konstantin Schneider-Heieck[1,2] and Christoph Handschin[1]

[1]Biozentrum, University of Basel
[2]Department of Biosystems Science and Engineering, ETHZ, Basel

Endurance exercise is among the most effective strategies for promoting health and longevity [1]. In skeletal muscle at the molecular level, the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) protein plays a crucial role in adaptive remodelling processes induced by endurance exercise, ranging from mitochondrial biogenesis to vascularization and fiber-type switching [2], [3]. Given its involvement in various complex processes, PGC-1α requires tight regulation, which is partially achieved through the transcription of multiple isoforms [4]. Several isoforms of PGC-1α exist, formed through a combination of two different mechanisms – alternative promoter usage [5] and alternative splicing [6], [7]. Despite their discovery decades ago, the functional consequences of PGC-1α isoforms have been largely underestimated, even with the discovery of PGC-1α4 a functional isoform regulating hypertrophy [6].

Our aim is to identify novel PGC-1α isoform-specific functions, which may arise from their differing affinities for interaction partners (proteins or RNAs). To achieve this, we will utilize IP/MS and seCLIP, along with ChIP-Seq and RNA-Seq assays for the individual isoforms, to provide in-depth insights into the specific processes regulated by each PGC-1α isoform and to identify the underlying molecular mechanisms.

[1] J. P. Thyfault and A. Bergouignan, 'Exercise and metabolic health: beyond skeletal muscle', Aug. 01, 2020, Springer. doi: 10.1007/s00125-020-05177-6.
[2] C. Handschin et al., 'Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1α muscle-specific knock-out animals', Journal of Biological Chemistry, vol. 282, no. 41, pp. 30014–30021, Oct. 2007, doi: 10.1074/jbc.M704817200.
[3] C. Handschin et al., 'Abnormal glucose homeostasis in skeletal muscle-specific PGC-1α knockout mice reveals skeletal muscle-pancreatic β cell crosstalk', Journal of Clinical Investigation, vol. 117, no. 11, pp. 3463–3474, Nov. 2007, doi: 10.1172/JCI31785.
[4] V. Martínez-Redondo, A. T. Pettersson, and J. L. Ruas, 'The hitchhiker's guide to PGC-1α isoform structure and biological functions', Sep. 07, 2015, Springer Verlag. doi: 10.1007/s00125-015-3671-z.
[5] J. Chinsomboon et al., 'The transcriptional coactivator PGC-1 mediates exercise-induced angiogenesis in skeletal muscle', 2009. [Online]. Available: www.pnas.org/cgi/content/full/
[6] J. L. Ruas et al., 'A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy', Cell, vol. 151, no. 6, pp. 1319–1331, Dec. 2012, doi: 10.1016/j.cell.2012.10.050.
[7] Y. Zhang et al., 'Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1α', Journal of Biological Chemistry, vol. 284, no. 47, pp. 32813–32826, Nov. 2009, doi: 10.1074/jbc.M109.037556.

Contact: alexia.boeck@unibas.ch

Generation of a fluorescent reporter mice model to study skeletal muscle's denervation response

Alexia Böck-Pereda, Eleonora Maino, Shuo Lin, Claude Feigenwinter and Markus A. Rüegg

Biozentrum, University of Basel

The acetylcholine receptor (AChR) is a key component of the neuromuscular junction (NMJ) which is the critical unit driving neuromuscular communication. Two variants of AChR exist: the fetal and adult forms, which differ by a single subunit. The fetal AChR contains the fetal Cholinergic receptor nicotinic gamma subunit (Chrng), whereas in adult life Chrng is substituted by the adult Cholinergic receptor nicotinic epsilon subunit (Chrne). In conditions where muscle-nerve communication is disrupted, such as aging, amyotrophic lateral sclerosis, myasthenia gravis, and other neuromuscular disorders, skeletal muscle reactivates the expression of Chrng as part of a denervation response.

To investigate this process, we have generated the *Chrng-KI* mouse model, in which a P2A-H2B-mCherry reporter is knocked into the *Chrng* locus, allowing for fluorescent visualization of Chrng expression in myonuclei. Our findings demonstrated that the *Chrng-KI* mouse model exhibits normal NMJ formation, muscle function, Chrng levels confirming its suitability for denervation studies. Using this model, we identified a spatiotemporal regulation of the denervation response, with slow-twitch fibers upregulate Chrng faster than fast-twitch fibers following both complete and transient nerve injuries. Furthermore, targeted genetic ablation of key NMJ genes, such as *Musk*, triggered robust mCherry fluorescence, confirming the applicability of the reporter as a robust and sensitive platform for the discovery of novel genes indispensable for NMJ function, stability and maintenance.

Contact: sandrine.quirim@unibas.ch

Duality of PGC-1α: transcription co-factor and RNA-binding protein

Sandrine Quirim, Ioannis-Marios Roussis, Christoph Handschin

Biozentrum, University of Basel

Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a key player in the regulation of cellular metabolism and mitochondrial biogenesis, and is easily inducible in response to physical stimuli (exercise, nutrients, etc.). Despite extensive research on its role in remodelling the metabolic landscape and its regulation in physiological contexts, an RNA-processing function has only recently been described and is attributed to the less-studied C-terminal domain (CTD) which was previously shown to interact with RNA1. The CTD was previously demonstrated to recruit PGC-1α in transcriptionally active condensates in muscle cells. So far, RNAs bound to PGC-1α have only been identified in vitro (1,2). RNA binding partners of PGC-1α will be identified in vivo in muscle tissue through single end enhanced crosslinking and immunoprecipitation (seCLIP) approaches and determine their relevance in gene regulation and the complex transcriptional response in exercised muscle. These targets will subsequently be used to functionally interrogate RNA binding with PGC-1α through gain- and loss- of function experiments using adeno-associated viral vectors. Moreover, predicted disordered regions of PGC-1α will be probed for how likely they are to form condensates and how these impact target gene expression. These insights will advance our understanding of PGC-1α-mediated control of gene expression in response to exercise.

1 Pérez-Schindler, J., Kohl, B., Schneider-Heieck, K., Leuchtmann, A. B., Henríquez-Olguín, C., Adak, V., Maier, G., Delezie, J., Sakoparnig, T., Vargas-Fernández, E., Karrer-Cardel, B., Ritz, D., Schmidt, A., Hondele, M., Jensen, T. E., Hiller, S., & Handschin, C. (2021). RNA-bound PGC-1α controls gene expression in liquid-like nuclear condensates. Proceedings of the National Academy of Sciences, 118(36), e2105951118. https://doi.org/10.1073/pnas.2105951118

2 Tavares, C. D. J., Aigner, S., Sharabi, K., Sathe, S., Mutlu, B., Yeo, G. W., & Puigserver, P. (2020). Transcriptome-wide analysis of PGC-1α–binding RNAs identifies genes linked to glucagon metabolic action. Proceedings of the National Academy of Sciences, 117(36), 22204–22213. https://doi.org/10.1073/pnas.2000643117bar

Contact: julia.schedel@unibas.ch

Lack of Laminin-α2 results in a strong perturbation of the myotendinous junction morphology

Julia Schedel, Shuo Lin and Markus A. Rüegg

Biozentrum, University of Basel

The myotendinous junction (MTJ) is a critical interface between muscle fibers and tendons, essential for force transmission between muscle and bone. The extracellular matrix (ECM) at the MTJ contains specific components, such as collagen XXII, and is a crucial mediator of force transmission at this interface. Laminin-α2 is the main constituent of the muscle fiber ECM. It accumulates at the MTJ and extends into the tendon. Mutations in the *LAMA2* gene cause LAMA2-related muscular dystrophy (LAMA2 MD), an early-onset severe congenital muscular dystrophy.

Here, we examined the MTJ in *dyW/dyW* mice, a mouse model for LAMA2 MD. We find a strong disruption of MTJ morphology, including altered muscle fiber tips, collagen XXII mislocalization, and reduced muscle tendon interface. As MTJ loading is altered in *dyW/dyW* mice and MTJ maintenance requires loading and unloading, we also examined MTJ structures upon denervation-induced unloading. While muscle fiber tip morphology resembled that of *dyW/dyW* mice, collagen XXII distribution was not affected and the muscle–tendon interface was preserved. Finally, proteomic profiling via laser capture microdissection and mass spectrometry revealed significant regional and global shifts in MTJ protein composition in *dyW/dyW* and denervated mice. These findings demonstrate that laminin-α2 is required for MTJ stability, and that mechanical unloading contributes to the observed phenotype. Importantly, our results suggest that disruptions in MTJ structure and protein composition may contribute to the muscle dysfunction and pathology observed in LAMA2-related muscular dystrophy.

Contact: claude.feigenwinter@stud.unibas.ch

Genetic approaches to investigate sub-synaptic myonuclei in vivo

Claude Feigenwinter[1], Eleonora Maino[1], Alexia Böck[1], Lena Jörin[1], Minchul Kim[2,3] and Markus A. Rüegg[1]

[1]Biozentrum,University of Basel
[2]IGBMC, Institut de Génétique et de Biologie Moleculaire et Cellulaire, Illkirch, France
[3]Universite´ de Strasbourg, Strasbourg, France

Autophagy, a central catabolic process involving lysosomes, is essential for skeletal muscle homeostasis (1). X-linked myopathy with excessive autophagy (XMEA) is a rare myopathy characterized by accumulation of autophagic vacuoles in skeletal muscle and caused by mutations in the *VMA21* gene (2). VMA21 is a chaperone of the vacuolar (v)-ATPase proton pump, required for lysosomal acidification (3). VMA21 deficiency would reduce v-ATPase activity and thereby increase lysosomal pH, ultimately leading to autophagy blockade. Interestingly, we recently showed that Vma21 muscle-specific knockout mice display early lethality marked by severe skeletal muscle degeneration. Moreover, besides the short, ubiquitous VMA21 isoform (VMA21a), our lab identified a long VMA21 isoform, predominantly expressed in skeletal muscle (VMA21b). The physiological roles of each VMA21 isoform and their contribution to XMEA remain unknown.

To better understand the roles of VMA21 isoforms, we modulated their expression in vivo using adeno-associated virus (AAV) vectors. Interestingly, acute expression of the Cre recombinase in skeletal muscle from *Vma21flox/flox* mice led to complete tissue degeneration, indicating that VMA21 is required for adult muscle homeostasis. Surprisingly, overexpression of VMA21a isoform also caused rapid, pronounced muscle degeneration. Partial and complete muscle regeneration was detected 4 and 8 weeks after AAV injection, respectively. In contrast, VMA21b overexpression did not lead to major histological defects, suggesting that the two isoforms have distinct pathophysiological roles in skeletal muscle. Ongoing experiments aim at understanding the mechanisms leading to muscle degeneration upon VMA21 depletion and VMA21a overexpression, and at further characterizing the effect of VMA21b overexpression on skeletal muscle (e.g., changes in fiber type/size, autophagic flux, mTORC1/Akt activity). Unveiling yet-unknown functions of VMA21 will help to better understand XMEA pathogenesis and identify therapeutic approaches to counteract muscle dysfunction in the disease.

1. Castets, P., et al., J. Neuromuscul. Dis., 2016. 3(2): p.127-155.
2. Hirano, M. and S. DiMauro, Cell, 2009. 137(2): p.213-5.
3. Ramachandran, N., et al., Acta Neuropathol., 2013. 125(3): p. 439-57.

Contact: hirotaka.araki@unibas.ch

Optimal protein intake and mTORC1 inhibition in aging mice: A strategy to prevent sarcopenia

Hirotaka Araki, Daniel Ham Lena Jörin and Markus A. Rüegg

Biozentrum, University of Basel

Dietary protein intake elevates circulating amino acid levels, activating mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signalling and stimulating muscle protein synthesis. In older individuals, however, this anabolic response is attenuated, possibly due to age-related changes such as impaired amino acid transport, altered BCAA catabolism, or other factors affecting mTORC1 activation in skeletal muscle. In contrast, studies in model organisms and emerging human data suggest that low-protein diets may support healthy aging through effects on metabolic and cognitive health. Recent human studies have even questioned the protective role of high protein intake against sarcopenia, suggesting either no association or a paradoxical positive correlation.

Previously, our lab showed that mTORC1 is hyperactivated in sarcopenic muscle and that chronic inhibition by rapamycin delays sarcopenia progression (Ham et al., Nat Commun. 2020). Furthermore, muscle-specific TSC1 knockout mice with constitutive mTORC1 activation exhibit premature sarcopenic phenotypes. Whether low-protein diets actively suppress sarcopenia and how optimal protein intake intersects with mTORC1 signalling in aging muscle remains unclear. To address this, we aim to determine the optimal protein intake (with or without mTORC1 inhibition) for sarcopenia prevention in aged mice. C57BL/6 mice aged 15 months were fed one of four diets differing in protein content (6%, 13%, 20%, or 40% of total energy) with or without eRAPA (Eudragit-encapsulated rapamycin), yielding eight groups. Treatment will continue until 28 months of age. During the intervention, we are monitoring body weight and food intake weekly, measuring body composition every two months, and assessing muscle function via grip strength, inverted grid test, and voluntary wheel running. As complementary analysis, we conducted a parallel intervention in young adult mice (4 to 10 months old) under the same dietary conditions to evaluate age-related changes in protein requirements and mTORC1 responses. While final tissue collection and metabolic assays in the old group are pending, preliminary data indicate that dietary protein levels affect muscle strength in aged mice and that mTORC1 signalling responses may differ across age groups.

This study aims to determine how dietary protein and mTORC1 inhibition interact to modulate sarcopenia progression, and whether optimal protein intake should be defined in an age-specific manner to promote healthy muscle aging.

Contact: lionel.tintignac@unibas.ch

Identification of new interactors of eIF3f by endogenous proximity-dependent biotin labelling in human muscle cells

Lionel Tintignac[1,5], Nitish Mittal[2,5], Shahidul Alam[1,2,3], Meric Ataman[2,4], Yusuf I. Ertuna[1,2], Thomas Bock[2], Beat Erne[1], Mihaela Zavolan[2,4] and Michael Sinnreich[1]

[1]Departments of Neurology and Biomedicine, Pharmazentrum, University and University Hospital of Basel
[2]Biozentrum, University of Basel
[3]Centre for Organismal Studies (COS), Heidelberg University, Heidelberg, Germany.
[4]Swiss Institute of Bioinformatics, Lausanne
[5]Equal contributors

Regulation of protein synthesis is central to maintaining skeletal muscle integrity and its understanding is important for the treatment of muscular and neuromuscular pathologies. The eIF3f subunit of the translation initiation factor eIF3 has a key role, as it stands at the crossroad between protein-synthesis-associated hypertrophy and MAFbx/atrogin-1-dependent atrophy.

To decipher the molecular mechanisms underpinning the role of eIF3f in regulating muscle mass, we established a cellular model that enables interrogation of eIF3f functionality via identification of proximal interactors. Using CRISPR-Cas9 molecular scissors, we generated single cell clones of immortalised human muscle cells expressing eIF3f fused to the BirA biotin ligase (eIF3f-BioID1 chimera) from the endogenous EIF3F locus. Biotinylated proteins, representing interactors of eIF3f in nanometer range distance, were identified by streptavidin pull-downs and mass spectrometry. In both proliferating and differentiated muscle cells, the eIF3f-BioID1 chimera co-sedimented with ribosomal complexes in polysome profiles and interacted mainly with components of the eIF3 complex, and with the eIF4E, eIF4G, and eIF5 initiation factors. Surprisingly, we identified several nucleus-localised interactors of eIF3f, and the immunofluorescence analyses revealed a previously unknown nuclear localization of eIF3f in both myoblasts and myotubes. We also identified novel cytoplasmic partners of eIF3f, responsible for the maintenance of skeletal muscle ultrastructure (sarcomeric/Z-disc (SYNPO2) bound proteins) and proteins of the lysosomal compartment (LAMP1). The established tagging system should be useful to further advance studies of eIF3f function in hypertrophic and atrophic conditions in skeletal muscle.

Contact: gaetan.lopez@unige.ch

Role of STIM1L in the muscle response to stress conditions

Gaëtan Lopez, Stephan Koenig, Florent Chabry, Olivier Dupont, Maud Frieden, Perrine Castets

Department of Cell Physiology and Metabolism, University of Geneva

Clinical studies show that patients with Duchenne muscular dystrophy (DMD) develop orofacial dysfunction. Currently, the majority of studies using DMD mouse models focus on limb and respiratory muscles and the craniofacial muscles are neglected. The primary aim of this pilot study was to investigate how the lack of dystrophin affects orofacial function, with emphasis on the changes in the masseter muscles. Two different mouse models for DMD, namely the *mdx*^{5Cv} model (8 mice, 13-week-old), representing mild disease, and the *D2/5Cv* model (8 mice, 13-week-old) representing severe disease, were used. Histological sections of masseter muscles and of two exemplary limb muscles (extensor digitorum longus and soleus) were examined for inflammation; frequency of centralised nuclei; presence of degenerative or regenerative fibres; and fibrosis. Observations were compared to wild-type mice in the respective genetic backgrounds.

Results suggest that masseter muscles in the mild *mdx*^{5Cv} model are more severely affected in term of myonecrotic lesions and inflammation when compared to the *D2/5Cv* model at 13 weeks. However, masseter muscles in *D2/5Cv* mice exhibit greater histopathological changes than those of *mdx*^{5Cv} mice, namely fibrosis, as determined by trichrome Masson and collagen staining. The pathological fibrosis of the masseter muscles is comparable to that of the limb muscles. In conclusion, the masseter muscles of DMD mice showed dystrophic changes, comparable to those in the limb muscles. These insights may be highly relevant for the future development of anti-fibrotic therapy for patients with DMD, where the target should not be limited to the skeletal but also to the craniofacial muscles.

Contact: samuele.metti@epfl.ch

Anthrax toxin receptor 2 (ANTXR2) as a novel regulator of muscle stem cell function

Samuele Metti, Arpine Grigoryan, Béatrice Kunz, F. Gisou van der Goot

Global Health Institute, Ecole Polytechnique Fédérale de Lausanne

Muscle stem cells (MuSCs) are the myogenic stem cell pool that guarantees the regenerative potential of myofibers. They are located in a specialized microenvironment in which the fine regulation of extracellular matrix organization influences their fate. Collagen VI (COL6) is a key component of MuSCs niche: it is highly expressed by quiescent stem cell and its deficiency leads to impaired MuSCs self-renewal capabilities. However, the surface receptor(s) transducing COL6 signals in MuSC remains elusive. *In silico* gene expression analysis revealed that, among all known COL6 interactors, Anthrax Toxin Receptor 2 (ANTXR2) is the best candidate in mediating COL6 outside-inside function.

We confirmed that Antxr2 is highly expressed in quiescent MuSCs and downregulated upon MuSCs activation. However, Antxr2 is re-expressed in myoblasts and partially in fusing myotubes, but it is completely absent in myonuclei. Thus, we generated a new MuSC-specific Antxr2 inducible knockout mouse model and found that Antxr2 ko MuSCs undergo premature activation in vivo and ex vivo. In addition, Antxr2-deficient muscle display a delayed regeneration upon muscle damage. These data candidate ANTXR2 as a new stemness factor and pave the way for dissecting the roles of COL6/ANTXR2 axis in MuSCs.

Contact: loann.laubry@unige.ch

STIM1 promotes sustained Ca^{2+} -entry via PMCA1 activation, enhancing skeletal muscle differentiation

Loann Laubry, Jessica Brunetti, Stéphane Koenig, Maud Frieden

Department of Cell Physiology and Metabolism, University of Geneva, Medical Centre

The Store Operated Calcium Entry (SOCE) is a ubiquitous mechanism occurring after the depletion of the endoplasmic reticulum (ER) Ca^{2+} stores. The main players of this mechanism are the stromal interaction molecule 1 (STIM1), a Ca^{2+} sensor located in the ER, and Orai1, a plasma membrane Ca^{2+} channel. In skeletal muscle, a longer isoform of STIM1 called STIM1L is highly expressed. Since Ca^{2+} signals are essential for proper muscle differentiation, our aim was to determine the specific functions of STIM1 and STIM1L during myogenesis.

To do so, we used a doxycycline-inducible shRNAmir expression to downregulate both STIM1/1L isoforms or only STIM1L. The knock-down was triggered simultaneously with the differentiation of the myoblasts into myotubes. Electrical stimulations-induced Ca^{2+} transients showed that STIM1/1L and STIM1L downregulation impacts the amplitude of Ca^{2+} transients. Moreover, the ability of the myotubes to sustain these transients was impaired in only minutes. Interestingly, the cytosolic Ca^{2+} extrusion was slowed down upon both down-regulations. After identifying PMCA1 as the main extrusion protein in human myotubes, co-immunoprecipitation experiments showed an interaction between PMCA1 and STIM1 but not STIM1L. Moreover, the downregulation of PMCA1 showed to not impact the first steps of differentiation but decreased the size of the myotubes.

Overall, our data reveal an alteration of the Ca^{2+} circuitry upon down-regulation of STIM1 and STIM1L. The Ca^{2+} clearance is impaired during myogenesis with evidence that only STIM1 can directly modulate Ca^{2+} extrusion. We are now investigating if STIM1-PMCA1 interaction can reduce the calcium-dependent inactivation of Orai1, allowing sustained Ca^{2+} entry and NFATc1 translocation for a better myotube maturation.

Contact: olivier.dupont@unige.ch

Distinct roles of STIM2.1 and STIM2.2 in human myotube formation and function

Olivier Dupont, Stéphane König and Maud Frieden

Department of Cell Physiology and Metabolism, University of Geneva

Store-operated Ca^{2+} -entry (SOCE) is fundamental during myogenesis and involves the Orai Ca^{2+} channel and the sarcoplasmic reticulum (SR) Ca^{2+} sensor STIM. Ca^{2+} store depletion activates the SR resident protein STIM to gate Orai. The resulting Ca^{2+} entry is eventually pumped back into the SR by the SERCA pumps. Skeletal muscles express STIM1 and STIM2, along with their respective splicing isoforms. STIM2.1 was shown to act as a negative regulator of Orai1, while STIM2.2 is an activator. qPCR analysis confirmed the presence of both STIM2 isoforms, with STIM2.1 being more expressed in myotubes as compared to myoblasts.

This study aims to understand the implications of STIM2.1 and STIM2.2 on human myogenesis, using an in vitro model of differentiated myotubes. No major changes in myotube formation were observed after siSTIM2.1 (same size and same MEF2C expression), while siSTIM2.2 led to the formation of larger myotubes. However, upon STIM2.1 knockdown only, we observed an elevated basal Ca^{2+} level, which was associated with the nuclear localization of NFATc1 at rest and an elevated Ca^{2+}-dependent calpain activity. The higher basal Ca^{2+} level was mainly due to an enhanced Ca^{2+} entry, as shown by the Mn^{2+} quench assay. Overexpression of STIM2.1 in cells treated with siSTIM2.1 restored basal Ca^{2+} entry, confirming the specific contribution of STIM2.1 to this process. To study the function of STIM2 isoforms during EC coupling, cells were stimulated with high extracellular K^+ concentration. The downregulation of either STIM2.1 or STIM2.2 reduced cytosolic peak Ca^{2+} levels, which is mirrored by reduced SR Ca^{2+} release. Compared to the full releasable Ca^{2+} pool, high K^+ induced around 60% of Ca^{2+} depletion in control and siSTIM2.2 conditions, while only 40% is released in siSTIM2.1 condition. This suggests a partial impairment of EC coupling, possibly due to a decrease in the mRNA levels of three key EC coupling proteins—DHPR, RyR1, and STAC3—only in the STIM2.1 downregulation conditions. The elevated calpain activity might also negatively impact the EC coupling.

In conclusion, during myotube differentiation, STIM2.1 is important in EC coupling protein expression and basal Ca^{2+} balance, whereas STIM2.2 contributes primarily to the regulation of myotube size.

Contact: diego.michel@unige.ch

Pharmacological AMPK activation promotes higher levels of Pax7 expression in human muscle stem cells.

Diego Michel[1,2], Axelle Bouche[1,2], Remi Mounier[3], Didier Hannouche[1,2] and Thomas Laumonier[1,2]

[1]Cell Therapy and Musculoskeletal Disorders Laboratory, Department of Orthopedic Surgery, Geneva University Hospitals and Faculty of Medicine
[2]Department of Cell Physiology and Metabolism, Faculty of Medicine, Geneva
[3]Institut Neuromyogène, CNRS 5261 – INSERM U1315 - UCBL1, Lyon, France

Muscle stem cells (MuSCs) have gained attention as a promising therapeutic intervention for degenerative muscle diseases. Previously, we demonstrated that human muscle reserve cells (MuRCs) closely resemble quiescent MuSCs and exhibit enhanced regenerative capacity in vivo. Transcriptomic analyses revealed heterogeneity in Pax7 expression among human MuRCs, identifying a Pax7-High subpopulation characterised by a deeper quiescent state and reduced myogenic priming. This subpopulation exhibits a metabolic preference for fatty acid uptake and mitochondrial β-oxidation, which are pathways closely linked to AMPK signalling.

In this study, we examined the impact of compound 991, an allosteric AMPK activator, on the differentiation of human myoblasts and the subsequent fate of human MuRCs. Treatment with compound 991 did not alter the differentiation capacity of human myoblasts, as indicated by comparable percentages of MEF2C-positive cells and of Pax7-positive MuRCs. Western blot analysis revealed that treatment with compound 991 led to a 70% increase in the pACC/ACC ratio in human MuRCs, indicating enhanced AMPK activation. Flow cytometry analyses showed that compound 991 increased Pax7 expression in human MuRCs by 1.4-fold. Interestingly, Pax7 upregulation in human MuRCs was reversed by etomoxir, an inhibitor of mitochondrial fatty acid uptake. This suggests a functional link between fatty acid metabolism and Pax7 expression in human MuRCs.

Our findings suggest that AMPK activation by compound 991 does not impair the differentiation capacity of human myoblasts but rather promotes the in vitro generation of human MuRCs with increased Pax7 expression. Ongoing experiments are assessing the kinetics of human MuRC reactivation, as measured by EdU incorporation, following 6, 12 or 24 hours in growth medium.

Contact: eleonore.parisel@unige.ch

Decoding the inflammatory role of human skeletal muscle in mRNA vaccines induced reactogenicity

Eléonore Parisel [1,2], Paul Desert[3], Margaux Hubert[3], Patrick Syntin[3], Arnaud M. Didierlaurent[1], Thomas Laumonier[2]

[1]Department of Pathology and Immunology, Faculty of Medicine, Geneva
[2]CTMD lab, Geneva University Hospitals and Faculty of Medicine, Geneva
[3]Research Department, Sanofi Vaccines R&D, Marcy L'Etoile, France

mRNA-based vaccines represent a powerful tool for preventing infectious diseases, largely due to their ability to trigger a transient inflammation that enhances and shapes the desired immune response. However, this process can lead to short-lived local and systemic side effects, collectively known as reactogenicity, in certain individuals. Since mRNA vaccines are typically administered intramuscularly, the skeletal muscle environment, composed of many cell types including muscle fibers, fibroblasts, immune cells, and endothelial cells, may play a pivotal role in initiating this inflammation. Yet, the specific contribution of muscle tissue to vaccine-induced reactogenicity in humans remains poorly understood.

This study aims to identify muscle-specific inflammatory biomarkers and pathways involved in mRNA vaccine-induced reactogenicity. A distinct blood inflammatory signature was observed in individuals experiencing reactogenicity after vaccination. To explore the skeletal muscle's role, human myoblasts, myotubes, and whole muscle biopsies were exposed to varying concentrations of mRNA vaccines for up to 24 hours. Flow cytometry revealed that mRNA vaccine stimulation upregulated MHC class I and ICAM1 expression in human myoblasts, while MHC class II levels remained unchanged. Additionally, stimulation of myotubes with mRNA vaccines for 24 hours led to an increase in myogenic differentiation, as evidenced by a higher MEF2C/DAPI ratio. Using Olink technologies, we also observed increased levels of IL-6 and CXCL1 in myoblasts or muscle biopsy supernatant 24 hours after exposure to the mRNA vaccine. Finally, preliminary bulk RNA-seq analyses of human muscle biopsies revealed an enrichment of type I and type II interferon-related pathways 6 and 18 hours after mRNA injection, compared to the control condition.

In conclusion, this study highlights the important role of skeletal muscle in shaping the inflammatory response to mRNA vaccines. A better understanding of these mechanisms is crucial for the development of next-generation mRNA vaccines that are less reactogenic yet still immunogenic. This work was funded by Sanofi and the University of Geneva. Paul Desert, Margaux Hubert, and Patrick Syntin are employees of former employees of Sanofi and may hold shares and/or stock options in the company. Eléonore Parisel, Arnaud M. Didierlaurent, and Thomas Laumonier declare no competing interests.

Contact: emira.koshi@unige.ch

Insights into tongue involvement in Duchenne Muscular Dystrophy: study using a mouse model

Emira Koshi, Aouatef Djeraba Ait-Lounis, Stavros Kiliaridis, Gregory Antonarakis

Department of Orthodontics, Faculty of Medicine, University of Geneva

Duchenne Muscular Dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration. Clinically, in boys with DMD, tongue hypertrophy contributes to significant orofacial abnormalities and severe malocclusions, yet the molecular and structural mechanisms underlying this involvement remain poorly understood. This study investigates tongue pathology in the *mdx5Cv* mouse model of DMD across three age groups (3, 6, and 12 months) compared with age-matched wild-type controls, with the aim of clarifying disease progression and identifying key periods for intervention. Tongues will be dissected and sectioned for histological and immunofluorescence analyses. Myosin heavy chain (MyHC) isoform expression will be assessed by RT-qPCR and immunofluorescence. Additional staining will be used to evaluate inflammation, necrosis, regeneration, fibrosis, and fat deposition. Quantitative morphometric analyses, including minimal Feret's diameter and variance coefficients of fibre size, will be combined with counts of centrally nucleated fibres to provide an integrated assessment of dystrophic changes.

Our preliminary data show that while overall body weight was no different between dystrophic and control mice, tongues from *mdx5Cv* mice at 6 and 12 months were significantly lighter than those of wild-type controls. Moreover, centrally nucleated fibres, absent in wild-type tongues, were detected in *mdx5Cv* tongues at 3, 6, and 12 months (2.6%, 9.5%, and 4%, respectively). By clarifying the mechanisms of tongue involvement in DMD, this study aims to provide novel insights into how this condition contributes to the development of orofacial abnormalities. Ultimately, the findings may help identify the right timing for intervention and prevention of dentofacial changes in affected boys, contributing to improved quality of life.

Contact: i.ceren@stud.unibas.ch

Development of a pharmacological therapy for patients with RYR1-related congenital myopathies: effect of drug interruption combined with exercise

Huriye İrem Ceren, Alexis Ruiz Velez, Teresa Ianiri, Francesco Zorzato, Susan Treves

Department of Biomedicine, University Hospital Basel

Congenital myopathies are genetically diverse non-progressive muscle disorders for which, to date, no therapies exist. Mutations in *RYR1* are the primary genetic cause of congenital myopathies, responsible for 30% of all cases. *RYR1* is the gene encoding the ryanodine receptor (RyR1) calcium channel of the sarcoplasmic reticulum, a major calcium channel involved in excitation contraction coupling. Not surprisingly, mutations in *RyR1* disrupt Ca^{2+} signalling, leading to severe congenital myopathies.

To better understand the pathophysiology of RYR1-related congenital myopathies with the aim of developing a therapy for affected patients, our laboratory created a mouse model (henceforth referred to as *dHT* mouse) knocked in for two *RyR1* mutations isogenic to those identified in a severely affected child. We also investigated human muscle biopsies from affected patients in order to identify intracellular pathways for therapeutic interventions. We found that in both human patients and *dHT* mice epigenetic enzymes are abnormally elevated. Furthermore, in a pre-clinical study using the *dHT* mouse model, we found that inhibiting epigenetic enzymes with small molecules, leads to improved muscle strength and restores RyR1 protein levels. With the aim of starting a phase 1 clinical trial in patients, in this study we explored whether the drugs beneficial effects persist after treatment interruption and/or if exercise enhances recovery, supporting translation toward therapy for recessive RYR1-related myopathies.

Contact: nadege.zanou@unil.ch

Development of a new technique based on CRISPRa to activate endogenous RYR1 expression in myopathy cells.

Jérémy Vidal, Gabriele Scaperrotta and Nadège Zanou

Institute of Sport Sciences, University of Lausanne

A specific class of myopathies, called recessive ryanodine receptor type 1 (RyR1)-related myopathies (RyR1-RM), is caused by recessive mutations in the RYR1 gene. These mutations decrease the levels of RyR1, a protein that functions as the calcium release channel in skeletal muscle during contraction. Increasing evidence from various studies, including research from my lab, indicates that 1) RyR1 levels are decreased in non-RyR1-RMs and 2) reduced RyR1 levels trigger a wide range of muscular disorders. We recently demonstrated that reduced RyR1 content triggers endoplasmic reticulum (ER) stress and its pro-apoptotic pathway in both in vitro and in vivo models.

Currently, we are working on two main areas: first, identifying the role of ER stress in disorders triggered by reduced RyR1 content and second, developing an innovative technique to activate endogenous RyR1 expression in muscle cells.

However, the size of the *RYR1* genes precludes their use in gene therapy with adeno-associated viruses (AAVs) in patients due to the 5–6 kb size limit of currently available AAV vectors.

Therefore, we developed a CRISPRa-based tool to re-express RYR1 from its endogenous locus. CRISPRa has an advantage over exogenous overexpression protocols because we can typically achieve physiologically relevant overexpression due to the epigenetic activation of the *RYR1* promoter. First, three gRNAs and a non-targeting control sequence that target the *RYR1* promoter are cloned into the lenti-SAMv2 plasmid and tested for RYR1 induction. Then, the two best-performing gRNAs and a non-targeting gRNA in combination with dCas9-VP64 are used to create lentiviruses that are applied to isolated human primary muscle cell cultures. Muscle cells infected with our CRISPRa construct were selected using blasticidin and hygromycin. After successfully reconstituting RYR1 expression in human-derived myoblasts, we differentiated the cells and performed cellular and expression phenotyping, which included qPCR, calcium handling assays, and ER stress investigation.

This technique's success opens new therapeutic perspectives for myopathies with reduced RYR1 content.

Contact: daria.barbash@bc.biol.ethz.ch

Therapeutic targeting of SRSF1 interactions with GC-rich RNA repeats in the context of C9-ALS/FTD

Daria Barbash, Noémie Kociolek, Antoine Cléry, Frédéric Allain

Institute of Biochemistry, ETH Zurich

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor system [1]. Frontotemporal dementia (FTD) is a disorder characterized by behavioural changes, aphasia [2]. The primary genetic cause of these diseases is the presence of (G4C2) hexanucleotide repeat expansion (HRE) in the first intron of *C9ORF72* gene, with more than 30 repeats deemed to be pathological [3]. Serine-arginine rich splicing factor 1 (SRSF1) in complex with NXF1 exports HRE RNAs from the nucleus into the cytoplasm, where they are translated into toxic peptides, which is seen as the main cause of C9-ALS/FTD [4]. SRSF1 is a protein composed by two RNA recognition motifs (RRM1+2) which are linked by a flexible linker, and a C-terminal disordered RS domain [5], [6]. SRSF1 localization and function are regulated by the RS domain phosphorylation [7]. HRE RNAs can condense into foci in the nucleus of patient neurons [8], and SRSF1 co-localizes with these foci [4]. The formation of SRSF1- HRE RNA foci may have a protective function against C9-ALS/FTD by decreasing HRE RNA export.

Here, we could demonstrate that short HRE RNA forms a G-quadruplex (G-Q) that is bound by RRM1 domain of phosphorylated SRSF1. Pathological (G4C2)43 formed heterogeneous, G-Q containing structures, bound by both RRM1 and RRM2. Unphosphorylated SRSF1 interacts with HRE RNA via RS domain in addition to RRMs. Both unphosphorylated and phosphorylated SRSF1 could phase separate with (G4C2)43 RNA. However, we show that increasing levels of (G4C2) HRE RNAs decrease their phase separation with phosphorylated rather than with unphosphorylated SRSF1 which may result in lower levels of HRE RNA retention in neuroprotective nuclear foci. We rationally developed small molecule and peptides to inhibit SRSF1 RRM2 domain binding to (G4C2)n RNA. We propose that this approach could restrict the nuclear export of HRE RNA, which depends on specific interactions between RRM domains and HRE RNA, while preserving the formation of neuroprotective nuclear foci, as this process may instead rely on the binding of the unphosphorylated RS domain to HRE RNA.

Contact: loris.levet@unige.ch

Modulation of VMA21 isoforms leads to skeletal muscle degeneration and impaired autophagy

Loris Levet, Ilaria Cocchiararo, Florent Chabry, Perrine Castets

Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva

Autophagy, a catabolic process involving lysosomes, is essential for skeletal muscle homeostasis (1). X-linked myopathy with excessive autophagy (XMEA) is a rare neuromuscular disorder characterized by the accumulation of autophagic vacuoles in skeletal muscle and caused by mutations in the *VMA21* gene (2). *VMA21* encodes a chaperone of the vacuolar (v)-ATPase proton pump, required for lysosomal acidification (3). In cells from XMEA patients, VMA21 deficiency reduces v-ATPase activity and thereby increases lysosomal pH, ultimately leading to autophagy blockade. We recently showed that skeletal muscle-specific Vma21 knockout mice (*Vma21mKO*) display early lethality at 4 weeks of age, associated with severe skeletal muscle degeneration. Moreover, in addition to the ubiquitous VMA21 isoform (VMA21a), our laboratory identified a long VMA21 isoform specific to skeletal muscle (VMA21b) (4). However, the physiological roles of each VMA21 isoform and their respective involvement in XMEA pathogenesis remain unknown.

To investigate their function, we modulated the expression of VMA21 isoforms in adult skeletal muscle. Injection of adeno-associated virus (isotype 9, AAV9) expressing the Cre recombinase in TA/EDL muscles of adult Vma21-floxed mice efficiently depleted *VMA21* and led to rapid and extensive degeneration of these muscles. As in *Vma21mKO mice*, muscle degeneration was preceded by the blockade of autophagic flux at the degradation steps and the accumulation of abnormal vesicles observed by electron microscopy. These findings highlight that VMA21 is essential for maintaining homeostasis in adult skeletal muscle. Unexpectedly, VMA21a overexpression also triggered a severe phenotype, marked by major muscle fibre death and inflammation. While autophagy induction was unchanged in VMA21a-overexpressing muscle, reduced LC3II levels and increased p62 accumulation suggest complex deregulation of autophagic degradation steps. Interestingly, VMA21b overexpression induced milder muscle alterations and did not impair autophagic flux. In line with these phenotypic differences, mass spectrometry analysis revealed that VMA21 isoforms interact with distinct proteins, suggesting non-redundant, isoform-dependent functions, beyond v-ATPase regulation in skeletal muscle. Deciphering the pathophysiological roles of each VMA21 isoform will provide new insights into XMEA pathogenesis and guide the development of therapeutic approaches for the disease.

[1] Castets, P., et al., J. Neuromuscul. Dis., 2016. 3(2): p.127-155.
[2] Hirano, M. and S. DiMauro, Cell, 2009. 137(2): p.213-5.
[3] Ramachandran, N., et al., Acta Neuropathol., 2013. 125(3): p. 439-57.
[4] Cocchiararo, I., et al., Hum Mol Genet., 2023. 32(24): p.3374-3389

Contact: patricia.lopeznaveira@unige.ch

Deciphering the roles of VMA21 in skeletal muscle to understand the pathogenesis of a rare Autophagic Vacuolar Myopathy

Patricia Lopez Naveira, Ilaria Cocchiararo, Loris Levet, Florent Chabry, Perrine Castets

Department of Cell Physiology and Metabolism, Faculty of Medicine

X-linked myopathy with excessive autophagy (XMEA) is a rare disease, characterized by muscle weakness and atrophy, with a severity ranging from neonatal lethality to adult forms. The disease is caused by mutations in the VMA21 gene, which encodes a chaperone protein essential for the assembly of the vacuolar ATPase (V-ATPase). This proton pump is required for lysosomal acidification and thereby autophagy, a catabolic process ensuring the degradation and recycling of cellular components. Studies in yeast and cells from XMEA patients revealed that VMA21 deficiency reduces V-ATPase assembly, leading to elevated lysosomal pH and impaired autophagic degradation.

To gain insights into XMEA pathogenesis, we generated skeletal muscle-specific Vma21 knockout mice (*Vma21mKO*) using an HSA-Cre-mediated deletion of *Vma21* exon 2. Strikingly, 40% of *Vma21mKO* mice die at 4 weeks of age, while the surviving mice develop a severe muscle dystrophy, with a mortality by 7-8 months. Skeletal muscle degeneration started at 26-28 days of age in *Vma21mKO* mice, with predominant alterations detected in diaphragm and regions enriched in type IIA fibers in tibialis anterior. In contrast, the soleus muscle was relatively spared at this age. We identified that autophagic flux is blocked at the degradation steps in mutant muscle as soon as day 23, i.e, before muscle degeneration. Importantly, we uncovered that a diet enriched in fat was sufficient to eliminate the early lethality, but not muscle degeneration. This suggested that muscle-specific VMA21 depletion triggers a lethal, whole-body metabolic crisis. In line with this, body weight, fat mass, as well as glycemia of *Vma21mKO* mice decreased between 25 and 28 days of age. In addition, we observed an accumulation of glycogen, an up-regulation of glucose transporters, and a down-regulation of genes implicated in fatty acid elongation and storage in mutant muscle at day 26.

We are currently analyzing the histology of liver and white/brown adipose tissues and evaluating the activity of metabolic pathways in these organs to assess the systemic impact of muscle-specific VMA21 depletion. These investigations will help us understand the early lethality of *Vma21mKO* mice and provide important insights into XMEA pathogenesis and severe forms of the disease.

Contact: huiying.li@unibas.ch

Functional consequences of MH-causative RYR1 mutations on the immune system

Huiying Li[1], Irem Huriye Ceren[1], Helga C. A. Silva[2], Mariz Vainzof[3], Alexis Ruiz[1], Hervé Meier[1], Brandow Willy Souza[3], Pamela Vieira Andrade[2], Thalles Sestokas Zorzeto[2], Murilo Fregoneze Farias[2], Francesco Zorzato[1,4], Susan Treves[1,4]

[1] Department of Biomedicine, University Hospital Basel
[2] Malignant Hyperthermia Unit, Federal University of São Paulo, Brazil
[3] Human Genome and Stem Cell Research Center, University of São Paulo, Brazil
[4] Department of Life Science and Biotechnology, University of Ferrara, Italy

Background: Over 700 RYR1 variants have been identified in humans, with more than 165 linked to malignant hyperthermia susceptibility (MHS) and related myopathies. Beyond skeletal muscle, gain-of-function RYR1 mutations appear to modulate immune activity by enhancing cytokine release and T-cell stimulation. We investigated whether RYR1 mutations also affect human B-cell calcium signalling and activation.
Methods: Peripheral blood and EBV-immortalized B cells were collected from 10 MHS patients with distinct RYR1 mutations and 12 healthy controls. Calcium flux was measured using Fluo-4 AM during 4-chloro-m-cresol (4-CMC) stimulation to calculate EC_{50} values. Surface markers (CD83, CD86, MHC-II, CD38, CD39, CD40) were analyzed by flow cytometry following caffeine or caffeine + tetracaine treatment. PBMC phenotypes and serum immunoglobulins were assessed by flow cytometry and TMT-MS proteomics.
Results: MHS B cells showed a lower EC_{50} for 4-CMC–induced Ca^{2+} release (≈ 630 µM vs > 800 µM; $p < 0.05$), indicating RyR1 hypersensitivity. Caffeine induced a time-dependent increase of CD86 in RYR1-mutated lines (24–72 h; $p < 0.05$), partially blocked by tetracaine, confirming RyR1 dependence. PBMCs from MHS patients contained a higher proportion of $CD86^+$ B cells than controls, showing enhanced activation in vivo. Serum proteomics revealed elevated IgA2 but otherwise comparable antibody profiles.
Conclusions: B cells carrying MH-causative RYR1 variants display increased Ca^{2+} sensitivity and enhanced CD86 expression both in vitro and ex vivo. These findings identify RyR1-mediated Ca^{2+} signalling as a novel regulator of human B-cell activation and suggest a broader immunomodulatory role for RYR1 beyond skeletal muscle.

Contact: m.mroczek888@gmail.com

Molecular and ultrastructural basis of the neuromuscular junction defect in PURA-congenital myasthenic syndrome

Magdalena Mroczek[1,2], Corinna Preusse[3], Michał Bielak[4], Adela Sobolewska[4], Adela Della Marina[5], Vera Dobelmann[6], Magdalena Chrościńska-Krawczyk[4], Hans-Hilmar Goebel[3], Werner Stenzel[3], Andreas Roos[5,6]

[1]Human Genomics Research Group, Department of Biomedicine, University of Basel
[2] Department of Consultation-Liaison-Psychiatry and Psychosomatic Medicine, University Hospital Zurich, University of Zurich
[3] Department of Neuropathology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany, Berlin
[4] University Children Hospital, Department of Child Neurology, Lublin, Poland
[5] University Duisburg-Essen, Department of Pediatric Neurology, Centre for Neuromuscular Disorders, Centre for Translational, Neuro- and Behavioral Sciences, Essen, Germany
[6]. Heinrich-Heine University Düsseldorf, University Hospital, Institute of Neurology

Background: PURA-related neurodevelopmental disorder is an ultrarare congenital genetic condition caused by pathogenic autosomal dominant variants in the *PURA* gene. Although the disease is primarily classified as a central developmental disorder, some phenotypic features such as positive effect of salbutamol/pyridostigmine bromide indicate an endplate defect.
Methods: We performed myopathological, ultrastructural, proteomic, qPCR studies on the muscle biopsy of one muscle biopsy derived from a 3-months-old patient carrying the pathogenic (c.159del; p.(Leu54Cysfs*)) PURA variant. In addition, proteomic signature of extracellular vesicles and Thrombospondin-4 (marker protein of neuromuscular junction function) level were examined in sera derived from 8 PURA-patients.
Results: Electron microscopy unraveled structural alterations in line with perturbed synaptic transmission including rarefication of postsynaptic clefts and vesicular alterations within endothelial capillary cells and the myofibres. Proteomics revealed dysregulation of structural proteins similar to those seen in congenital myopathies where treatment with endplate stimulators is frequently successful. Therefore, we suspect a defect in the vesicular transport of proteins from muscle to endplate. Proteomics on vesicles purified from sera showed significant increase of 44 and decrease of 82 proteins with marked vulnerability of histones and NOTCH2. ELISA-based Thrombospondin-4 quantification revealed significant increase in patients.
Conclusions: In PURA syndrome, a functional NMJ-defect might be based on pathological vesicle transport accompanied by dysregulation of structural proteins and altered protein composition of extracellular vesicles. Thrombospondin-4 is the first introduced blood biomarker for PURA syndrome.

Contact: elif.sakiri@unibas.ch

Molecular mechanisms of autoantibody-mediated complement activation in myasthenia gravis

Elif Sakiri, Sebastian Holdermann, Nicholas Sanderson, Tobias Derfuss

Department of Biomedicine, University of Basel

Antibody-mediated complement activation is the main driver of myasthenia gravis (MG), as demonstrated by the beneficial response of MG patients to complement-inhibitory therapies. The main complement inducing autoantibodies in MG are specific for the acetylcholine receptor, a pentameric ion channel.

In this project, we isolated monoclonal antibodies from the peripheral blood of MG patients to investigate their pathogenicity. We observed a striking synergistic effect on complement activation when two distinct monoclonal IgG antibodies were combined, whereas each antibody alone failed to induce complement activation on acetylcholine receptor expressing cells, both in vitro and in vivo. The aim of this study is to understand why only the combination of these antibodies, rather than each one alone, triggers this response, thereby elucidating the structural mechanisms of the classical complement pathway.

We demonstrated that bivalent binding is critical for this synergistic effect, as replacing the naturally bivalent IgGs with engineered monovalent IgGs diminishes complement activation. Using high resolution microscopy we showed that the strongly complement activating antibody combination promotes clustering of acetylcholine receptors. To further understand this phenomenon, we measured the antibody binding kinetics using a real-time interaction assay based on live cells. With this method, we showed that addition of a second IgG stabilizes the binding of the first, as reflected in a reduced dissociation rate. This phenomenon of cooperative affinity was not observed on cells with immobilized antigen, demonstrating that it depends on the mobility of antigen in the membrane, which allows an increased proportion of bivalent binding of the IgGs. These studies aim to elucidate the structural and functional parameters that enable high-potency complement activation

Notes

Notes