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Report and Abstracts of the 19th Meeting of the Interuniversity Institute of Myology: Assisi, October 20-23, 2022

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Abstract

After two years of conferences on a virtual platform due to the Covid-19 pandemic, finally, the 19th annual meeting of the Interuniversity Institute of Myology (IIM) has returned to the heart of central Italy, in Assisi, an important cultural hub, which boasts a wide range of historic buildings and museums. This event brought together scientists from around the world providing a valuable opportunity to discuss scientific issues in the field of myology. Traditionally, the meeting particularly encourages the participation of young trainees, and the panel discussions were moderated by leading international scientists, making this a special event where young researchers had the opportunity to talk to prestigious scientists in a friendly and informal environment. Furthermore, the IIM young researchers' winners for the best oral and poster presentations, became part of the IIM Young Committee, involved in the scientific organization of sessions and roundtables and for the invitation of a main speaker for the IIM 2023 meeting. The four keynote speakers for the IIM Conference 2022 presented new insights into the role of multinucleation during muscle growth and disease, the long-range distribution of giant mRNAs in skeletal muscle, human skeletal muscle remodelling from type 2 diabetic patients and the genome integrity and cell identity in adult muscle stem cells. The congress hosted young PhD students and trainees and included 6 research sessions, two poster sessions, round tables and socio-cultural events, promoting science outreach and interdisciplinary works that are advancing new directions in the field of myology. All other attendees had the opportunity to showcase their work through poster presentations. The IIM meeting 2022 was also part of an advanced training event, which included dedicated round tables and a training session of Advanced Myology on the morning of 23 October, reserved for students under 35 enrolled in the training school, receiving a certificate of attendance. This course proposed lectures and roundtable discussions coordinated by internationally outstanding speakers on muscle metabolism, pathophysiological regeneration and emerging therapeutic approaches for muscle degenerations. As in past editions, all participants shared their results, opinions, and perspectives in understanding developmental and adult myogenesis with novel insights into muscle biology in pathophysiological conditions. We report here the abstracts of the meeting that describe the basic, translational, and clinical research and certainly contribute to the vast field of myology in an innovative and original way.

Key Words: Muscle development; adult myogenesis; metabolism; epigenetics; omics; metabolism; 3D cell models; organoids; extracellular vesicles: homeostasis; neuromuscular features; Duchenne muscular dystrophy; cachexia; sarcopenia; muscle stem cells; clinical studies; personalized medicine.

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The 19th annual meeting of the Interuniversity Institute of Myology (IIM), founded in 2004 by several Italian universities, was held from 20 to 23 October 2022. Due to the greatly improved COVID-19 pandemic situation, the meeting was held in Assisi in the Umbria region of central Italy. The desire to meet in person in the official headquarters of the last IIM meetings attracted many

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participants, which were 118 scientists coming from different countries, including Italy, Belgium, France, Denmark, USA and Brazil. This year we had four main talks and 33 talks selected from the submitted contributions and 42 posters were the basis for a lively scientific debate, mainly aimed at promoting the active participation of the young participants, as typical of all IIM meetings. Keynote speakers were Doug Millay (Department of Pediatrics, University of Cincinnati Cincinnati, OH, USA); Scott Q. Harper (Nationwide Children's Hospital, Ohio State University College of Medicine Columbus, OH, USA); Jean Farup (Dept of Biomedicine, Aarhus University Aarhus, Denmark); Chiara Mozzetta (Institute of Molecular Biology and Pathology, National Research Council of Italy, Rome, Italy). The 4th edition of the Advanced Training Course in Advanced Myology within the IIM meeting was organized through a collaboration with the University of Perugia, Italy. During the training course, fourteen participants under the age of 35 attended lectures and round tables with the international speakers, in addition to be exposed to the fully immersion the IIM meeting itself. This year, the panel discussions were on skeletal muscle aging (discussants: Doug Millay, Sestina Falcone, and Antonio Musarò) and environment (discussants: Jean Farup and Scott Q. Harper). To further stimulate the involvement of the young participants, one of the keynote speakers and the theme of the roundtables were selected by the IIM Young Committee, composed by trainees who won prizes in past editions of the IIM meeting. Also in this IIM edition, Gloria Antonini, Parent Project Italy, brought the voice of patients affected by Duchenne and Becker Muscular Dystrophies. The industry was represented by Barbara Canonico who spoke about the world of extracellular vesicles1 and their cargos, a technical talk promoted by Prodotti Gianni. Both Parent Project and Prodotti Gianni sponsored the meeting. The scientific topics of the presentations in an interdisciplinary fashion covered several aspects of the skeletal muscle biology including, muscle genetics and epigenetics, stem cells and regenerative medicine, muscle and exercise, signaling and metabolism, clinical studies and therapeutic approaches to muscle diseases. The first keynote lecture was given by Douglas P. Millay, Professor at Department of Pediatrics, University of Cincinnati, USA. The title of his talk was: Mechanisms of myoblast fusion and role of multinucleation during muscle development, growth, and disease. He presented the latest study of his team about the biochemical activities of the myogenic fusogens, deciphering the role of multinucleation for myofiber growth and adaptations. These results include the recent results on myomerger fusogenic activity that requires its two ectodomain helices, responsible for the membrane-stressing activity during the final step of myoblast fusion spatially and temporally regulated2. The second keynote lecturer, Dr. Harper's primary research focus at Nationwide Children's has been developing gene therapies to treat neuromuscular and neurological disorders. Scott Harper is Professor of Pediatrics at the Ohio State University College of Medicine, USA and serves as chief scientific adviser at Armatus Bio, a gene therapy startup company in Columbus, Ohio. He was invited by the IIM Young Committee and his talk was entitled: Development of DUX4 inhibition therapies for Facioscapulohumeral muscular dystrophy (FSHD). He presented recent data from his lab on a meticulous screening to identify a subset of serine/threonine phosphomimetic mutants and an arginine methylation null mutant which protected cells against double homeobox 4 protein (DUX4) -mediated toxicity in FSHD patients3. In follow-up studies, they demonstrated the therapeutic promise of targeting DUX4 post-translational modifications that could protect against DUX4-induced cell death in FSHD myoblasts. The third Keynote lecture was presented by Jean Farup, assistant professor at Dept of Public Health Aarhus University, Denmark. In his talk entitled "Human skeletal muscle fibro-adipogenic progenitors in degeneration and regeneration", he focused on the human muscle microenvironment and the role of fibro-adipogenic progenitors (FAPs) in skeletal muscle homeostasis. His work highlighted the important role of FAP subsets in human skeletal muscle during chronic muscle degeneration, focusing on the crosstalk between FAPs and macrophages in regulating immune cell infiltration and local tissue inflammation. Indeed, type 2 diabetes mellitus (T2DM) is associated with impaired skeletal muscle function and FAPs$CD90^+$ hyperproliferative population and production of extracellular matrix. In general, FAP levels return to the baseline upon resolution of the muscle injury and FAP$CD90^+$ proliferation was reduced by in vitro treatment with metformin.[4] These data provided new insight into the dynamics and multiple roles of FAPs in human T2DM skeletal muscle. The fourth keynote lecture was given by Chiara Mozzetta, a group leader at Institute of Molecular Biology and Pathology (IBPM) of the National Research Council of Italy, located at University Sapienza of Rome, Italy. The title of her talk was Role of peripheral heterochromatin in the maintenance of fibro-adipogenic progenitors' genome integrity and cell identity and she gave an overview on the heterochromatic lamina modulation during cell division. She showed that PR domain containing 16 (Prdm16) mediates FAP developmental capacities through the lamina-associated domain organization and heterochromatin sequestration at the nuclear periphery. Prdm16 cooperates with the H3K9 methyltransferases G9a/GLP to mediate tethering and silencing of myogenic genes, thus repressing the myogenic fate in FAPs5. This information could be relevant for FAP reprogramming during muscle regeneration in dystrophic muscles paving the way of possible therapeutic approach. Also in this edition, the voice of patients affected by Duchenne and Becker Muscular Dystrophy (DMD and BMD) was brought by Gloria Antonini, from the Parent Project

association that it is indeed a social promotion association (aps) founded in Italy in 1996 (after the USA and the Netherlands) and currently is in touch with over 800 families of DMD and BMD patients. This relatively young aps economically supports research and dissemination of the multidisciplinary approaches to treat patients that so far doubled the life expectancy of DMD patients improving overall the quality of life. A technical talk promoted by the sponsor Prodotti Gianni introduced the world of extracellular vesicles, highlighting recent results and troubleshooting in emerging technologies. Among the submitted abstracts, 33 talks were selected covering all the topics of the meeting, ranging from basic muscle function and repair to the effects of exercise, molecular mechanisms, and possible therapeutic approaches to muscle degeneration. The speakers came from Italy, Belgium, France and Brazil. The quality of the interventions was very high creating intense discussions among the participants of all ages. Overall, 42 posters were discussed with good interactions between presenter and attendees. At the end of the meeting, prizes were awarded to young researchers (less than 35 years old) based on the evaluation of groups of scientists selected by the IIM scientific board. For the best talks the prizes were awarded to David Osamwonuyi Amadsun and Laura Yedigaryan, while Federica Esposito won the prize for best poster and the one for the best active participation was awarded to Sara Roccabianca. Finally, on 22/10/2022, during the 19th annual IIM conference, the elections for the renewal of the Director and the scientific council were held and all conference participants had the right to vote. Sorci Guglielmo, Musarò Antonio, Falcone Sestina, Latella Lucia, Penna Fabio, Mocciaro Emanuele, Urciuolo Anna, Palacios Daniela, Riuzzi Francesca were elected by secret ballot. The scientific council has elected Doctor Guglielmo Sorci director of the IIM for the three-year period 2022-2025. Subsequently, pursuant to Art. 5 of the Statute, Alessandra Sacco, Davide Gabellini, Maurilio Sampaolesi, and Stefania Fulle joined the IIM Scientific Council as associate members. After the pandemic period, the 19th IIM Meeting managed once again highlighted the participation of young researchers, bringing together many mythologists, with interests from basic science to preclinical and clinical studies formulation of new hypotheses and new international collaborations. Without a doubt, the young researchers of IIM meeting, the young clinicians of Padua Muscle Days (PMD, a meeting more oriented towards advanced translational myology) and the young authors of articles printed electronically in the European Journal of Translational Myology (EJTM) shape the future of research in myology. Here, the abstracts of the 19th IIM meeting show the relevant research contribution of this community of myologists in anticipation of the 20th IIM Congress, scheduled for October 12-15, 2023, to be held in Assisi, Italy.

List of acronyms

IMM - Interuniversity Institute of Myology
PMDs - Padua Muscle Days
COVID-19 - Disease caused by SARS_CoV-2
DMD - Duchenne muscular dystrophy
E-C coupling - excitation-contraction coupling
FSHD - Facioscapulohumeral muscular dystrophy
T2DM - type 2 diabetes mellitus

Contributions of Authors

Authors equally contributed to write the manuscript. They also approved the final version.

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Conflict of Interest

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Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Disclaimer

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Interuniversity Institute of Myology

19th IIM Meeting

Assisi, Italy • 20-23 October 2022

Pathogenesis and Therapies of Neuromuscular Diseases

Programme & Abstracts

https://IIM2022.azuleon.org

Topics

Biophysics and E-C coupling

Genetics and epigenetics

Muscle stem cells and regenerative medicine

Muscle wasting and cachexia

Exercise

Signaling and metabolism

Therapeutic approaches

Keynote Lectures

Jean
Farup

Scott
Harper

Doug
Millay

Chiara
Mozzetta

Scientific Committee

Sestina **Falcone**

Stefania **Fulle**

Davide **Gabellini**

Cesare **Gargioli**

Francesca **Grassi**

Antonio **Musarò**

Fabio **Penna**

Pier Lorenzo **Puri**

Alessandra **Sacco**

Maurilio **Sampaolesi**

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Duchenne Parent Project aps

PRODOTTI GIANNI

BCC DI SPELLO E DEL VELINO
CREDITO COOPERATIVO ITALIANO
Territorio Umbro e Sabino

Programme

11:30–14:30 **Registration**

14:45–15:00 **Welcome and opening of the meeting**

15:00–15:40 **Lecture 1**

Chair: Sestina Falcone

Doug Millay *(Cincinnati, OH, USA)*

Mechanisms of myoblast fusion and role of multinucleation during muscle development, growth, and disease

15:40–17:10 **Session 1: Signaling in muscle growth, homeostasis and diseases**

Chairs: Laura Yedigaryan and Valeria Runfola

Roberta Andreotti *(Padua, Italy)*

The interplay between polyglutamine-expanded AR and its transcription co-factors in skeletal muscle

Yvan Torrente *(Milan, Italy)*

Defective dystrophic thymus determines degenerative changes in skeletal muscle

Cosimo De Napoli *(Padua, Italy)*

The pathophysiology of the Super Relaxed state of myosin in skeletal muscle

Martina Esposito *(Padua, Italy)*

MUSA1 is a novel critical regulatory element for Z-line homeostasis and skeletal muscle function

Luca Giacomelli *(Urbino, Italy)*

EPS induces the secretion of different subpopulation of extracellular vesicles in C2C12 myotubes

17:10–17:30 *Coffee break*

17:30–17:50 **Welcome from Authorities**

17:50–19:05 **Session 2: Satellite cells and muscle regeneration in healthy muscle and in diseases**

Chairs: Beatrice Biferali and Elena Ruggieri

Vittoria Marini *(Leuven, Belgium)*

Duchenne muscular dystrophy cardiomyopathy modelling using patient-derived cardiac organoids

Claudia Fuoco *(Rome, Italy)*

Perivascular stem/progenitor cells, up-and-coming tissue regenerating route

Alessio Rotini *(Créteil, France)*

Sympathetic innervation is required to maintain the quiescent muscle stem cell pool via perivascular-derived Angpt-1

19:30–21:30 ***Aperitivo Umbro*** **and *(only for participants registered to the High Training formula)*Rountable Discussions: D. Millay, S.Q. Harper, J. Farup, A. Musarò**

9:00–9:40 **Lecture 2**

Chair: Emanuele Mocciaro

Scott Q. Harper *(Columbus, OH, USA)*

Development of *DUX4* inhibition therapies for Facioscapulohumeral muscular dystrophy (FSHD)

9:40–10:40 **Session 3: Biophysics and E-C coupling in the pathophysiology of neuromuscular diseases**

Chairs: Dario Coletti and Chiara Novello

David Osamwonuyi Amadsun *(Siena, Italy)*

Junctophilins and CLIMP63: a novel interaction in skeletal muscle

Gloria Antonini *(Parent Project aps)*

The voice of patients affected by Duchenne and Becker Muscular Dystrophy

Barbara Canonico

The world of extracellular vesicles: results and troubleshooting *(Technical talk promoted by Prodotti Gianni)*

10:40–11:10 *Coffee break*

11:10–12:40 **Session 4: Genetic and epigenetic alterations in muscle dystrophies and myopathies**

Chairs: Gabriella Dobrowolny and Nicoletta Filigheddu

Giuseppina Caretti *(Milan, Italy)*

BET inhibitors rewire metabolism in the aged skeletal muscle

Emanuele Mocciaro *(Milan, Italy)*

A novel therapeutic perspective for FSHD muscular dystrophy

Valeria Runfola *(Milan, Italy)*

Identification of the first direct endogenous inhibitor of DUX4 in FSHD muscular dystrophy

Claudia Fornelli *(Turin, Italy)*

The epigenetic regulator JQ1 targets fat metabolism and counteracts diet-induced obesity

Laura Yedigaryan *(Leuven, Belgium)*

Investigating the therapeutic potential of extracellular vesicle-derived microRNAs circulating in murine models of muscle hypertrophy

13:00 *Lunch*

14:30–16:00 **Poster discussion (Session 1, odd numbers)**

16:30 *Bus departure to Perugia: guided tour and Dinner*

9:00–9:40 **Lecture 3**

Chair: Pier Lorenzo Puri

Jean Farup *(Aarhus, Denmark)*

Human skeletal muscle fibro-adipogenic progenitors in degeneration and regeneration

9:40–11:10 **Session 5: Muscle fibrosis, sarcopenia and cachexia**

Chairs: Sara Chiappalupi and Rosanna Piccirillo

Louise Medaer *(Leuven, Belgium)*

Employing isogenic cell models to study the underlying mechanism of cystinosis myopathy

Chiara Noviello *(Paris, France)*

Exploring the protective role of GDG5 against skeletal muscle disuse atrophy

Luca Madaro *(Rome, Italy)*

Spatially resolved transcriptomics reveals innervation-responsive functional clusters in skeletal muscle

Giulio Masiero *(Padua, Italy)*

A new murine model for skeletal disuse muscle atrophy with a 3D-printed cast

Camilla Pezzini *(Padua, Italy)*

Understanding BMP signaling in cancer cachexia

11:10–11:30 *Coffee break*

11:30–13:00 **Poster discussion (Session 2, even numbers)**

13:00 *Lunch*

15:00–15:40 **Lecture 4**

Chair: Pier Lorenzo Puri

Chiara Mozzetta *(Rome, Italy)*

Role of peripheral heterochromatin in the maintenance of fibro-adipogenic progenitors' genome integrity and cell identity

15:40–17:15 **Session 6: Therapeutic approaches for muscle diseases**

Chairs: Marta Morotti and Rosa Mancinelli

Gaia Laurenzi *(Rome, Italy)*

The role of protein-kinase C theta (PKCθ) in ALS disease progression

Silvia Casati *(Milan, Italy)*

Impact of Drp1 activation and fission induction in the pathogenesis of DMD progression

Paulina Roux-Biejat *(Milan, Italy)*
A novel alternative therapeutic strategy for Duchenne Muscular Dystrophy

Emilie Venereau *(Milan, Italy)*
Rebalancing HMGB1 redox isoforms expression to counteract muscular dystrophy

Valentina Di Felice *(Palermo, Italy)*
Physiactisome: a new nanovesicle drug containing heat shock protein 60 for treating muscle wasting and cachexia

Marilia Seelaender *(São Paulo, Brazil)*
Post-COVID syndrome: inflammation and the muscle

17:15–17:45 *Coffee break*

18:00–19:00 **IIM General meeting**

20:30 *Social Dinner - Awards and prizes*

22:00 *Dance party*

Reserved to participants registered to the
High Training Course in ADVANCED MYOLOGY

10:00 *Bus departure to Palazzo Bernabei*

10:30–12:30 **Dedicated lessons**

D. Millay, S.Q. Harper, J. Farup, C. Mozzetta

12:30 *Light lunch*

Odd numbers: Friday, 21 October (14:30-16:00)
Even numbers: Saturday, 22 October (11:30-13:00)

P.2 Pathogenic epigenetic reprogramming of stromal cells contributes to adverse cardiac remodeling
Valeria Bianconi *(Rome, Italy)*

P.3 Safety and efficacy of a possible gene therapy approach for FSHD muscular dystrophy
Beatrice Biferali *(Milan, Italy)*

P.4 Targeting IL-6 for the stabilization of skeletal muscle in diseases
Caterina Boccia *(Rome, Italy)*

P.5 Impaired myoblast differentiation in C2C12 cells with genetic knockdown of phosphomannomutase 2 (*PMM2*) gene
Matteo Bocconcelli *(Urbino, Italy)*

P.6 Modulation of the cyclin inhibitor p27 to ameliorate Merosin Deficient Congenital Muscular Dystrophy (MDC1A)
Rosa Bonaccorso *(Milan, Italy)*

P.7 Unveiling the HDAC4 functions in mediating the cross-talk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy
Giorgia Cavioli *(Rome, Italy)*

P.8 Ablation of RAGE (receptor for advanced glycation end-products) translates into reduced tumorigenic and cachectic potential in LLC-tumor bearing mice
Sara Chiappalupi *(Perugia, Italy)*

P.10 Study of extracellular vesiscles (EVs) protein content released by dystrophic fibro-adipogenic progenitors (FAPs) treated with HDAC inhibitors (HDACi)
Federica Esposito *(Rome, Italy)*

P.11 A new way of studying the muscular secretome in a prematurely aged model
Giulia Ferrarese *(Padua, Italy)*

P.12 Pharmacological targeting of the receptor for advanced glycation end-products (RAGE) to counteract cancer cachexia
Giulia Gentili *(Perugia, Italy)*

P.14 Characterization of collagen turnover pathways in primary muscle fibroblasts derived from the mdx mouse model of Duchenne Muscular Dystrophy
Matteo Giovarelli *(Milan, Italy)*

P.15 Mitochondrial complex II is tightly related to augmented peripheral fatigue
Gaia Giuriato *(Verona, Italy)*

P.17 Effects on muscle stem cells of exosomes derived from both untrained young and trained old subjects
Lorenzo Marramiero *(Chieti, Italy)*

P.18 MyomiR regulation by S1P signalling in skeletal muscle atrophy
Elisabetta Meacci *(Florence, Italy)*

P.20 Cytoplasmic HDAC4 delivery as a potential therapeutic approach for Duchenne Muscular Dystrophy
Viviana Moresi *(Rome, Italy)*

P.21 Calcium-activated K^+-channels contribute to muscle damage in dystrophic mdx mice
Marta Morotti *(Rome, Italy)*

P.22 A common calpain-3 variant explains a significant number of LGMD R1 calpain3-related cases in Eastern and Central Europe
Magdalena Mroczek *(Zurich, Switzerland)*

P.23 Identification of natural products able to counteract the formation of advanced glycation end-products (AGEs) sustaining muscle atrophy
Martina Paiella *(Novara, Italy)*

P.25 Combining vitamin E-functionalized CHOcolate with exercise to reduce the risK Of protein-energy malnutrition in pre-dementia AGEd people - The Choko-AGE study
Anna Pedrinolla *(Verona, Italy)*

P.26 Loss of Jab1 in muscle lineage causes a muscular dystrophy that resembles LAMA2 disease
Emanuela Porrello *(Milan, Italy)*

P.27 Effects of environmental pollutions exposure on human muscle stem cells
Cristina Purcaro *(Chieti, Italy)*

P.28 Vitamin D binding protein affects neuromuscular junction
Tommaso Raiteri *(Novara, Italy)*

P.29 Apelin resistance contributes to muscle loss during cancer cachexia in mice
Andrea David Re Cecconi *(Milan, Italy)*

P.30 The effects of vitamin D binding protein on skeletal muscle mitochondria
Simone Reano *(Novara, Italy)*

P.31 MICAL2 modulation for hampering Rhabdomyosarcoma progression
Lorenza Rinvenuto *(Leuven, Belgium)*

P.32 Effects of chronic nitrate supplementation on exercise training outcomes in old mice: a neuromuscular perspective
Maira Rossi *(Pavia, Italy)*

P.33 Overexpression of sAnk1.5 does not alter glucose homeostasis in a transgenic mouse model
Egidio Maria Rubino *(Siena, Italy)*

P.35 *Equisetum arvense* standardized extract hinders age-related sarcopenia
Laura Salvadori *(Novara, Italy)*

P.37 Characterization of a novel *in vitro* model of sarcopenia
Andrea Scircoli *(Novara, Italy)*

P.38 Mass spectrometry-based characterization of proteome and acetylome landscape of murine myotubes treated with givinostat
Valeria Spadotto *(Cinisello Balsamo, Italy)*

P.39 Duchenne's muscular dystrophy involves a defective transsulfuration pathway activity
Valentina Vellecco *(Naples, Italy)*

P.40 SRT2104, a new specific SIRT1 activator, promotes muscle recovery enhancing mitochondrial metabolism in DMD
Silvia Zecchini *(Milan, Italy)*

P.41 Testing a new therapeutic approach for cancer cachexia using X-MET as a 3D skeletal muscle model
Mariam Zouhair *(Rome, Italy)*

P.42 STAT3-mediated autophagy drives muscle regeneration
Lucia Latella *(Rome, Italy)*

Invited Speakers

Doug MILLAY

Department of Pediatrics, University of Cincinnati
Cincinnati, OH, USA

Dr. Millay has a long-standing interest in various aspects of skeletal muscle biology. His training has integrated several research areas including molecular, cellular, and developmental biology, and biochemistry. The Millay laboratory at Cincinnati Children's Hospital Medical Center aims to identify the factors that regulate cell-cell fusion, using skeletal muscle as a model system, delineate their biochemical functions and the biophysical properties associated with membrane coalescence, and then ultimately translate that information to augment pathological conditions. He discovered the muscle-specific proteins (Myomaker and Myomerger) that are necessary and sufficient for myoblast fusion. The group has a wide-range of projects that encompass basic and translational biology related to these fusogens and the consequences of fusion, namely multinucleation that controls muscle development and adaptations. The basic science projects in the laboratory are attempting to biochemically elucidate the activities of Myomaker and Myomerger, which would allow for a complete understanding of the mammalian myoblast fusion reaction through biochemical and cell biological approaches. Additionally, the lab is interested in understanding the role of the hundreds of myonculei within the muscle syncytium. For this research area, they generated mouse models where the number of myonuclei are titrated and also performed transcriptional profiling of myonuclei from mice. They developed a publicly searchable site (https://research.cchmc.org/myoatlas/) where users can determine expression of genes within myonuclei across the lifespan of the mouse. Our goal here is to understand the role of muscle stem cell fusion during various phases of muscle development, growth, and disease. Finally, the translational work relates to transferring the function of Myomaker and Myomerger onto various membrane vectors to enhance delivery of therapeutic material to skeletal muscle.

Mechanisms of myoblast fusion and role of multinucleation during muscle development, growth, and disease

Douglas P. Millay[1,2]
[1]Division of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center
[2]Department of Pediatrics, University of Cincinnati

Fusion of plasma membranes is essential for skeletal muscle development, regeneration, exercise-induced adaptations, and results in a cell that contains hundreds to thousands of nuclei within a shared cytoplasm. The differentiation process in myocytes culminates in their fusion to form a new myofiber or fusion to an existing myofiber thereby contributing more synthetic material to the syncytium. The choice for two cells to fuse and become one could be a dangerous event if the two cells are not committed to an allied function. Thus, fusion events are highly regulated and controlled by numerous proteins and lipids that help prepare the cells to fuse. Once ready to fuse, the membrane remodeling component of the myoblast fusion reaction is driven by muscle-specific proteins Myomaker and Myomerger. These transmembrane proteins function to independently modify membranes to drive fusion, where Myomaker functions at or before the outer leaflets of the two membranes mix (hemifusion) and Myomerger controls pore formation and thus fusion completion. Here, I will discuss our efforts to: a) mechanistically dissect the biochemical activities of the myogenic fusogens b) harness the activities of the fusogens for muscle therapies and c) decipher the role of multinucleation for myofiber growth and adaptations.

HARPER

Nationwide Children's Hospital, Ohio State University College of Medicine
Columbus, OH, USA

Scott Harper, PhD, is a Principal Investigator in the Center for Gene Therapy at the Abigail Wexner Research Institute at Nationwide Children's Hospital and a Professor of Pediatrics at the Ohio State University College of Medicine. In addition to his primary academic positions, Dr. Harper serves as Chief Scientific Adviser at Armatus Bio, a gene therapy startup company in Columbus, Ohio. Dr. Harper is also a member of the Scientific Advisory Boards of the Charcot-Marie-Tooth Association (CMTA) and Andelyn Biosciences, and a standing member of the NIH Neurological Sciences and Disorders B (NSD-B) study section.

Dr. Harper earned a PhD in Cellular and Molecular Biology from the University of Michigan Medical School, where he worked in the lab of Dr. Jeff Chamberlain to develop the first generation of micro-dystrophin gene therapies for Duchenne Muscular Dystrophy (DMD). He then completed postdoctoral training in the lab of Beverly Davidson, PhD, at the University of Iowa, where he developed gene therapies to treat dominant neurodegenerative diseases, including Huntington's Disease.

Dr. Harper's primary research focus at Nationwide Children's has been developing gene therapies to treat neuromuscular and neurological disorders, including FSHD. He was recipient of the 2014 "Outstanding New Investigator" Award from the American Society of Gene and Cell Therapy.

Development of *DUX4* inhibition therapies for Facioscapulohumeral muscular dystrophy (FSHD)

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Skeletal muscle myofibers are large and elongated cells with multiple and evenly distributed nuclei. Nuclear distribution suggests that each nucleus influences a specific compartment within the myofiber and implies a functional role for nuclear positioning. Compartmentalization of specific mRNAs and proteins has been reported at the neuromuscular and myotendinous junctions, but mRNA distribution in non-specialized regions of the myofibers remains largely unexplored. We report that the bulk of mRNAs are enriched around the nucleus of origin and that this perinuclear accumulation depends on recently transcribed mRNAs. Surprisingly, mRNAs encoding large proteins - giant mRNAs - are spread throughout the cell and do not exhibit perinuclear accumulation. Furthermore, by expressing exogenous transcripts with different sizes we found that size contributes to mRNA spreading independently of mRNA sequence. Both these mRNA distribution patterns depend on microtubules and are independent of nuclear dispersion, mRNA expression level and stability, and the characteristics of the encoded protein. Thus, we propose that mRNA distribution in non-specialized regions of skeletal muscle is size selective to ensure cellular compartmentalization and simultaneous long-range distribution of giant mRNAs.

FARUP

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Jean Farup did his graduate studies in human exercise physiology at Dept of Public Health Aarhus University from which he obtained his PhD in stem cell physiology in 2014. He then continued into a postdoc position at Dept of Clinical Medicine, Aarhus University, and later joined the lab of Thomas Rando at Stanford University. During this time, he studied muscle stem cell quiescence and the role of the muscle microenvironment in chronic diseases such as type 2 diabetes. In 2019 he joined the Dept of Biomedicine at Aarhus University as an assistant professor where he has been establishing a research group focusing on the muscle microenvironment in human skeletal muscle. His work focuses on the fibro-adipogenic progenitors (FAPs) and their role in maintenance and degeneration in human skeletal muscle. Most recently, his work has highlighted the important role of FAP subsets in human skeletal muscle during chronic muscle degeneration. Current studies focus on the cross-talk between FAPs and macrophages and the role for FAPs in regulating immune cell infiltration and local tissue inflammation in human muscle.

Human skeletal muscle fibro-adipogenic progenitors in degeneration and regeneration

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Skeletal muscle is a multicellular organ responsible for insulin stimulated glucose disposal and solely responsible for human locomotion. Accumulating evidence suggest that maintenance of muscle function is dependent on a number of non-myogenic progenitor cells including the mesenchymal fibro-adipogenic progenitors (FAPs). Here we present data on how fibro-adipogenic progenitors (FAPs) are intimately involved in both de-and regeneration of human skeletal muscle. We show that non-injured human skeletal muscle from type 2 diabetic patients (T2D) display an extensively remodelled niche characterized by fibrosis and adipocyte accumulation. We prospectively identify the cellular source of this (i.e. human FAPs) and show that T2D is associated with an altered content and functionality of these niche cells. Interestingly, using single cell RNA-seq and FACS we identified subpopulations of FAPs with diverse transcriptional and functional phenotypes. Using Thy1 (CD90) as a separation marker of the two populations we show that the FAP^{CD90+} display an increased clonality and enrichment in extra-cellular matrix (ECM) production compared to FAP^{CD90-}. Importantly, the FAP^{CD90+} is the major cell population that specifically increases in T2D and is thus likely responsible for the extensive remodelling of the skeletal muscle niche. Conversely, in human regeneration, FAPs, and in particular FAP^{CD90+}, temporarily expand. Here we find that FAPs likely play a pivotal role for mediating immune cell infiltration in the early phase of muscle regeneration. Specifically, FAPs secrete high levels of Complement 3, a central protein in the complement system, and provide the basis for phagocytosis of damaged muscle fibers. In contrast to muscle degeneration in T2D, we find that FAP levels return to baseline upon resolution of the muscle injury. These data provide new insight into the dynamics and multiple roles of FAPs in human skeletal muscle.

MOZZETTA

Institute of Molecular Biology and Pathology (IBPM),
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Chiara Mozzetta is a researcher and Group Leader at Institute of Molecular Biology and Pathology (IBPM) of the National Research Council of Italy, located at University Sapienza of Rome. The major focus of the Mozzetta lab is to study how histone modifying enzymes control stem cell's fate plasticity by shaping three-dimensional genome organization, with the goal to understand how this contributes to stem cells' lineage restriction. Particularly, the Mozzetta lab is currently devoting its efforts in deciphering how heterochromatin is dynamically re-shaped to coordinate cell-type specific gene expression patterns to establish and maintain cell identity during tissue regeneration, aging and disease. Because a causal link between changes in genome architecture and pathological de-regulation of transcriptional control is emerging, the Mozzetta lab aims to study how, and to what extent, these processes are altered in muscle disorders, such as muscular dystrophies and malignancies of muscular origin. Since chromatin modifying enzymes offer a reservoir of possible druggable targets, the research team of the Mozzetta lab anticipates that expanding the knowledge on the epigenetic mechanisms dictating stem cells fate decisions will contribute to develop next-generation selective approaches to modulate their developmental capacities therapeutically.

Role of peripheral heterochromatin in the maintenance of fibro-adipogenic progenitors' genome integrity and cell identity

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Spatial genome organization is crucial to maintain proper gene expression patterns in a cell-type specific manner. Silent heterochromatin is spatially confined towards the nuclear periphery and anchored to the nuclear lamina (NL) by lamina-associated domains (LADs), in which alternate fate genes are embedded to maintain cell identity. However, how heterochromatic lamina-associated regions are inherited after every cell division to maintain cell identity and whether re-establishment of peripheral heterochromatin organization is disrupted as a pre-requisite of malignant cell transformation is poorly studied.
Here, we will discuss our latest data reporting on how peripheral heterochromatin preserves genome integrity and cell identity of fibro-adipogenic progenitors (FAPs). Following up our recent study in which we identified Prdm16 as a FAPs-specific regulator of heterochromatin assembly to the nuclear envelope, we will present data showing its role in mediating LADs' deposition and maintenance of nuclear architecture and genome stability. We will provide evidence of how Prdm16 absence can promote genomic abnormalities in FAPs by impairing stable LADs-NL interaction during cell division, thus driving mitotic errors. Finally, we will discuss how FAPs-specific loss of Prdm16 might predispose these mesenchymal progenitors to the acquisition of (pre)-malignant features.

Selected Talks Abstracts

The interplay between polyglutamine-expanded AR and its transcription co-factors in skeletal muscle

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SBMA is an X-linked, late-onset, and progressive neuromuscular disease caused by a CAG expansion coding for glutamine in the androgen receptor (AR) gene, and its dependence on androgens restricts its full manifestation to males.
For a long time, SBMA has been thought to primarily be a motor neuron disorder. However, in the last decade a key emerging aspect of this disease is the primary involvement of peripheral tissues, such as skeletal muscle.
A crucial event for AR function is the interaction with its co-regulators that is necessary to assemble a functional transcription complex, but how these coregulators are recruited is not clear yet.
Using different *in vivo* and *in vitro* models we have found that specific AR co-regulators are overexpressed in SBMA skeletal muscle in an androgen-dependent manner and these AR co-activators enhance polyglutamine-expanded AR toxic gain-of-function and toxicity.
These results support the idea that changes in the expression of these co-regulators could lead to differences in the expression of AR-target genes in skeletal muscle.
Androgens binding to polyQ-expanded AR triggers SBMA through a combination of toxic gain- of-function (GOF) and loss-of-function (LOF)mechanisms.
The different results that we obtained suggest that targeting overexpressed AR native co-regulators could be a possible therapeutic approach for SBMA to attenuate the AR toxic gain of function (given by the polyQ-expanded AR) without exacerbating the AR loss of function.

Defective dystrophic thymus determines degenerative changes in skeletal muscle

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In Duchenne muscular dystrophy (DMD), sarcolemma fragility and myofiber necrosis produce cellular debris that attract inflammatory cells. Macrophages and T-lymphocytes infiltrate muscles in response to damage-associated molecular pattern signalling and the release of TNF-α, TGF-β and interleukins prevent skeletal muscle improvement from the inflammation. This immunological scenario was extended by the discovery of a specific response to muscle antigens and a role for regulatory T cells (Tregs) in muscle regeneration. Normally, autoimmunity is avoided by autoreactive T-lymphocyte deletion within thymus, while in the periphery Tregs monitor effector T-cells escaping from central regulatory control. Here we report impairment of thymus architecture of mdx mice together with decreased expression of ghrelin, autophagy dysfunction and AIRE down-regulation. Transplantation of dystrophic thymus in recipient nude mice determines the up-regulation of inflammatory/fibrotic markers, marked metabolic breakdown that leads to muscle atrophy and loss of force. These results indicate that involution of dystrophic thymus exacerbates muscular dystrophy by altering central immune tolerance.

The pathophysiology of the Super Relaxed state of myosin in skeletal muscle

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In the skeletal muscle, myosin is the most abundant protein and it is responsible for the basal metabolism of the tissue. Active skeletal muscle consumes a large amount of energy, while relaxed muscle is adapted to save energy. The relaxed myosin exists in two states: a disordered state (DRX) that exhibits a relevant ATPase activity and the myosin structure is not properly defined, and a Super Relaxed State (SRX) in which the myosin structure is ordered and that has an extremely low ATPase activity. Despite the very low specific energy consumption, skeletal muscle constitutes around 40% of human body mass, thus myosin could play a role in whole body basal metabolism. Controlling the equilibrium between myosin in SRX and DRX could potentially modulate daily energy consumption.
Recently, we developed a new class of molecules that are able to modulate the stability of myosin SRX in both directions, so these molecules could induce an increase energy expenditure or could reduce it. These molecules are effective in rabbit, mouse and human skeletal muscle. In this project, we want to explore myosin SRX/DXR states in response to pharmacological modulation, evaluating muscle contractility and energy consumption. We will also analyze a possible contribution of the SRX destabilization in the muscular hypermetabolism of the Amyotrophic Lateral Sclerosis (ALS), both in mice models and in human biopsies from ALS patients, and the effect of pharmacological modulators on those samples.

MUSA1 is a novel critical regulatory element for Z-line homeostasis and skeletal muscle function

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Skeletal muscle health and function is guaranteed by a finely regulated balance between protein synthesis and degradation of damaged structures and organelles. In the search for new players of skeletal muscle degradative systems, in the past years we identified MUSA1 as a novel E3 ubiquitin ligase sitting at the crossroad of two fundamental pathways controlling muscle mass; indeed, MUSA1 expression is driven by FoxO3 activity during catabolic conditions, while it is suppressed by the BMP signalling, a well-known anabolic pathway. To better characterize MUSA1 role in skeletal muscle physiology, we generated muscle-specific knock-out mice. MUSA1 deletion leads to a progressive sarcomeres structure degeneration in knock-out muscles, which is dramatically exacerbated with aging, as well as under catabolic conditions such as starvation and denervation. Histological and electron microscope analyses revealed the presence of wide areas within muscle fibers - especially oxidative ones - characterized by undigested sarcomeric components, as well as the aggregation of Z-line structures resembling nemaline rods. In line with this evidence, knock-out muscles proteomes reveal a progressive accumulation of proteins important for sarcomeres and cytoskeleton assembly and stabilization, together with Z-disc components. As a consequence, the ultrastructural alteration leads to a reduction of muscle force in knock-out animals together with a progressive myopathic-like phenotype. Concluding, our data support MUSA1 as a critical player in controlling Z-disc homeostasis together with muscle function. However, further investigations are needed to better characterize MUSA1 substrates and to precisely dissect the molecular pathogenic mechanisms involved in the perspective of therapeutic intervention.

EPS induces the secretion of different subpopulation of extracellular vesicles in C2C12 myotubes

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Skeletal muscle (SkM) has emerged as an important secretory organ releasing myokines and, as demonstrated in recent literature, also extracellular vesicles (EVs) during exercise. Accumulating evidence suggests that these signals largely contribute to exercise-induced muscle adaptations in an autocrine, paracrine or endocrine manner. Currently, there is a substantial interest in how acute and chronic exercise can promote EV release and its role in mediating the systemic effects of SkM activity. Nevertheless, most of the previous studies about EV secretion in acute and chronic exercise have been focused on EVs isolated from serum and plasma, which comprise a whole mixture of vesicles derived from circulating cells and other secretory tissues besides skeletal muscle. For these reasons, the present study aims to clarify if a prolonged acute session of EPS in C2C12 myotubes, mimicking a high-intensity exercise bout, can promote the secretion of both large and small extracellular vesicles. High (10K-) and low (100K-) density EVs, secreted from contracting myotubes, have been quantified and characterized for vesicular marker content. The obtained data clearly showed an increase in the number of 10K-EVs coupled with a decrease in CD81+ and an increase in ADAM-10+ particles. Differently, we found no apparent accumulation of 100K-EVs following EPS. In more detail, CD81 was expressed at similar levels in both control and EPS conditions, whereas these vesicles resulted negative for ADAM-10. Finally, we investigated the ability of EPS-derived EVs to trigger inflammatory activation in RAW-264.7 macrophages. IL-1beta and IL-6 mRNA quantification in RAW 264.7 cells treated with EPS-derived EVs and soluble proteins displayed a pro-inflammatory activation of target macrophages mainly with 10K-EVs. Altogether, the data suggest that EVs could have a role in regulating physical adaptations to high-intensity exercise.

Duchenne muscular dystrophy cardiomyopathy modelling using patient-derived cardiac organoids

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Duchenne muscular dystrophy (DMD) is a monogenic muscle-wasting disease caused by mutations in the X-linked dystrophin gene. In recent years, the use of respiratory assist devices and non-invasive positive pressure ventilation increased the life expectancy of DMD patients, thus contributing to the rise of late-stage DMD complications such as dilated cardiomyopathy (DCM). Dystrophic hearts are characterized by premature cell death of cardiomyocytes leading to fibrosis, reduction in cardiac contractility, left ventricle dilatation, and ultimately congestive heart failure. Despite DCM affecting up to 90% of late-stage DMD patients and becoming their major cause of death, the biology underlying the derangement observed in the myocardium has been poorly investigated. This is mainly due to a shortage of human cardiac biopsies and the lack of preclinical human models that recapitulate the DCM phenotypes. Hence, to bridge this scientific gap and investigate whether DMD-related cardiomyopathy occurs in 3D models, we developed DMD patient-derived 3D cardiac organoids (DMD-COs) and CRISPR/Cas9 isogenic-corrected controls (DMD-Iso-CO). By culturing the COs up to 93 days, only the DMD-COs showed cardiomyocyte degeneration, loss of sarcoglycans, higher cell death rate, and endoplasmic reticulum stress followed by the formation of fibrotic and adipose tissue. Additionally, the bulk RNA sequencing analysis performed on COs at 56 days revealed the enrichment in hypertrophy/dilated cardiomyopathy, arrhythmia, adipogenesis, and fibrosis pathways only in DMD-COs. Furthermore, by means of in silico analyses, we pointed out five crucial miRNAs involved in this dysregulated gene network that could represent therapeutic targets. In conclusion, we generated patient-derived cardiac organoid models that displayed DMD-related cardiomyopathy features and disease progression phenotypes in long-term cultures.

Perivascular stem/progenitor cells, up-and-coming tissue regenerating route

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Pericytes, at first named Rouget cells for C.M. Banjamin Rouget, French histologist first describing a population of contractile cells with spreading branched soma processes wrapping blood vessel and being in intimate contact with the endothelium (Rouget, 1873). Pericytes are defined as perivascular stem/progenitor cells contributing to the vascular basal membrane constitution and still able to communicate with endothelial cells. They are present in all organs and tissue, although in different proportion, and beside contribution to blood vessel architecture, stability and permeability, they concur in different biological processes, showing great plasticity. Pericytes are similar to mesenchymal stem cells able to differentiate into muscle, cartilage, fat and bone, and attend physiological tissue regeneration. It is still unknown the real differentiative potential of pericytes isolated from different tissue, nevertheless it is much more demonstrated and substantiated their angiogenic capabilities.
Hence the combination of plasticity and angiogenic capability elevate the pericytes in a privileged position as promising cell population for tissue regeneration and repair. In this regard, we have: isolated human derived muscle pericytes, characterized by mass cytometry analysis and tested their potentiality *in vitro*and *in vivo*, exploiting novel 3D based technologies, for skeletal muscle tissue reconstruction.

Sympathetic innervation is required to maintain the quiescent muscle stem cell pool via perivascular-derived Angpt-1

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In the vascular niche, perivascular cells are known to modulate the behaviour of adult muscle-specific stem cells, named satellite cells (SCs), by releasing ANGPT-1 and therefore maintaining SCs in a quiescent state.
Here, we have unveiled the sympathetic innervation as crucially implicated in the maintenance of SCs both at steady state and during muscle regeneration. We show that sympathetic nerve fibers innervate the SC niche by targeting β-adrenergic receptors (β-ARs) on VSM/perivascular cells and, via the release of perivascular-derived ANGPT-1, ensure the maintenance of SCs in a quiescent, undifferentiated state. In response to 6-OHDA-mediated sympathetic nerve ablation, we observe vascular remodelling and, therefore, perivascular cells reduce expression of SC maintenance factor ANGPT-1. Accordingly, SCs exit from quiescence, activate and rapidly fuse giving rise to new myofibers. This premature differentiation finally leads to a reduced quiescent SC pool. Finally, in vivo ANGPT-1 depletion in perivascular cells is able to recapitulate the 6-OHDA-mediated loss of quiescent SCs, highlighting the pivotal role of ANGPT-1 as mediator of sympathetic nervous system signalling to SCs. In addition, in vitro stimulation of β-adrenergic signaling in perivascular cells leads to Angpt-1 downregulation and concomitant increased SC fusion, suggesting that ANGPT-1 acts downstream of the β-adrenergic signaling in perivascular cells.
Altogether, our results provide novel and strong evidences that sympathetic nervous system regulates ANGPT-1 release via modulation of β-adrenergic signalling in support perivascular cells, thus being able to maintain the adjacent SCs in a quiescent and undifferentiated state. Remarkably, the modulation of this neuro-vascular circuitry will have important implications for the understanding of SCs in neuromuscular diseases, such as DMD, where both sympathetic innervation and vascular niche signals are dysregulated, finally impairing SC function.

Junctophilins and CLIMP63: a novel interaction in skeletal muscle

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Triads, skeletal muscle specific structures made by the juxtaposition of a T-Tubule and two terminal cisternae of the sarcoplasmic reticulum, represent the central hub for the Excitation- Contraction Coupling mechanism. Multiple proteins are involved in triad biogenesis and maintenance including Caveolin 3, amphipysin2/Bridging integrator-1 and Junctophilins(JPHs). JPHs are directly responsible for the assembly of SR-T-tubule junctions thanks to their molecular structure composed by eight lipid binding motifs that allow interaction with T-Tubules and a C-terminal transmembrane domain that anchors the protein to the sarcoplasmic reticulum. At triads, JPHs form homo- and heterodimers that interact with several proteins, such as the ryanodine receptors and dihydropyridine receptors, thus also supporting the assembly of proteins of the calcium release complex. Studying protein interactions at triads is a crucial step in the further characterization of this structure. Results from studies performed in our laboratory offer intriguing perspectives in this regard thanks to the identification of the cytoskeleton-linking membrane protein 63 (CLIMP63) as a novel interactor of JPHs. CLIMP63 is a transmembrane protein, composed by a cytosolic region containing a microtubule-binding domain and a luminal segment presenting coiled-coil domains that mediate protein oligomerization; CLIMP63 oligomers act as ER shapers, helping in maintaining the correct luminal spacing between opposite membranes within ER cisternae. Previous data from the literature demonstrated that CLIMP63 interacts with triadin, a transmembrane protein localized at triads. We report here on results from BioID proximity dependent labelling and co-immunoprecipitation experiments showing that CLIMP63 can also bind JPHs. Real-time PCR and Western blot analysis showed that expression of CLIMP63 mirrors the mains steps in triad maturation, suggesting a potential role of this protein in triad assembly and maintenance.

The voice of patients affected by Duchenne and Becker Muscular Dystrophy

Gloria Antonini
Parent Project aps

Parent Project aps is an association of patients and parents of children affected by Duchenne and Becker Muscular Dystrophies (DMD and BMD), considered the most common among rare diseases and for which there is still no cure. The association is committed to funding research and disseminating the multidisciplinary approach that has so far enabled doubling the patients' life expectancy and improving their quality of life. Parent Project Scientific office manages all the activities related to the support of research and the dissemination of scientific information to patients, families and the outside world. The office also manages the Italian DMD/BMD patient registry, collecting demographic, genetic and clinical information about patients with DMD/BMD, necessary for the recruitment of patients for clinical trials, but also to improve the epidemiological information about these pathologies. Parent Project aps is committed to build a future of quality for thousands of children and young people living with DMD/BMD and identifies information as one of the key tools to achieve this important goal. For this reason, the association runs an educational program that includes an annual international conference which is a unique opportunity to raise awareness, to share experiences and to learn about the latest progresses in the fight to end Duchenne and Becker. Families, physicians, researchers, caregivers, industry partners and patients living with Duchenne and Becker gather to connect, to share information and to discuss and debate the latest news and opportunities in DMD and BMD research. The next conference is scheduled from the 17th to 19st of February 2023.
For more information, please visit our websites:
http://parentproject.it
http://strategieterapeutiche.parentproject.it/

BET inhibitors rewire metabolism in the aged skeletal muscle

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Aging is associated with a progressive decline of muscle mass and strength, that is observed among healthy adults, with an acceleration in the rate of decline past middle age. The pathological loss of muscle mass associated with aging, known as sarcopenia, negatively affects the quality of life and leads to increased occurrence of falls, hospitalization, and to decreased independence. Previous reports from our group showed that the bromodomain and extra-terminal domain (BET) protein BRD4 plays a role in promoting muscle wasting in experimental models of cancer cachexia and muscular dystrophy. Here, we evaluated the impact of pharmacological blockade of BET proteins in the skeletal muscle of 24-month-old mice.
Mice were treated with the BET inhibitor $JQ1^{+}$ (20mg/kg) or the inactive enantiomer $JQ1^{-}$ daily, for 24 days. During treatment, mice were weighed, and muscle performance was evaluated through the treadmill and grip tests. After sacrifice, different muscles and several tissues were isolated and collected for morphological and molecular analysis, including RNA- seq, Western Blot, and IHC.
Our data show that JQ1 treatment induced weight loss in old mice and BET blockade also displayed a beneficial effect on muscle performance, and it was associated with a marked reduction in fibrosis.
Following JQ1 treatment, RNA-seq assays highlighted an enrichment in the level of key transcripts involved in fatty acid oxidation in skeletal muscle. Metabolomic and immunoblot analysis revealed a reduced reliance on glycolysis and an increase in fatty acid oxidation.
In conclusion, our data suggest that $JQ1^{+}$ treatment ameliorates mitochondrial fatty acid metabolism in old mice, improves muscle function and it may be beneficial in the treatment of sarcopenia.

A novel therapeutic perspective for FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) represent one the most common neuromuscular disorder affecting both children and adults regardless of gender. FSHD is caused by aberrant re-activation of the transcription factor DUX4, which is physiological restricted in the early stage of embryo development. DUX4 aberrant expression triggers a pro-apoptotic transcriptional program leading to muscle wasting and disease progression. As today, no cure or therapeutic option is available to FSHD patients. Given its pivotal role in FSHD, blocking DUX4 expression with small molecule drugs is an attractive solution.

We previously demonstrated that the long non-coding RNA DBE-T is essential for aberrant DUX4 expression in FSHD. Using affinity purification followed by proteomics, we identified the chromatin remodeling protein WDR5 as a novel DBE-T interactor and a key player required for the biological activity of the lncRNA. We found that, in FSHD muscle cells, WDR5 is necessary for the expression of both DUX4 and its target genes. Moreover, WDR5 silencing rescues both cell viability and myogenic differentiation of FSHD muscle cells as efficiently as silencing directly DUX4. Remarkably, we obtained analogous result by WDR5 pharmacological inhibition. Intriguingly, RNA-seq indicates that the WDR5 inhibitor globally blocks the DUX4-induced gene expression, which is the major molecular signature in FSHD skeletal muscle. Notably, the treatment causes minimal effects to healthy muscle cells.

These results not only support a crucial role of WDR5 in the regulation of DUX4 expression but also provide a novel druggable target opening a promising therapeutic path to the treatment of FSHD.

Identification of the first direct endogenous inhibitor of DUX4 in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular diseases, affecting children and adults of all ages and both sexes. Unfortunately, no treatment is currently available. FSHD is caused by gain of expression of the double homeobox 4 (DUX4) gene, encoding for a transcription factor normally silent in most adult somatic tissues. In FSHD, DUX4 aberrant activation triggers a pro-apoptotic transcriptional program resulting in muscle wasting. DUX4 has been recently implicated also in the pathogenesis of solid tumors, leukemia and herpes viral infection. Hence, blocking DUX4 activity is a plausible therapeutic option for FSHD and other diseases associated with aberrant DUX4 expression or activity.
We have identified MATR3 as the first direct endogenous inhibitor of DUX4. MATR3 is a nuclear protein mutated in ALS and dominant distal myopathy, diseases displaying molecular overlaps with FSHD. We found that MATR3 directly binds to DUX4 DNA-binding domain and blocks DUX4-mediated gene expression. As a result, MATR3 administration rescues cell viability and myogenic differentiation of FSHD muscle cells, while it does not affect healthy muscle cells. Notably, we characterized a shorter MATR3 fragment that is necessary and sufficient to directly block DUX4-induced toxicity to the same extent of the full-length protein by significantly impairing DUX4-binding to its genomic targets. Genome-wide experiments confirmed MATR3 proficiency in decreasing DUX4 bona fide target genes.
In summary, we identified the first endogenous inhibitor of DUX4 for the treatment of FSHD, that in perspective might be applied to a spectrum of related and currently incurable DUX4- associated diseases.

The epigenetic regulator JQ1 targets fat metabolism and counteracts diet-induced obesity

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Sarcopenia is a degenerative state of the skeletal muscle characterized by a progressive decline of muscle mass and strength due to fiber atrophy. Aging has been recognized as a causative factor for sarcopenia. Furthermore, during aging total fat mass frequently increases, affecting the metabolic homeostasis and playing a central role in the occurrence of many chronic diseases, such as type II diabetes, obesity and finally sarcopenia. Our data demonstrated the relevant role of the bromo and extra-terminal domain (BET) proteins inhibitor JQ1 in attenuating inflammation and fibrosis in sarcopenic mice. Moreover, we observed that JQ1 administration to old mice markedly reduced white adipose tissue mass compared to untreated mice, suggesting a potential role of JQ1 on visceral fat deposition during aging. Based on our preliminary data, the aim of this study was to investigate the role of JQ1 in decreasing fat deposition in a chronic diet-induced obesity (DIO) model of C57BL/6J mice, mimicking human metabolic syndrome. Data collected from mice showed that JQ1 administration reduced fat mass accumulation, preserving skeletal muscle mass and function. These results were supported by *in vitro* experiments, demonstrating a JQ1 lipolytic effect on mature adipocytes. Moreover, the *in vivo* fat loss did not induce systemic inflammation or lipid over-accumulation in muscle and liver. In order to understand if muscle plays a leading role in the modulation of adipose tissue fatty acid metabolism, we evaluated JQ1 effects on skeletal muscle metabolism and oxidative capability. Biochemical and molecular analysis revealed a not significant impact on mitochondrial mass and biogenesis, but suggested a switch from carbohydrate to fatty acid oxidation due to JQ1 administration. Further analyses are ongoing, with the goal to decipher the pathways responsible for JQ1 regulation of adipose tissue metabolism and to provide new targets for the treatment of sarcopenia and sarcopenic obesity.

Investigating the therapeutic potential of extracellular vesicle-derived microRNAs circulating in murine models of muscle hypertrophy

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Skeletal muscle exudes the irrefutable capability of maturation and regeneration under physiological and pathological settings. Loss of muscle mass is a worrisome characteristic of muscle diseases, as well as the typical process of aging. The emergence of the role of paracrine factors in both the pathogenesis of muscle disorders and homeostatic processes has propelled the importance of extracellular vesicles (EVs) as modulators of such processes, as well as potential therapeutic agents. The cargo of EVs typically includes proteins, lipids, and nucleic acids such as messenger RNAs (mRNAs) and non-coding RNAs, for instance, microRNAs (miRNAs). miRNAs have been thoroughly shown to be potential modulators of stem cell differentiation, as their binding to target mRNAs may lead to translational repression and/or mRNA degradation. In this study, by screening the mRNA, protein, and miRNA content of EVs derived from murine models of muscular dystrophy, aging, and muscle hypertrophy, we overall identified a hypertrophic miRNA combination suitable for ameliorating muscle wasting. We tested this miRNA combination among others *in vitro*, on human mesoangioblasts (hMABs) and found an improvement in myogenic differentiation.
Furthermore, the combination of these miRNAs was tested *in vivo* with injections of miRNA-treated hMABs in cardiotoxin-injured aged mice. Features such as muscle weight and cross-sectional area were enhanced compared to relevant controls. Overall, we provide evidence that the EV-derived miRNA combination which we identified enhances the myogenic proclivity of myogenic stem cells.

Employing isogenic cell models to study the underlying mechanism of cystinosis myopathy

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Cystinosis is a rare, autosomal recessive metabolic disease caused by lack or dysfunction of cystinosin (CTNS) leading to lysosomal cystine accumulation. This systemic disease affects several organs, including the muscles. Cystinosis myopathy forms a major concern leading to life-threatening events like swallowing difficulties and respiratory insufficiency. These complications affect over 80% of the untreated patients by the age of 40 and still affect cysteamine-treated patients although the rate of myopathy is decreased. The etiology of cystinosis myopathy remains to be elucidated. This project aims to shed light on the role of CTNS in a human muscle model to better understand the pathophysiology of cystinosis myopathy with the potential to develop new therapies. Human immortalized myoblasts together with CRISPR-nanoblade and viral vector technology are used to install isogenic muscle models and to assess the potential of a gene addition approach, respectively. In a first step, CRISPR technology was employed to generate a polyclonal, isogenic human *CTNS* knock-out (KO) myoblasts, corroborated at genomic DNA level and by elevated cystine levels in metabolomic analysis. *CTNS* cDNA addition using lentiviral vectors reverted the cystine accumulation seen in the *CTNS* KO model. To further evaluate the cystinosis muscle phenotype, differentiation analysis of the myoblasts was assessed for *CTNS* KO, showing no significant difference in the fusion index compared to the wild-type (WT) myoblasts. We assessed several key regulators of myogenic differentiation, and observed decreased RyR1 expression, a Ca^{2+} channel located within the sarcoplasmic reticulum, in *CTNS* KO cells compared to WT cells. Interestingly, *CTNS* cDNA addition rescued this phenotype. As a next step, we are further investigating the role of RyR1 and are establishing 3D spheroid models to better mimic the *in vivo* pathophysiology of cystinosis myopathy.

Exploring the protective role of GDG5 against skeletal muscle disuse atrophy

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Skeletal muscle is a high plastic tissue able to change its mass upon different stimuli accordingly with environmental changes. Its adaptability depends on many factors and is based on complex mechanisms. Among the process that could alter muscle mass homeostasis, disuse and inactivity induce strong muscle mass and function decrease, having heavy impact on life quality and requiring long time to recover.
Growth Differentiation Factor 5 (GDF5) is a crucial player in muscle homeostasis, shown to counteract both denervation- and age-related muscle wasting by limiting the activation of catabolic signals. However, its effects on disuse atrophy following muscle immobilization has to be investigated. In order to establish a potential therapeutic tool having a wide relevance, ranging from disease to microgravity exposure (space flight), we evaluated the consequences of GDF5 overexpression after 10 days of immobilization and 3 weeks of release of hind limb mouse muscles. We observed that local GDF5 overexpression in posterior limbs improved muscle mass loss during immobilization..
We aim to better characterize the effect of GDF5 treatment on several morphological and functional parameters of skeletal muscle upon immobilization/release, in order to establish if GDF5-based treatment could be proposed for optimal muscle recovery after disuse.
In parallel, a study of microgravity exposure was carried on a muscle cell line. We showed that, in the absence of gravity, myotube formation was inhibited, suggesting that this condition could impact cytoskeleton and fusion capability. We will establish if GDF5 treatment might be beneficial for myoblast fusion and myotube morphology during microgravity exposure.
In conclusion, our preliminary results suggest that a treatment based on GDF5 could have a therapeutic potential to ameliorate the pathophysiology of muscle during disuse condition to be applied also to space flight and microgravity exposure.

Spatially resolved transcriptomics reveals innervation-responsive functional clusters in skeletal muscle

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Striated muscle is a highly organized structure composed by well-defined anatomical domains with integrated but distinct assignments. So far, the lack of a direct correlation between tissue architecture and gene expression has limited our understanding of how each unit responds to physio-pathologic contexts.
Here, we show how the combined use of spatially resolved transcriptomics and immunofluorescence can bridge this gap by enabling the unbiased identification of such domains and the characterization of their response to external perturbations. Using a spatiotemporal analysis, we followed the changes in the transcriptomics profile of specific domains in muscle in a model of denervation. Furthermore, our approach allowed us to identify the spatial distribution and nerve dependence of atrophic signalling pathway and polyamine metabolism to glycolytic fibers. Indeed, we demonstrate a pronounced alteration of polyamine homeostasis upon denervation. Our dataset will serve as a resource for future studies of the mechanisms underlying skeletal muscle homeostasis and innervation.

A new murine model for skeletal disuse muscle atrophy with a 3D-printed cast

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Disuse skeletal muscle atrophy is a condition characterized by a decrease in muscle mass and strength caused by an imbalance in protein synthesis and protein degradation. This process naturally occurs upon a reduced or absent physical activity and it is often related to illness, forced bed rest, or unhealthy lifestyles. Currently, no treatment is available for disuse atrophy and it can only be prevented by overloading exercise, causing severe problems for patients who are not able to exercise due to chronic diseases, disabilities, or forced bed rest. Current murine models for muscle atrophy are mainly of two kinds: hindlimb suspension and ankle joint immobilization. Both methods can induce disuse atrophy in hindlimbs, however, they also come with criticalities. Hindlimb suspension is very effective, reproducible, and well- characterized, preserves the integrity of the animals but induces atrophy in both limbs, depriving of the possibility to use the contralateral muscles as control, and control legs are missing. The common ankle joint immobilization methods maintain a control leg but they are variable in the degree of atrophy induced and are typically even more invasive. Often they severely damage the animal's skin in a way that does not allow to prolong the immobilization for several days and therefore affect the ability to perform a recovery phase. The lack of treatments and relevance of this atrophic process requires a unilateral, safe, and robust model. We developed and optimized a new model for disuse skeletal muscle atrophy consisting of a 3D-printed cast that can be applied to a single limb in a non-invasive way and produces little to no swelling and skin damage, inducing 25% of atrophy (measured in terms of muscle wet weight and cross-sectional area) and the 37% of reduction in the specific force. The mice recover effectively mass and force in 3 weeks after the cast removal.

Understanding BMP signaling in cancer cachexia

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Cancer cachexia is a multi-factorial metabolic syndrome characterized by excessive body weight loss due to progressive fat and lean mass catabolism occurring in more than half of cancer patients. The loss of skeletal muscle mass and strength are considered as the most relevant features of cancer cachexia and predictors of poor outcomes.
Bone Morphogenetic Protein (BMP)/Smad1/5/8 pathway is a positive regulator of muscle mass homeostasis. We demonstrated that diminished Smad1/5/8 signalling in muscles plays a critical role for the onset of cancer cachexia and pharmacological reactivation of the BMP pathway in the muscles of tumor-bearing mice prevents muscle wasting. The transcriptional activity of Smad1/5/8 is specifically required to mediate a beneficial effect on muscle mass.
Moreover, several components of the BMP pathway are transcriptionally modulated in cachectic muscles.
Using an unbiased genome-wide approach to investigate the chromatin remodeling that may occur in cancer cachexia, we performed ChIP-seq experiments in muscles to characterize histone modifications.
We focused on H3K27Ac, a histone mark of active regulatory elements. An increase of acetylated histones was detected in muscles of C26- tumor bearing mice, compared to the control group. Process enrichment analysis reported the BMP pathway among the top 20 pathways with hyperacetylated histones. We searched for potential transcription factors binding sites in the hyperacetylated promoters of different BMP pathway components that are transcriptionally modulated in the context of cancer cachexia.
Our aim is to understand how cancer stimulates transcriptional changes leading to BMP-Smad1/5/8 inhibition in skeletal muscles to favor cachexia onset in order to identify new potential therapeutic targets.

The role of protein-kinase C theta (PKCθ) in ALS disease progression

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Neuromuscular junction (NMJ) represents the morphofunctional interface between muscle and nerve. The impairment of muscle-nerve communication is a typical characteristic of several diseases, including Amyotrophic Lateral Sclerosis (ALS). ALS is characterized by NMJs dismantlement, myofiber type switch, metabolic defect and immune cell infiltration that leads to spinal cord inflammation and finally to motor neuron degeneration. In a previous work we have characterized a transgenic mouse model that over-expresses the ALS human mutant gene, the $SOD1^{G93A}$ one, selectively in the skeletal muscle ($MLC/SOD1^{G93A}$ mice). These mice exhibit several features of the pre-symptomatic phase of ALS disease, including the impairment of muscle metabolism and NMJs defects. Recently, we have demonstrated a causal link between the aberrant activation of the Protein kinase C-θ (PKCθ) and the NMJ dismantlement in the skeletal muscle of the $MLC/SOD1^{G93A}$ mice. We then extended the aim to clarify the role of PKCθ activity in the mouse model of ALS disease represented by the $SOD1^{G93A}$ mice, that overexpress ubiquitously the human SOD1 mutant gene and to verify whether we can counteract different pathological aspects of ALS diseases modulating PKCθ -mediated pathways.

Impact of Drp1 activation and fission induction in the pathogenesis of DMD progression

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Loss of function mutations in *DMD* gene encoding for dystrophin protein cause Duchenne Muscular Dystrophy (DMD) a severe progressive neuromuscular disease. Despite remarkable progress has been made in genetic approaches to restore dystrophin, or its function, new therapeutic strategies are needed. In this view, muscle weakness in DMD is thought to be dependent, at least in part, on damaged mitochondria and compromised bioenergetics.
Consistently mitochondria are an attractive target for therapeutic interventions. Dystrophic fibers show marked mitochondria fragmentation, however, few studies have addressed the relevance of mitochondrial shape in the muscle damage progression.
Accordingly, we generated a DMD mouse model with intrinsically fluorescent mitochondria, the *mdx*-PhAM mouse, to precisely define mitochondrial dynamics during DMD progression and we confirmed the existence of a less interconnected mitochondrial network in *mdx* single fibers by 3-dimensional reconstruction. In agreement, Western blot experiments showed a significant upregulation of pro-fission proteins, Drp1 and its receptors, in *mdx* muscles starting from 3 months of age, suggesting the shifting of mitochondrial dynamics towards Drp1-mediated mitochondrial fission. This can potentially contributes to DMD pathological fibrosis and inflammation by triggering the activation of specific signaling pathways, such as inflammation by DAMPs (mtDNA) release and UPR response.
Therefore, to assess the relevance of Drp1-dependent fission enhancement in DMD pathogenesis we treated *mdx* mice with MDIVI-1, a specific Drp1 inhibitor. We have obtained encouraging results as for muscle functionality and phenotype, thus confirming the relevance of Drp1 as a therapeutic target in DMD.

A novel alternative therapeutic strategy for Duchenne Muscular Dystrophy

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The loss-of-function mutation in Duchenne Muscular Dystrophy (DMD) triggers a cascade of pathological events leading to muscle fiber degeneration. As inflammation and oxidative stress are two hallmarks of this disease, we investigated the possibility to counteract these processes by administering a selective serotonin reuptake inhibitor (SSRI) known to have anti-oxidative/inflammatory properties (Fluoxetine or Sertraline) or an antioxidant (Plumbagin, Quercetin, N-Acetylcysteine) to the dystrophic *mdx* mice. Our results showed that all the drugs improved the phenotype of *mdx* mice. They alleviated oxidative stress and inflammation shifting macrophages, the essential coordinators of inflammation and myogenesis, towards the M2 phenotype, known to improve tissue repair. We identified Fluoxetine and Plumbagin as the most efficient in their class, notably due to their ability to activate the Nrf2 pathway, which plays a pivotal role in the anti-oxidant/inflammatory response and muscle regeneration. Noteworthy, this is the first time Plumbagin is tested in muscular dystrophy. Moreover, promising data were obtained in a *Drosophila melanogaster* model of dystrophy. We then combined these two molecules so that they could benefit from each other in an additive/synergistic manner. We found that the combination significantly improved muscular performance *in vivo* in addition to its capacity to modulate inflammation and oxidative stress. There is a pressing need for an alternative therapeutic strategy for DMD, as in the absence of a definite cure, corticosteroids are part of care recommendations, but they delay the progression of the disease at the cost of severe side effects. As such, drug repurposing could be a solution with time- and cost-savings advantages. Indeed, antioxidants are available as dietary supplements and present none or few side effects; their combination with SSRIs, already renowned as safe, would in turn, further reduce adverse events by allowing a reduced dosage.

Rebalancing HMGB1 redox isoforms expression to counteract muscular dystrophy

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High Mobility Group Box 1 (HMGB1) protein acts as a DNA chaperone in the nucleus and as a signal of tissue damage when extracellularly released, contributing to inflammation. We identified HMGB1 as a functional link between oxidative stress and inflammation in muscular dystrophies. The oxidation of HMGB1 cysteines switches its extracellular activities from the orchestration of tissue regeneration to the promotion of inflammation. Extracellular HMGB1 is present at high levels and undergoes oxidation in dystrophic patients and in mouse models of Duchenne Muscular Dystrophy (DMD) and Limb-Girdle Muscular Dystrophy type 2d.
Genetic ablation of HMGB1 in muscles of DMD mice leads to an amelioration of the dystrophic phenotype associated to decreased inflammation and muscle degeneration, indicating that HMGB1 release and oxidation is a detrimental process in muscular dystrophy. Pharmacological treatment with a non-oxidizable variant of HMGB1, called 3S, improves muscle performance, regeneration and satellite cell engraftment in dystrophic mice, while reducing inflammation and fibrosis. Overall, our data demonstrate that the balance between HMGB1 redox isoforms dictates whether skeletal muscle is in an inflamed or regenerating state, and that providing a non-oxidizable form of HMGB1 is a possible therapeutic approach to counteract the progression of the dystrophic phenotype.

Physiactisome: a new nanovesicle drug containing heat shock protein 60 for treating muscle wasting and cachexia

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Physiactisome is a new anti-cachectic drug, based in the secretion of vesicles (exosomes) produced by genetically modified muscle cells. The final product seems to mimic what occurs naturally in an organism following physical exercise. These vesicles contain substances with beneficial properties for muscle tissue and can be used to counteract severe forms of muscular atrophy, such as those observed in conditions of chronic diseases such as cancer, Alzheimer's and kidney or heart dysfunction. Physiactisome, currently validated in animal testing, aims to be the first specific drug against muscular atrophy, with the particularity of being customizable. The methos used to obtain Physiactisome may be applied to muscle stem cells of the patient himself, obtainable by simple biopsy. Since muscle atrophy, in its most severe form (cachexia), is often the cause of death because it interferes with therapy, Physiactisome, by contrasting muscle atrophy, can not only increase patients' survival and quality of life , but also to improve the effectiveness of other therapies.

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Post-COVID syndrome: inflammation and the muscle

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COVID-19 firstly envisaged a disease affecting the respiratory system, was demonstrated to impact other organs and tissues during acute manifestation- including the muscle- inducing myositis in a considerable number of patients. While disease severity has been blunted by vaccination, major individual and public health concerns have arisen following the pandemic, as most of former COVID-19 patients (up to 85%) present persisting or novel symptoms (Post- acute COVID syndrome-PACS). We shall discuss the results obtained with 389 formerly hospitalized COVID patients, who show, after 6 to 11 months, alterations that allow the isolation of specific phenotypes of long-term response to the disease. In the study we found that a) muscularity at admission to hospital was associated with outcome in acute COVID and frequency of PACS; b) alteration of circulating inflammatory markers in patients with long COVID was concomitant to significant muscle loss, functional impairment and fatigue; c) men and women show different PACS profiles regarding muscle mass and function loss.

Poster Abstracts

P.2

Pathogenic epigenetic reprogramming of stromal cells contributes to adverse cardiac remodeling

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The cardiac stroma has a critical role in heart homeostasis and tissue remodeling after damage. Heart-resident stromal cells, known as cardiac fibro/adipogenic progenitors (cFAPs), have been recently implicated as the source of fibro-fatty infiltration that replace myocardium after cardiac damage. Thus, the elucidation of the epigenetic mechanisms that modulate their function is key to understand how to prevent pathogenic cardiac remodeling.
Here, we investigated the role of PRDM16 in modulating the fate of cFAPs. PRMD16 has been implicated in the epigenetic repression of hypertrophic genes in the heart and we recently reported that its repressive function is achieved by sequestration of peripheral heterochromatin at the nuclear lamina. Our data indicate that cFAPs-specific genetic ablation of PRDM16 induces cFAPs to contribute to the cardiac myogenic lineage. In particular, by the use of $Prdm16^{flox/flox}$; $PDGFR\alpha^{CreERT/+}$; $R26^{EYFP}$ transgenic mice, we demonstrate a significant contribution of YFP+/cFAPs to myocardium after ablation of PRDM16, in line with de- repression of cardiac specific transcripts in cFAPs. Upon heart damage, this culminates with cardiac hypertrophy and higher susceptibility to failure.
Mutations in *Prdm16* gene have been associated to the pathogenesis of cardiomyopathy in 1p36 deletion syndrome as well as in a proportion of nonsyndromic left ventricular non-compaction (LVNC) and dilated cardiomyopathy (DCM) patients. Our data suggest that pathogenesis of these diseases might be associated to aberrant remodelling of cardiac stroma imposed by pathogenic epigenetic reprogramming of cFAPs.

P.3

Safety and efficacy of a possible gene therapy approach for FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common neuromuscular diseases, affecting an estimated 1 in 8,000 individuals. FSHD is caused by aberrant myogenic expression of the transcription factor double homeobox 4 (DUX4). In FSHD, DUX4 mis- expression activates a pro-apoptotic transcriptional program leading to muscle wasting and weakness.
For long time, the lack of an animal model with FSHD features in which to study DUX4- mediated muscle pathology and test therapeutic interventions has been a major roadblock in developing treatments. We used FLExDUX4 mice which, when crossed to the skeletal muscle- specific and tamoxifen (TMX) inducible ACTA1-MCM driver, express DUX4 selectively in skeletal muscle upon TMX treatment. We conducted an in-depth analysis of FLExDUX4 mice disease over time and identify several pathogenic features that can be used to obtain a quantitative assessment of treatment efficacy.
In our lab, MATR3 was identified as the first endogenous protein inhibitor of DUX4. MATR3 binds DUX4 directly and block DUX4-induced toxicity in cell culture by counteracting DUX4 dependent gene expression. We identified a short MATR3 fragment that is sufficient to directly block DUX4-induced toxicity. We generated adeno associated viral vectors expressing full-length MATR3, the minimal MATR3 fragment inhibiting DUX4 or a control reporter gene under the control of a muscle-specific promoter. The constructs have been packaged in the highly myotropic AAVMYO serotype to maximize delivery to skeletal muscles while detargeting unwanted tissues upon systemic delivery in FLExDUX4 and control animals. Mice treated with AAVMYO-MATR3 constructs are currently analyzed at molecular, functional and histological levels to evaluate safety and efficacy of MATR3 delivery in vivo. Results from this study could open a new possibility to future therapeutic development for FSHD patients.

P.4

Targeting IL-6 for the stabilization of skeletal muscle in diseases

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Skeletal muscle tissue is known to influence the homeostatic balance of the entire organism, by releasing specific signalling molecules. Among them, IL-6 is a myokine exerting pleiotropic functions with hormetic effects. Indeed, a low and/or controlled release of IL-6 is associated with anti-inflammatory, anti-oxidant and pro-myogenic actions. In contrast, uncontrolled increased levels of IL-6 can act as a pro-inflammatory, pro-oxidant and pro-fibrotic mediator, contributing to muscle wasting. We addressed the specific physio-pathological role exerted by IL-6 in the maintenance of differentiated phenotype and defined the role of increased plasma levels of IL-6 on muscle homeostasis and the mechanisms contributing to muscle loss.
Moreover, we provided a therapeutic proof of concept that IL-6 signalling is a targetable pathway for muscle diseases, based on the evidences that interfering with IL-6 signaling conferred on pathologic muscles resistance to degeneration, guaranteeing a functional homeostatic maintenance of pathologic muscle.

P.5

Impaired myoblast differentiation in C2C12 cells with genetic knockdown of phosphomannomutase 2 (*PMM2*) gene

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A wide spectrum of neuromuscular syndromes, from muscle dystrophies to congenital myopathies/myastenic syndromes have been associated to Congenital Disorders of Glycosylation (CDG) caused by genetic N-glycosylation defects. PMM2-CDG is the most common form of CDG and is caused by mutations of the phosphomannomutase-2 (*PMM2*) gene, which encodes an enzyme essential for catalysing an early step of the N-glycosylation pathway. The rate and severity of myopathy in PMM2-CDG is still unexplored. Here, we used the C2C12 cells to investigate the effects of genetic knockdown of *PMM2* gene by siRNA or CRISPR/Cas9 systems on myoblast differentiation. Both transient (siRNA) and stable (CRISPR/Cas9) *PMM2* gene knockdown, markedly reduced *PMM2* mRNA expression (5.7±1.7 and 16.9±4.0 fold reduction, respectively), although the down-regulation of *PMM2* mRNA persist only in early phases of myoblast differentiation (day 3) in siRNA-transfect cells. Down- regulation of *PMM2* gene inhibited myoblast differentiation and disrupted the coordinated temporal expression of myogenic regulator genes *Ccnd1*, *MyoD*, *Myogenin* and *Mrf4*. The myotube formation marker MF20 was decreased in PMM2 siRNA-transfected cells and PMM2-CRISPR clones. Control myotubes also showed an increase of high mannose (ConA), complex mannose (PHA-L) and fucose (AAL) reactivities compared to myoblasts, while lectin binding decreased in CRISPR-PMM2 myotubes indicating a N-glycosylation deficiency.
Finally, N-glycosylation inhibition by *PMM2* knockdown decreased insulin-like growth factor 1 receptor (IGF-1R), downstream activation of Akt and dysregulated IGF-1 production compared to control cells. In conclusion, a proper N-glycosylation is crucial for C2C12 myoblast differentiation and for a correct IGF-1R signalling pathway activation. These results suggest that studying muscle protein glycosylation might help to explain the aetiology of muscle diseases commonly found in CDG and in other related disorders of N-glycosylation.

P.6

Modulation of the cyclin inhibitor p27 to ameliorate Merosin Deficient Congenital Muscular Dystrophy (MDC1A)

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MDC1A (or LAMA2 disease) is a severe disorder, mostly presenting within the first decade of life, and characterized by a progressive impairment of motor functions due to muscular dystrophy and dysmyelinating neuropathy.

MDC1A is due to recessive mutations in the *LAMA2* gene causing lack of laminin211 in Schwann cell and muscle basal lamina. Lack of laminin211 results in tissue degeneration resulting in relentless neuropathy and muscular dystrophy.

The disease is recapitulated in *Lama2* mouse models: the Lama2$^{dy2J/dy2J}$ mouse, characterized by a milder phenotype and the Lama2$^{dy3k/dy3k}$ mouse that presents a very severe phenotype. Our previous findings suggested that tissue degeneration in *Lama2* mice might be due to dysregulated, increased, levels of the cyclin inhibitor p27^{KIP1}. For this reason, we evaluated consequences of p27^{KIP1} down-regulation by genetic deletion of p27^{KIP1} in Lama2 mice by cross-breeding Lama2$^{dy2J/dy2J}$ into p27-null background in muscle lineage. The aim was to observe a rescue the muscular phenotype.

Our results show amelioration of motor performances (treadmill test) in double mutants (Lama2/p27KO) as compared to Lama$^{2dy2J/dy2J}$ mice. Pathology revealed a significant reduction of necrotic fibers and fibrosis in double mutant mice. Inflammatory cells were also reduced in double mutants as compared to Lama2$^{dy2J/dy2J}$ mice. Finally, we observed in double mutants a significant increase of regenerating fibers after damage and of (pax7 positive) satellite cells. Preliminary studies show an amelioration of survival when p27 is deleted in the very severe Lama2$^{dy3k/dy3k}$ genotype.

Our data suggest that muscle degeneration in LAMA2 disease could be, at least in part, consequence of dysregulated high level of the cyclin inhibitor p27^{KIP1}, and that p27^{KIP1} downregulation might constitute a valuable pharmacological target to ameliorate the disease.

P.7

Unveiling the HDAC4 functions in mediating the cross-talk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a fatal inherited muscle disease caused by the absence of dystrophin and characterized by muscle fragility, necrosis, and inflammation. As the disease progresses, myofibers are replaced by fibrotic and adipose tissues, leading to muscle weakness and eventually paralysis. The muscle-resident stem cells responsible for the fibro-fatty infiltration, while influencing the muscle stem cell (MuSC) differentiation, are the fibro-adipogenic progenitors (FAPs). One of the pharmacological approaches for the treatment of DMD, based on FAPs-targeting, is the pan-histone deacetylase inhibitor Givinostat, despite presenting several important limitations. Among the members of the histone deacetylase (HDAC) family, HDAC4 is crucial for the maintenance of skeletal muscle tissue upon different stimuli. Recently, we identified new protective functions of HDAC4 in the cytoplasm of dystrophic muscles. Skeletal muscle-specific deletion of HDAC4 in mdx mice (mdx;KO mice) worsens the pathological features of DMD, increasing muscle damage and compromising muscle regeneration, overall affecting muscle function.
My Ph.D. project is focused on further investigating HDAC4 functions in DMD. We found increased fibro-fatty infiltration in mdx;KO muscles. Although expressing similar levels of HDAC4, as expected, FAPs from mdx;KO mice showed increased pro-adipogenic potential, compared to FAPs from mdx littermates, accompanied by a higher ability to negatively influence mdx MuSC differentiation and survival. Such paracrine effects on mdx FAPs were mediated by HDAC4 from dystrophic skeletal muscle fibers, unveiling new HDAC4 functions in the crosstalk between the skeletal muscle cells and the muscle interstitial cells. Defining the secretome modulated by HDAC4 in DMD skeletal muscle will shed light on the mechanisms underpinning this disease and provide the experimental basis for new therapeutic approaches to counteract the pathological features of DMD.

P.8

Ablation of RAGE (receptor for advanced glycation end-products) translates into reduced tumorigenic and cachectic potential in LLC-tumor bearing mice

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Cachexia is a highly debilitating multifactorial syndrome affecting advanced cancer patients, and characterized by body weight loss and skeletal muscle atrophy [1]. We reported that genetic ablation of RAGE (receptor for advanced glycation end-products) in mice translated into delayed loss of muscle mass and strength, reduced tumor progression, and increased survival, suggesting a major role of RAGE in the cachectic syndrome [2]. To elucidate the role of RAGE in the tumor environment promoting cachexia, we generated LLC (Lewis lung carcinoma) clones stably transfected with expression vectors for RAGEΔcyto (non-transducing RAGE), full-length RAGE or empty vector. We found that LLC/RAGEΔcyto cells showed reduced migration in *in vitro* assay and almost inability to form colonies in soft agar. When injected s.c. in WT and *Ager*$^{-/-}$ (RAGE-KO) mice, LLC/RAGEΔcyto cells formed smaller masses than LLC/RAGE cells, with the smallest and biggest tumor masses found in *Ager*$^{-/-}$ mice injected with LLC/RAGEΔcyto cells and in WT mice injected with LLC/RAGE cells, respectively. Tumor masses from *Ager*$^{-/-}$ mice showed reduced amounts of myeloid-derived suppressor cells (MDSCs), which promote cancer progression and cachexia [3]. In line, i) the expression of the atrogene *Fbxo32* was found at the lowest levels in *tibialis anterior* muscles of *Ager*$^{-/-}$ mice injected with LLC clones, especially LLC/RAGEΔcyto; ii) LLC/RAGEΔcyto- conditioned medium was unable to induce C2C12 myotube atrophy *in vitro*. Our data suggest a critical role of RAGE expressed by LLC cells, in addition to RAGE expressed by the host animal, in sustaining tumor progression and cancer cachexia. Molecular targeting of RAGE emerges further as a promising approach to counteract muscle wasting in cancer patients.

1. Schmidt SF *et al.*, *Trends Cancer* 2018;4(12):849-60. 2. Chiappalupi S *et al. J Cachexia Sarcopenia Muscle* 2020;11(4):929-46. 3. Gabrilovich DI *et al.*, *Nat Rev Immunol* 2012;12(4):253-68.

P.10

Study of extracellular vesiscles (EVs) protein content released by dystrophic fibro-adipogenic progenitors (FAPs) treated with HDAC inhibitors (HDACi)

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Duchenne muscular dystrophy (DMD) is a rare neuromuscular disease caused by mutations in the dystrophin gene that lead to the absence of the homonymous protein which causes progressive muscle degeneration. There is no cure for DMD but only treatments that aim to improve the DMD patients' lifespan. Our group revealed the potential of epigenetic drugs, such as Trichostatin A which, by the inhibition of the Histone Deacetylases, improve muscle regeneration and counteract DMD progression. In the last few years, the potential of using Extracellular Vesicles (EVs) as cell-free treatment has emerged. We demonstrated that in dystrophic mice the communication between fibroadipogenic progenitors (FAPs), and muscle stem cells (MuSCs), is mediated by EVs and that the pharmacological treatment with TSA fine-tunes FAPs-derived EV microRNAs content, making them pro-regenerative. We observed that this genetic material can be transferred to MuSCs affecting their activation and differentiation and counteracting the DMD muscle degeneration. We deepened the pro- regenerative signature of EVs exploring the protein content of FAPs-derived EVs after TSA treatment. We revealed Integrin Beta 1 (Itgb1) as the most up-regulated protein in TSA-EV which works in a dimeric form with the a7 chain and ensures protection against muscle damage. It is known its role in the maintenance of MuSCs quiescence and in driving their proliferation and self-renewal during muscle regeneration. The link between Itgb1 and DMD is strong infact dystrophic MuSCs have unbalanced integrin dimer a7b1 with a decrease of b1 chain. Our results revealed that Itgb1 loaded in TSA-EVs is necessary to prevent the spurious MuSCs proliferation and differentiation, probably acting on their division. We evaluated that Itgb1 released by EVs to MuSCs contributes to the beneficial effect of TSA-EVs on the muscle's environment. In the future, we aim to deepen the mechanism by which the Itgb1 loaded in TSA-EVs acts on the MuSCs.

P.11

A new way of studying the muscular secretome in a prematurely aged model

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Mitochondrial dysfunction is a recognized contributor to the pathogenesis of sarcopenia and aging. Tezze et al. (2017) showed that in adult mice acute muscle-specific blocks of fusion, throw the deletion of mitochondrial protein optic atrophy 1 (OPA1), induce a profound muscle loss, metabolic changes, systemic inflammatory response, and precocious epithelial senescence that culminates with animal death. Mitochondrial dysfunction, caused by Opa1 ablation, triggers a dramatic increase in muscle fibroblast growth factor 21 (FGF21) expression and secretion from the skeletal muscle tissue. Impressively, the double muscle- specific ablation of Opa1/Fgf21 reverted completely the aging phenotype and prevented precocious death in mice. Importantly, the block of fission (Drp1 ko) or the concomitant block of fusion and fission (Drp1/Opa1 ko) showed higher FGF21 levels but not the precocious aging phenotype and the precocious death. These observations support the concept that FGF21 needs the presence of other factors that synergize, enhance, or counteract the trigger of the precocious senescence phenotype of OPA1 KO mice. For this reason, it is therefore very important to discover the other factors that may modulate FGF21 action in the target tissue and consequently promote its effect. For this purpose, we decided to generate Cre- recombinase inducible muscle-specific OPA1 knockout animals, expressing a mutant methionyl-tRNA synthetase that enables the labeling of muscular secretome with the non- canonical amino acid. This amino acid can be conjugated to different affinity tags and the labeled proteins can be identified by tandem mass spectrometry (MS/MS) both in the circulation and in the target tissue.

P.12

Pharmacological targeting of the receptor for advanced glycation end-products (RAGE) to counteract cancer cachexia

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Cachexia is a debilitating syndrome affecting more than 50% of advanced cancer patients and is directly responsible for about 20% of all cancer-associated deaths. Its major clinical feature is skeletal muscle atrophy leading to pronounced weight loss, reduced quality of life, and poor prognosis (1). Genetic ablation of RAGE (receptor for advanced glycation end-products) in tumor-bearing mice translates into reduced serum levels of cachexia-induced factors, delayed loss of muscle mass and strength, reduced tumor progression and increased survival. In cancer conditions, RAGE over-stimulated by high serum levels of its ligand, S100B, emerged as a determinant player in inducing cachexia, thus representing a promising target in therapeutic strategies (2). Here, we tested different pharmacological inhibitors (3,4) for their ability to reduce RAGE activity and counteract cancer cachexia. We treated Lewis lung carcinoma (LLC)-bearing C57Bl/6 mice with i.p. injections of the RAGE inhibitors, FPS-ZM1, Azeliragon (TTP488), RAP (RAGE antagonist peptide) and papaverine; and the S100B inhibitor, pentamidine, starting at the day of tumor appearance until 25 days post-tumor injection. We found that: i) Azeliragon and pentamidine showed toxic effects inducing death of mice before reaching the cachectic stage; ii) FPS-ZM1 and RAP did not counteract muscle wasting (in terms of weight loss and activation of the proteolytic systems); iii) papaverine reduced hallmarks of atrophy (body and muscle weight loss, protein degradation, and activation of the proteolytic systems) and prolonged survival. Thus, papaverine appears as the most efficacious pharmacological strategy targeting RAGE for anti-cachectic treatment, which is of great importance since an efficacious therapy for cachexia is still lacking.

1) Webster et al., *Front Physiol.* 2020; 2) Chiappalupi et al., *J Cachexia Sarcopenia Muscle.* 2020; 3) Singh & Agrawal, *Drug Dev Res.* 2022; 4) Saglam et al., *J Neuroimmunol.* 2021.

P.14

Characterization of collagen turnover pathways in primary muscle fibroblasts derived from the mdx mouse model of Duchenne Muscular Dystrophy

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In Duchenne muscular dystrophy (DMD), skeletal muscles undergo a progressive degeneration and fibrosis. We aimed at characterizing COL turnover pathways in cultured primary muscle fibroblasts obtained from the quadriceps of young and adult wild type (wt) and mdx mice (the most employed model for preclinical DMD research). Muscle fibroblasts were obtained from young (1 month) and adult (5 months) mdx mice. COL-I, COL-III and matrix metalloproteinases (MMP)-1 levels and MMP-2 activity were assessed, respectively, by Slot blot and SDS-zymography in cell culture supernatants. Gene expression for lysyl oxidase (LOX) and lysyl hydroxylase 2 (LH2b), and TIMP-1 and 2 were analyzed by real time PCR. COL-I and COL-III secreted by wt and mdx fibroblasts were not significantly affected by mdx in young and adult mice. LOX tended to be up-regulated whilst LH2b mRNA levels were significantly increased in both young and adult mdx compared to wt mice. MMP-1 and 2 were not affected, but TIMP-1 and 2 increased in fibroblasts of 5-month-old mdx mice. We show that increased collagen cross-linking and MMPs inhibition could contribute to muscular fibrosis. Interestingly, these two mechanisms could differently contribute to COL accumulation in the progression of DMD. In fact, in young mice fibrosis seems be dependent on increased collagen-crosslinking. Conversely, in adult mice, both increased collagen cross-linking as well as the inhibition of its degradation could act as major molecular mechanisms to furtherly favour the expansion and the persistence of the fibrotic remodeling of dystrophic muscles. These findings could offer new insight in understanding the mechanisms responsible for muscular fibrosis in DMD in order to find new therapeutic targets to effectively prevent its progression and potentially reverse its course.

P.15

Mitochondrial complex II is tightly related to augmented peripheral fatigue

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Mitochondria play an essential role during endurance performance. The intramuscular metabolic perturbations and the force-generating capacity are closely related to peripheral fatigue development. Studies have focused on the contraction cost during fatigue, ignoring the importance of the link between neuromuscular fatigue development and the mitochondrial complexes.
Purpose: To determine the mitochondrial contribution to neuromuscular fatigue.
Methods: 20 young healthy individuals (10M+10F) performed constant-load single-leg knee extension (85% of peak power output) to exhaustion (TTE). Fatigue was assessed with the interpolated twitch technique, via changes in supramaximal electrical stimulation pre- *vs.* post-exercise, during isometric maximal voluntary contractions (MVC) and at rest. A skeletal muscle biopsy was donated, and intrinsic mitochondrial respiration was assessed in permeabilized fibers (results showed as flux control ratio).
Results: Although the power-output normalized for the weight and the TTE were not different between groups (p=0.35), the peripheral fatigue (M: -63±12 *vs.* F: -31±21%, p=0.001) and the MVC (-40±14 *vs.* -26±13%, p=0.04) decreased significantly more in males. The voluntary activation (central fatigue index) was equal in the groups. Males showed lower net OXPHOS capacity (29.1±16.7 *vs.* 44.8±20.2, p=0.01) and higher Complex II contribution (CII; 0.58±0.1 *vs.* 0.49±0.1, p=0.01) compared to females. Moreover, a tight relationship was observed between the contribution of CII and the decrease in resting twitch (p=0.019) and MVC (p=0.013).
Conclusion: Females developed less peripheral fatigue and had higher net OXPHOS capacity, with a reduced involvement of CII compared to aged-matched males. This highlights the importance of CII in the development of peripheral muscle fatigue. Indeed, CII is not included in mitochondrial supercomplexes (formed to produce ATP efficiently), which might explain the differences in intramuscular metabolism.

P.17

Effects on muscle stem cells of exosomes derived from both untrained young and trained old subjects

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The sarcopenic process is dependent on many factors, as the activity of satellite cells(SCs), muscle stem cells that are activated (becoming myogenic precursor MPs) for muscle regeneration.Both fibers and MPs are important sites for the release of nanovesicles as exosomes (EXOs), suggesting that skeletal muscle plays a role of secretory organ. The EXOs can represent a communication way between close cells but even between distant cells playing an important role of cross-talking. Recent studies suggest that proper physical activity decelerates sarcopenia progression and exercise has great impact on the EXOs biogenesis. The aim of the present study is to elucidate the role of EXOs released by myoblasts and myotubes pre- and post-training and those present at the systemic level, as well as those released from MPs of untrained young people, on muscle regeneration in the elderly. To achieve this goal, healthy male untrained young (26±5 years;n=9) and elderly subjects (69±6 years;n=15) volunteers were recruited. The elderly were randomly assigned to different training (endurance or resistance) and control groups. Moreover, at the volunteer was done Vastus Lateralis skeletal muscle biopsies: before (Pre-) and after (Post-) training for elderly subjects, only one biopsy for young subjects. In detail: 1)MPs of young and elderly (pre- and post-) were isolated and characterized; 2)young and elderly EXOs were isolated and characterized by culture media, together to blood serum collected Pre- and Post- training; 3)microRNAs were analyzed in isolated EXOs; 4) proliferative rate and differentiation ability of sedentary elderly MPs, were evaluated after incubation with EXOs isolated from culture media of elderly Post-training and young subjects. Our data demonstrated that miRNAs transported by the EXOs, having a different expression both in young and elderly subjects trained. A positive effects on muscle regeneration seems to be related to EXOs released following endurance training.

P.18

MyomiR regulation by S1P signalling in skeletal muscle atrophy

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Skeletal muscle (SkM) atrophy arises as a physiological consequence of aging, for disuse, and as a consequence of pathologies, such as some neuromuscular disorders, or as a result of cancer (cachexia) (Larsson et al., 2019). Apart from the triggering events, which always implies multifactoriality, SkM atrophy is characterized by a decrease in SkM mass and function (*Furrer, et al., 2019, Sandri et al., 2016*). Although the signaling pathways leading to SkM atrophy have been extensively investigated, the key molecular processes driving myogenic cell degeneration requires further clarification. SkM specific miRNAs, so-called MyomiRs—have been proved to play important roles in maintaining SkM homeostasis (*Singh et al., 2020,* Cannataro et al., 2021; Casola et al 2021). Less is known on the regulation of MyomiR expression, expecially by bioactive lipids. Sphingolipids (SLs) represent a class of bioactive molecules capable of modulating the fate of many cell subtypes (*Cartier et al., 2019*), including SkM cells (reviewed in *Meacci et al., 2019, 2021*). In particular, sphingosine 1-phosphate (S1P) is crucial mediator in SkM cell biology due to its dual action: intercellular regulator as well as ligand of specific G-protein coupled S1P receptors. Some experimental evidence demonstrates a beneficial action of S1P in vivo (*Pantoja et al., 2013, Laurilia et al., 2022).* Recently, we showed the ability of S1P and ceramide to limit atrophy in mice bearing the C26 colon adenocarcinoma (*Pierucci et al., 2018 and 2021*), and a patent has been deposited for a pharmaceutical composition based on compounds able to affect the sphingolipid metabolism and S1P signalling (A_A) able to affect cell atrophy. Here, we investigated the involvement of A-A on the expression of several MyomiRs in C2C12 myotubes evaluating these effects together with those promoted by the glucocorticoid Dexamethasone, known to promote devastating effects on the musculoskeletal system.

P.20

Cytoplasmic HDAC4 delivery as a potential therapeutic approach for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a genetic disorder characterized by progressive muscle wasting and fatigue. Despite multiple research efforts and clinical trials, no effective treatment is available for DMD. Among pharmacological approaches, pan-Histone Deacetylase Inhibitors (HDACi) showed beneficial effects on muscle mass and function in preclinical studies. By epigenetically regulating the chromatin structure of muscle-resident cells and myofibers, HDACi promote muscle regeneration and oxidative metabolism while inhibiting fibrosis in mdx mice, a murine model of DMD. However, several limitations have been described for pan-HDACi. We have recently identified a new protective function for a specific isoform of HDAC, i.e. HDAC4, in dystrophic skeletal muscle. When localized in the cytoplasm, HDAC4 facilitates the membrane repair mechanism, a conserved mechanism triggered by membrane damage, thereby protecting myofibers and muscle stem cells from death. HDAC4 deletion increases myofiber degeneration and hampers muscle regeneration, overall decreasing mdx muscle function. These findings prompted us to investigate whether the cytoplasmic-restricted form of HDAC4 can be used as a genetic tool to ameliorate the pathological features in mdx mice. To this aim, we delivered a GFP-tagged HDAC4 L175A- expressing plasmid, and a GFP plasmid as control, in mdx muscles by electroporation. We found that delivering HDAC4 L175A improved mdx muscle architecture, by decreasing myofiber necrosis and enhancing muscle regeneration, overall leading to a functional amelioration. We also confirmed higher levels of proteins involved in the membrane repair response, pointing to the membrane repair mechanism as a mechanism underpinning the HDAC4-mediated rescue of the dystrophic phenotype. The fact that the over-expression of the cytoplasmic form of HDAC4 is beneficial for mdx muscles, provides a new perspective on nucleic acid-based therapeutic approaches to treat DMD.

P.21

Calcium-activated K^+-channels contribute to muscle damage in dystrophic mdx mice

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Inflammation causes a substantial part of muscle damage in Duchenne muscular dystrophy (DMD) and anti-inflammatory treatments are able to slow the course of the disease, in humans and mice.
In the disease, there is a complex cross-talk between cells of the immune system, in particular macrophages, and fibroblasts.
Pro-inflammatory macrophages (MΦs), required to clear debris of dead cells, are constitutively present in dystrophic muscles and contribute to muscle damage through the release of nitric oxide (NO). Alternatively-activated anti-inflammatory MΦs are less harmful, as they reduce NO release by pro-inflammatory MΦs and promote muscle regeneration. In the context of DMD, the main drawback in pushing MΦs towards an anti-inflammatory phenotype is that they release TGFβ1, promoting fibroblast proliferation and muscle fibrosis. Indeed, in DMD patients, abundance of anti-inflammatory MΦs correlates with enhanced fibrosis and poor prognosis.
Both MΦ phenotype and fibroblast proliferation critically depend on the activity of the Ca^{2+}-activated K^+ channel KCa3.1 (KCNN4), which therefore could represent a key factor in determining muscle damage in DMD. We therefore tested the effects of the selective channel blocker TRAM-34 on disease progression in mdx mice. Channel blockade favored the acquisition of an anti-inflammatory phenotype by tissue MΦs and reduced collagen deposition in muscles at all ages, while muscle damage was reduced only when treatment occurred during the early degenerative phase. No improvement in muscle performance was observed in vivo, likely because our mice had little functional deficits. The typical fragmented appearance of the endplate was not influenced by TRAM-34, suggesting that the process is not uniquely due to muscle fiber damage.
In summary, our data support the hypothesis that KCa3.1 channel is a key actor in several processes underlying muscle damage in DMD and can represent a useful target in the therapy of the disease.

P.22

A common calpain-3 variant explains a significant number of LGMD R1 calpain3-related cases in Eastern and Central Europe

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Introducion. A significant number of patients showing reduction of calpain-3 in the western blot and a phenotype corresponding to autosomal recessive calpainopathy have only a single pathogenic *CAPN3* variant identified. The investigated intronic *CAPN3* variant c.1746- 20C>G occurs in the Central and Eastern Europe with a frequency of >1% and currently there are conflicting interpretations on its pathogenicity.

Methods. We collected clinical data on 14 patients carrying the *CAPN3* c.1746- 20C>G variant in trans position with another *CAPN3* pathogenic/likely pathogenic variant. RT PCR and RNA-Seq were performed. The allelic frequency of the c.1746-20C>G variant was calculated from population studies in Russia, Latvia and Poland

Results. The patients compound heterozygous for the *CAPN3* c.1746-20C>G variant present a phenotype consistent with calpainopathy of mild/medium severity. We report five unaffected individuals homozygous for c.1746-20C>G and three affected, prevalently showing a late- onset, mild calapinopathy phenotype. Molecular studies showed that different splicing isoforms are produced in the muscle. We hypothesize that c.1746-20C>G is a hypomorphic variant with a specific reduction of RNA and protein expression and only individuals having a higher ratio of abnormal isoforms are affected. The variant is most frequent in the North/West regions of Russia and may originate from that area.

Conclusion. Reclassification of the *CAPN3* variant c.1746-20C>G from variant with a conflicting interpretation of pathogenicity to hypomorphic variant explains a large number of unidentified cases of LGMD R1 calpain3-related in Eastern and Central Europe. The very high frequency of the variant c.1746- 20C>G may result in the pseudo-dominant inheritance.

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P.23

Identification of natural products able to counteract the formation of advanced glycation end-products (AGEs) sustaining muscle atrophy

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AGEs are a heterogeneous group of non-enzymatically glycated molecules, especially proteins or lipids, usually resulting in fluorescent derivatives. Endogenous AGE formation naturally occurs during aging or in conditions of chronic hyperglycemia and oxidative stress, whereas intake of exogenous AGEs is linked to food consumption in Western diets (1). AGE accumulation in muscle, blood and skin is associated with reduced muscle mass (atrophy) and strength, and AGEs induce atrophy *per se* by interaction with the receptor for advanced glycation end-products (RAGE) (2). We tested thirty standardized dry extracts (from officinal plants and mushrooms), purified active compounds, and a food supplement (KYMASIN UP) for their ability in counteracting glyceraldehyde-derived fluorescent AGE formation by using a well-characterized albumin glycation assay kit (3), in which aminoguanidine solution was used as an anti-AGEs control. We found that: i) seven herbal extracts (*Withania somnifera, Equisetum arvense, Vaccinium macrocarpon, Euterpe oleracea, Camellia sinensis, Rhodiola rosea, Lepidium meyenii*), a mushroom (*Cordyceps sinensis*), three active compounds (lycopene, alpha lipoic acid, chlorophyll), and KYMASIN UP were able to inhibit AGE formation (fluorescence intensity) at 100 µg/ml in 24h; ii) *V. macrocarpon, Camellia sinensis* and chlorophyll showed a surprising ability in counteracting AGE formation in a dose-dependent manner starting from 3h; and iii) *Camellia sinensis* 500 µg/ml completely abolished AGE-derived fluorescence starting from 6h. Thus, we have identified natural products able to prevent AGE formation/accumulation to be tested in *in vitro* and *in vivo* experimental models of muscle atrophy for their potential in reducing the AGE-dependent detrimental effects in muscles.

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P.25

Combining vitamin E-functionalized CHOcolate with exercise to reduce the risK Of protein-energy malnutrition in pre-dementia AGEd people - The Choko-AGE study

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Objective: The aim of this study will be to verify the combined effect of supplementation with chocolate with a high content of polyphenols added to Vit-E and the regular practice of exercise on muscle mass in elderly subjects with cognitive decline, a population at high risk of malnutrition and fragility.
Materials and methods: Subjects recruited in the study, will be randomly assigned to one of the three groups for a duration of 6 months: A) control group that will maintain the diet with correct protein intake and will follow the 3 days/week high intensity exercise program; b) experimental group that will combine diet and exercise and will take 30 g/day of chocolate containing polyphenols and Vit-E; c) experimental group that will combine diet and exercise and will take chocolate without Vit-E.
Primary outcomes of this study will be muscle mass, muscle strength, and the ability to express force. A muscle biopsy will be taken and analyzed for: mitochondria respiration, muscle fiber morphology, single fiber force, Vit-E content, proteomics and cytokines expression.
Expected results: Considering the main outcome "muscle mass" in the target population the loss of muscle mass is assumed to be 1.0-1.5% (+/- 0.5%) in 6 months. In Group A, the expected increase is 2% (+-0.5%); in Groups B and C the expected increase is 4% (+-0.5%), and 1.5% (+-0.5%) respectively.
The greatest ameliorations are expected in the Group B due to the synergic effect of VIT-E, polyphenols and exercise. Vit-E with its cytoprotective and antioxidant effect, reduces ROS production supporting a more efficient mitochondrial metabolism. Polyphenols affect cortisol secretion, further reducing the catabolic action of this hormone, over secreted in this population. Exercise increases of muscle mass and strength.

P.26

Loss of Jab1 in muscle lineage causes a muscular dystrophy that resembles LAMA2 disease

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Recessive mutation in LAMA2 gene, encoding for the α2 chain of Laminin211, cause congenital muscular dystrophy with dysmyelination neuropathy (MDC1A or LAMA2 disease). Laminin-211 is a heterotrimer, composed by alpha2, beta1 and gamma1 chain, constituting the primary component of the Schwann cell and muscle basal lamina.
Previously, we demonstrated that Jab1 regulates levels of $p27^{Kip1}$ in Schwann cells, controlling cell cycle progression and differentiation. Jab1 deletion in Schwann cell causes a dysmyelinating peripheral neuropathy, characterized by an arrest in Schwann cell proliferation, axonal sorting defects and hypomyelination. All these pathologic features phenocopy the neuropathy of LAMA2 mutants.
To assess whether Jab1 plays also a role in the pathogenesis of LAMA2 muscular dystrophy, we generated mice with conditional inactivation of *Jab1* in the muscle cell lineage by the use of the MyoDi-cre transgene (Jab1-MscKO mice).
Jab1-MscKO mice showed reduced lifespan, failure to growth and significant motor function impairment. Muscles showed myopathic features such as reduced fiber diameter, degenerating and atrophic fibers, presence of necrotic fibers, inflammatory cells and fibrosis, similarly to what observed in Lama2 mutants.
Jab1-MscKO muscles showed increased of $p27^{Kip1}$ and $p21^{Cip1}$ expression, two cell cycle inhibitors, suggesting a possible impact on the mitogenic activity of quiescente satellite cells. Actually, the evaluation of satellite cell number showed a reduction of proliferating satellite cells, suggesting a defect of cell cycle progression.
Genetic deletion of $p27^{Kip1}$ in muscles of *Jab1-MscKO* mice ameliorates satellite cell cycling, slightly improved the muscle regeneration and some aspect of the muscular dystrophy like motor performances and muscle pathology.
Our results suggest that Jab1 and $p27^{Kip1}$ are involved in the pathogenesis of LAMA2 muscular dystrophy.

P.27

Effects of environmental pollutions exposure on human muscle stem cells

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Environmental pollution, together with predisposing genetic factors, play an important role in determining adverse effects on human health, both at short and long term. Recent literature reveals the role of environmental agents on human health with increased levels of oxidants that interfere with the regenerative capacity of stem cells in several tissues. Among other tissues, muscle tissue could also be affected. The aim of this study is to verify the interaction between atmospheric pollutants and adult muscle stem cells (MSCs), that, if altered in their metabolisms, may no longer be functional and capable of performing the normal task in the body by ensuring tissue turnover. To achieve this goal, healthy male subjects between 20 and 82 years; n=15 volunteers were recruited to undergo muscle biopsy to obtain the MSCs. In detail: 1)MSCs were isolated and characterized; 2)MSCs were exposed into Reaction Chamber to ozone (O_3) at 120Ppb, to evaluate the cell growth capacity and the differentiation process; 3)different sizes of polystyrene nanoparticles at different concentrations on MSCs proliferation effects were tested; 4)the effect of the smaller nanoparticles at two different concentrations on MSCs differentiation were assessed; 5)O_3 and nanoparticles were tested together on SCs proliferation; 6) superoxide anion levels in MSCs, after 24h of exposure to the various pollutants, were evaluated. In conclusion, we found that O_3 and smaller nanoparticles used at higher concentrations seem to negatively affect the proliferative capacity of both young and old people already in the first 24h of exposure. Moreover, MSCs incubated with nanoparticles at a lower concentration appear to have less inhibitory effect on differentiation while the incubation with O_3 induces a lower differentiation capacity than controls. In addition, in MSCs the levels of superoxide anion increase after 48h of exposure to ozone while with small nanoparticles the levels increase already after 24h.

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P.28

Vitamin D binding protein affects neuromuscular junction

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Vitamin D binding protein (VDBP) is a multifunctional serum glycoprotein belonging to the albumin gene family, whose main function is the transport of vitamin D (VD) in the bloodstream. Interestingly, high levels of VDBP have been reported in biological fluids of patients affected by pathologies associated with muscle wasting and weakness, suggesting that VDBP could contribute to this phenotype. Preliminary data from our lab demonstrate that VDBP has direct biological effects on skeletal muscle cells, in which it damages mitochondria and causes muscle atrophy. Since a growing body of evidence suggests that the neuromuscular junctions (NMJs) might be a critical mediator of muscle wasting, we investigated whether VDBP could affects NMJs. Our data show that VDBP impairs the formation and stability of agrin-induced AChR clusters in C2C12 myotubes, an in vitro model of NMJ. To assess if VDBP had a causative role in NMJ homeostasis deregulation also *in vivo*, we experimentally induced the expression of VDBP in VDBP knock-out (KO) mice by adeno- associated virus (AAV)-mediated gene expression. The reduction of muscle performances and mass in VDBP-injected mice was accompanied by a striking morphological damage in NMJ, seen as a reduction of AChR clusters and endplates surface area, and increased fragmentation. To determine whether VDBP contributes to cause NMJ dysfunction also in cancer cachexia-associated muscle wasting, we induced cancer cachexia in VDBP KO mice by inoculation of LLC cells, and we demonstrated that, while cachexia in wild-type mice is characterized by NMJ dismantling and denervation, NMJ architecture was mainly maintained in tumor-bearing VDBP KO mice. Although the primary function of VDBP is to transport VD metabolites, we showed that VDBP acts as a hormone per se, having a direct pro-atrophic activity on skeletal muscle and we can speculate that VDBP-induced deterioration of NMJs could represent a mechanism mediating DBP-induced muscle wasting *in vivo*.

P.29

Apelin resistance contributes to muscle loss during cancer cachexia in mice

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Cancer cachexia consists of dramatic body weight loss with rapid muscle depletion due to imbalanced protein homeostasis. We found that the mRNA levels of apelin decrease in muscles from cachectic hepatoma-bearing rats and three mouse models of cachexia.
Furthermore, apelin expression inversely correlates with MuRF1 in muscle biopsies from cancer patients. To shed light on the possible role of apelin in cachexia in vivo, we generated apelin 13 carrying all the last 13 amino acids of apelin in D isomers, ultimately extending plasma stability. Notably, apelin D-peptides alter cAMP-based
signaling in vitro as the L-peptides, supporting receptor binding. In vitro apelin 13 protects myotube diameter from dexamethasone-induced atrophy, restrains rates of degradation of long-lived proteins and MuRF1 expression, but fails to protect mice from atrophy. D-apelin 13 given intraperitoneally for 13 days in colon adenocarcinoma C26-bearing mice does not reduce catabolic pathways in muscles, as it does in vitro. Puzzlingly, the levels of circulating apelin seemingly deriving from cachexia-inducing tumors, increase in murine plasma during cachexia. Muscle electroporation of a plasmid expressing its receptor APJ, unlike apelin, preserves myofiber area from C26-induced atrophy, supporting apelin resistance in vivo.
Altogether, we believe that during cachexia apelin resistance occurs, contributing to muscle wasting and nullifying any possible peptide-based treatment.

P.30

The effects of vitamin D binding protein on skeletal muscle mitochondria

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Vitamin D binding protein (DBP) is a serum glycoprotein synthesized by hepatocytes, whose main function is vitamin D transport in the bloodstream. Proteomic analyses of samples from patients affected by pathologies characterized by cachexia, including several types of tumors, have shown an upregulation of DBP. Moreover, the upsurge of DBP in the two commonly used models of cancer cachexia, C26 colon carcinoma and Lewis Lung Carcinoma (LLC) tumor- bearing mice, led to the hypothesis that DBP may contribute to cancer cachexia-associated muscle loss.
In vitro, treatment of C2C12 myotubes with DBP induced atrophy without the involvement of the ubiquitin-proteasome system and likely through exacerbation of mitophagy. Indeed, DBP causes mitochondrial dysfunction, seen as a reduction of oxygen consumption rate, augmented reactive oxygen species (ROS) production, and increased mitochondrial fission. The intramuscular administration of DBP in mice lacking DBP (DBP KO) confirmed the detrimental effects on mitochondria homeostasis and led to a decrease in the muscular oxidative metabolism, as highlighted by the reduction of the fraction of oxidative fibers.
To verify if DBP has a role in the mitochondrial alterations usually observed in cachectic skeletal muscles, we induced cancer cachexia in WT and DBP KO mice by inoculation of LLC cells. The muscles of tumor-bearing DBP KO mice were partially protected from wasting compared to WT mice and displayed preservation of mitochondrial respiration.
These findings suggest that DBP is a mediator of mitochondrial damage and a crucial player in the establishment of muscle wasting.

P.31

MICAL2 modulation for hampering Rhabdomyosarcoma progression

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma occurring in both paediatric and young adults. RMS is characterized by malignant myoblasts unable to exit the cell cycle and form syncytial muscle. To date, the current multimodal treatments consisting of chemotherapy, surgery and/or radiation are ineffective for the 30% of RMS cases requiring higher doses of chemotherapy and irradiation leading to life threatening side effects. In order to prevent resistance and relapses, it is necessary to develop novel strategies by targeting crucial mediators involved in RMS pathogenesis. We recently provided evidence that calponin and lim domain containing 2 (MICAL2) protein affects muscle filament dynamics as well as the regeneration of muscle tissues[1]. Physiologically, MICAL2 stimulates F-actin depolymerization by promoting actin turnover and actin subunit modification. Furthermore, MICAL2 acts as a regulator of mesenchymal to epithelial transition and of myogenic differentiation. However, MICAL2 overexpression correlates to cancer progression and malignance in epithelial cancers[2]. Interestingly, MICAL2 RNA and protein are also increased in murine and human RMS cell lines. Thus, we aim to unravel the role of MICAL2 in proliferation, migration and hence the myogenic differentiation incapability in RMS cells.
Hence, MICAL2 loss of function studies have been performed on both murine and human RMS cells showing a negative effect on the RMS proliferation ability. Taken together these data demonstrate that modulations of MICAL2 have an impact on RMS cells, remarking the importance of its balance for cancer progression.

P.32

Effects of chronic nitrate supplementation on exercise training outcomes in old mice: a neuromuscular perspective

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Ageing is well known to cause sarcopenia, a loss of muscle mass and function, which in turn impairs exercise performance and neuromuscular stability, decreeing a reduced quality of life. Exercise training can counteract sarcopenia. Nitrate supplementation can boost physical performances and reduce time to exhaustion, ameliorating mitochondrial responses. The aim of the present study was to assess whether chronic nitrate supplementation enhances the impact of exercise training on sarcopenia.
22-months-old C57BL/6 male mice were assigned as follows: old controls (Old-CTRL, n=7); old supplemented with 1.5 mM inorganic $NaNO_3$ in drinking water (Old-N, n=7); old subjected to medium-high intensity exercise protocol (Old-EX, n=7); old underwent combined interventions (Old-Ex+N, n=7). The interventions lasted for 2 months. All mice were sacrificed at 24 months and skeletal muscles dissected for *ex-vivo* determinations.
Locomotor activity and mitochondrial respiration (e.g. OXPHOS coupling efficiency) were similarly enhanced in both Old-EX and Old-Ex+N groups. Furthermore, neuromuscular junction (NMJ) profile underwent positive remodelling in all the treated groups compared to the Old-CTRL, at different rates. Fibers innervation was even ameliorated, as suggested by a decreased postsynaptic fragmentation and a reduced number of NCAM-positive fibers in all experimental groups. NMJ morphology improvements were associated with the overexpression of neurotrophins (BDNF and NT-4) in the Old-EX group and with a higher anabolic response (increased P70S6K and S6 phosphorylation) in the Old-N group, in agreement with higher Gastrocnemius fibers cross-sectional area when compared to the Old- CTRL.
The present data indicate that both exercise and nitrate supplementation have the potential to counteract aged-induced neuromuscular deterioration. Combining exercise and nitrate supplementation did not show an additive effect compared to single treatment.

P.33

Overexpression of sAnk1.5 does not alter glucose homeostasis in a transgenic mouse model

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Genome-Wide Association Studies identified a novel SNP (rs508419) associated with T2D in the internal promoter of the *ANK1* gene, which drives the expression of sAnk1.5, a striated muscle-specific small ANK1 isoform. The rs508419-C risk allele was shown to result in higher transcriptional activity of the ANK1 internal promoter with respect to the rs508419-T allele. Accordingly, skeletal muscle biopsies of individuals homozygous for the rs508419-C risk allele presented higher levels of sAnk1.5 mRNA and protein compared to the alternative (C/T or T/T) genotypes. To investigate whether sAnk1.5 overexpression in skeletal muscle might represent a predisposing factor to T2D susceptibility, we generated a transgenic mouse model where the coding sequence of the murine sAnk1.5 is under the transcriptional control of the skeletal muscle-specific rat myosin light chain promoter, $Tg^{sAnk1.5}$. Skeletal muscles of $Tg^{sAnk1.5}$ mice expressed up to 52% as much sAnk1.5 protein as wild-type muscles, mirroring the difference observed between individuals with C/C or T/T genotype.
Basal glucose levels, glucose, and insulin tolerance were monitored over a period of twelve months in $Tg^{sAnk1.5}$ mice fed with a standard or with a high-fat diet. $Tg^{sAnk1.5}$ mice fed a standard diet showed no significant alterations in weight gain, calorie intake, glucose disposal, and insulin tolerance compared to control mice. In addition, $Tg^{sAnk1.5}$ mice fed with a high-fat diet, although presenting an increased calories intake, did not show evidence of altered glucose homeostasis. Altogether these results suggest that sAnk1.5 overexpression does not predispose, *per se*, to T2D susceptibility inmice.

P.35

Equisetum arvense standardized extract hinders age-related sarcopenia

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The age-related progressive loss of skeletal muscle mass and function, called sarcopenia, predisposes the elderly to frailty, fractures, loss of independence, morbidity, and mortality. Sarcopenia is an unresolved social burden due to growing healthcare costs associated with a super-aging society (1,2). Multiple factors contribute to muscle wasting during aging, including imbalanced protein turnover which results in an excessive myofibrillary protein breakdown (especially, type II myosin heavy chain, MyHC-II), changes in myofiber type composition, reduced regenerative capacity, increased reactive oxygen species, and low- grade chronic inflammation. Considering the growing interest in identifying natural active compounds useful in treating or preventing sarcopenia (3), we focused on the *Equisetum arvense* (EQ) medical plant containing anti-oxidants and anti-inflammatory metabolites. Using several *in vitro* models mimicking muscle atrophy, we found that a standardized extract of EQ counteracted myotube diameter reduction and MyHC-II degradation blunting the activity of different catabolic pathways depending on the applied atrophying stimulus. Here, we investigated the effects of EQ administration on pre-geriatric (21-month-old) C57BL/6 mice by melting the extract (500 mg/kg/die) directly into the drinking water for 3 months. We found that EQ consumption during aging: i) improved muscle performance; ii) preserved muscle mass and myofiber area; iii) maintained the expression of fast MyHC-II balancing the shift towards slow MyHC-I; iv) preserved the levels of genes involved in the maintenance of muscle mass and fatty acid metabolism. Mechanisms underlying the effects of EQ consumption were also investigated. Our data suggest that EQ might be proposed as a new non-pharmacological treatment to preserve muscle functionality in sarcopenia conditions.

1. Cruz-Jentoft & Sayer, *Lancet* 2019; 2. Wiedmer *et al*., *Ageing Res Rev*. 2021; 3. Qu Z *et al*., *J Nutr Biochem*. 2021.

P.37

Characterization of a novel *in vitro* model of sarcopenia

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Sarcopenia is the age-related progressive loss of skeletal muscle mass and functionality, which finally leads to poor physical performance and frailty. It results from the interaction of many mechanisms, such as the establishment of an imbalance between protein synthesis and breakdown in favor of the latter, and the impairment of the muscle regeneration process, which may be due to the increased number of senescent satellite cells (SCs) observed in sarcopenic patients. To date, there are different models of sarcopenia *in vivo*, which, involving the use of aged or genetically modified mice, are time- and resource-consuming.
However, there are not any *in vitro* models of sarcopenia able to mimic such a complex phenomenon. Here, we propose a novel *in vitro* model of sarcopenia, which could allow to better characterize the mechanisms underlying this condition and to investigate potential therapies.
To this aim, we reproduced an *in vitro* system of skeletal muscle from the accelerated aging phenotype obtained in mice by transplanting senescent preadipocytes (Xu M, et al. *Nature Medicine*, 2018). In detail, we induced a sarcopenic phenotype in C2C12 myotubes through incubation with the conditioned medium of doxorubicin-induced senescent 3T3-L1 preadipocytes (SCM), which causes myotube atrophy seen as both reduction of their diameter and induction of the ubiquitin-proteasome system. In addition, treatment with SCM significantly reduced both differentiation and fusion ability of C2C12 myoblasts, according to the reduced myogenic capability of sarcopenic SCs.
We developed a new *in vitro* tool that could accelerate the research in the field of sarcopenia, allowing not only a deeper understanding of mechanisms underlying this condition, but also the screening and the characterization of potential effects that molecules or compounds could have on aged or aging skeletal muscle *in vitro*, thus, reducing the time, costs, and the number of animals required in the study of sarcopenia.

P.38

Mass spectrometry-based characterization of proteome and acetylome landscape of murine myotubes treated with givinostat

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Protein lysine side chain acetylation is a post translational modification that regulates a wide range of cellular functions. Accumulating evidence shows that in skeletal muscle, acetylation is involved in gene expression, cell signaling, energy metabolism and muscle mass regulation. Histone deacetylases (HDACs) have thus emerged as key regulators of skeletal muscle in physiological and pathological conditions. Indeed, in a phase 2 clinical trial, HDACs inhibition with the pan-inhibitor givinostat reduced inflammation, necrosis, and fibrosis in muscle tissue of Duchenne Muscular Dystrophy patients. Moreover, a recently concluded phase 3 clinical trial has demonstrated that givinostat counters muscle deterioration leading to a reduced decline of muscle functions.
Since acetylation is a widespread modification that occurs both on histone and non-histone proteins, HDAC inhibition elicit global changes at the proteome level and on the acetylome. However, at present the overall impact of givinostat on muscle cell proteome and acetylome is still uncharacterized. Thus, we set up mass spectrometry-based global analyses of in vitro differentiated murine myotubes treated with givinostat. This approach allowed the quantification of changes induced by the inhibitor on both protein expression and acetylation levels. Preliminary results showed that givinostat equally induced the up and downregulation of protein expression. Upregulated proteins were mainly structural proteins or proteins involved in muscle contraction, while we observed the downregulation of proteins regulating the cell cycle and DNA replication. We found that acetylation changes occur rapidly after givinostat treatment. As expected, histone acetylation was increased but modulated acetyl- sites were identified also on many other types of proteins, including transcriptional regulators, metabolic enzymes and muscle structural proteins.

P.39

Duchenne's muscular dystrophy involves a defective transsulfuration pathway activity

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Duchenne muscular dystrophy (DMD) is the most frequent X chromosome-linked disease caused by mutations in the gene encoding for dystrophin, leading to progressive and unstoppable degeneration of skeletal muscle tissues. Despite recent advances in the understanding of the molecular processes involved in the pathogenesis of DMD, there is still no cure. In this study, we aim at investigating the potential involvement of the transsulfuration pathway (TSP), and its by-end product namely hydrogen sulfide (H_2S), in primary human myoblasts isolated from DMD donors and skeletal muscles of dystrophic (mdx) mice. In myoblasts of DMD donors, we demonstrate that the expression of key genes regulating the H_2S production and TSP activity, including cystathionine γ lyase (CSE), cystathionine beta-synthase (CBS), 3 mercaptopyruvate sulfurtransferase (3-MST), cysteine dioxygenase (CDO), cysteine sulfonic acid decarboxylase (CSAD), glutathione synthase (GS) and γ -glutamylcysteine synthetase (γ-GCS) is reduced. Starting from these findings, using Nuclear Magnetic Resonance (NMR) and quantitative Polymerase Chain Reaction (qPCR) we show that the levels of TSP-related metabolites such as methionine, glycine, glutathione, glutamate and taurine, as well as the expression levels of the aforementioned TSP related genes, are significantly reduced in skeletal muscles of mdx mice compared to healthy controls, at both an early (7 weeks) and overt (17 weeks) stage of the disease. Importantly, the treatment with sodium hydrosulfide (NaHS), a commonly used H_2S donor, fully recovers the impaired locomotor activity in both 7 and 17 old mdx mice. This is an effect attributable to the reduced expression of pro-inflammatory markers in skeletal muscle tissues. In conclusion, our study uncovers a defective TSP pathway activity in DMD and highlights the role of H_2S-donors for novel and safe adjuvant therapy to treat symptoms of DMD.

P.40

SRT2104, a new specific SIRT1 activator, promotes muscle recovery enhancing mitochondrial metabolism in DMD

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Duchenne Muscular Dystrophy (DMD) is an X-linked degenerative genetic disease caused by mutations of the DMD gene encoding dystrophin protein. While remarkable progress has been made in genetic approaches to restore dystrophin or its function, targeting secondary pathological mechanisms remains an important issue to address. SIRT1 belongs to a class of NAD+-dependent class III deacetylase that controls several key cellular processes. Different attempts have been done to increase SIRT1 activation in mdx mice, however, despite the initial promise, the current opinion reveals the need for developing better and more selective activators of sirtuins. Among these, the SRT2104 molecule is the most advanced in clinical studies. We have challenged the effects of SRT2104 administration in mdx mice and 12 weeks of SRT2104 supplementation with the diet improved muscle performance and muscle phenotype. SRT2104 administration also boosted muscle OxPhos capacity, as further confirmed by respiratory complexes' activity, supporting the idea of SRT2104 as a good metabolic enhancer.
To mechanistically characterize the SRT2104 mode of action, a series of molecular dynamics simulations have been performed on the available structures of SIRT1. They support the idea that a conformational selection mechanism is responsible of the activity of SRT2104, i.e., the open inactive conformation of the protein explores a more compact intermediate state that is stabilized by the drug, then converted into its active form.
We have further investigated SRT2104 action by exploring the proteomic profiles of muscles through quantitative mass spectrometry, revealing the SRT2104-dependent enhancement of the muscle contraction system. Moreover, we have also characterized the acetylated landscape of mdx muscle after SRT2104 administration pointing out some interesting deacetylated metabolic enzymes, therefore both approaches proved muscle improvements and specific metabolic effects of the drug.

P.41

Testing a new therapeutic approach for cancer cachexia using X-MET as a 3D skeletal muscle model

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Cancer cachexia is a complex metabolic syndrome characterized by body weight loss, atrophy, and a consequent decline in muscle force-generating capacity. The underlying causes and mechanisms of cachexia are not completely understood, and currently a therapeutic approach that can completely reverse this syndrome is still lacking (Baracos et al. 2018) . In this context, IL-6 has been found to be a cancer disease prognostic marker, and it is also increased in patients with cancer induced cachexia. The aim of this study is to verify whether IL-6 transignalling can contribute to the induction of the cachectic phenotype using X-MET as a 3D skeletal muscle model (Carosio et al. 2013). The X-MET is an engineered vascularized skeletal muscle construct able to recapitulate the complex morphological properties, architecture and function of skeletal muscle with the advantage of enabling the monitoring of muscle contraction properties over time. To investigate the specific contribution of the alternative signalling of IL-6 to cachexia-associated alterations, a selective inhibition of IL-6 transignalling has been performed. Overall, our results highlighted the prominence of IL-6 transignalling in contributing to pathological changes associated with cancer cachexia and demonstrates how the X-MET muscle construct can represent a useful model to study skeletal muscle alterations.

P.42

STAT3-mediated autophagy drives muscle regeneration

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Age-related reduced regenerative potential and muscle wasting are associated with the decline of muscle stem cells (MuSCs) number and function, due to intrinsic and systemic alterations. STAT3 holds a role in regulating MuSCs expansion and lineage progression. Likewise, the autophagic process regulates MuSCs activation toward efficient muscle regeneration. The established role of autophagy in maintaining muscle mass and tissue homeostasis together with the emerging role of STAT3 in regulating the autophagic process inspired this work. Our hypothesis is that STAT3 regulates muscle regeneration by affecting autophagy thereby restoring the bioenergetic demand of the old myogenic niche toward efficient skeletal muscle repair.
We show that STAT3 inhibitor (STAT3i) treatment induces the autophagic process upon muscle regeneration both *in vitro* and *in vivo*. STAT3-mediated autophagy during muscle regeneration is associated with eIF2???? phosphorylation that is conceivably achieved by PKR that is no longer sequestered by STAT3. Intriguingly, STAT3i treatment is able to resume the autophagic process in old MuSCs, otherwise characterized by low levels of autophagy, pushing toward muscle regeneration. Our findings highlight the key role of the STAT3- dependent autophagy in driving muscle regeneration, revealing potential biological targets in MuSCs that may restart an efficient regenerative response in aged mice.

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