12TH ANNUAL METNET INTERNATIONAL MEETING

FROM METABOLIC CROSSTALK TO THERAPEUTIC INNOVATION

BARCELONA, SPAIN

OCTOBER 29-30, 2026

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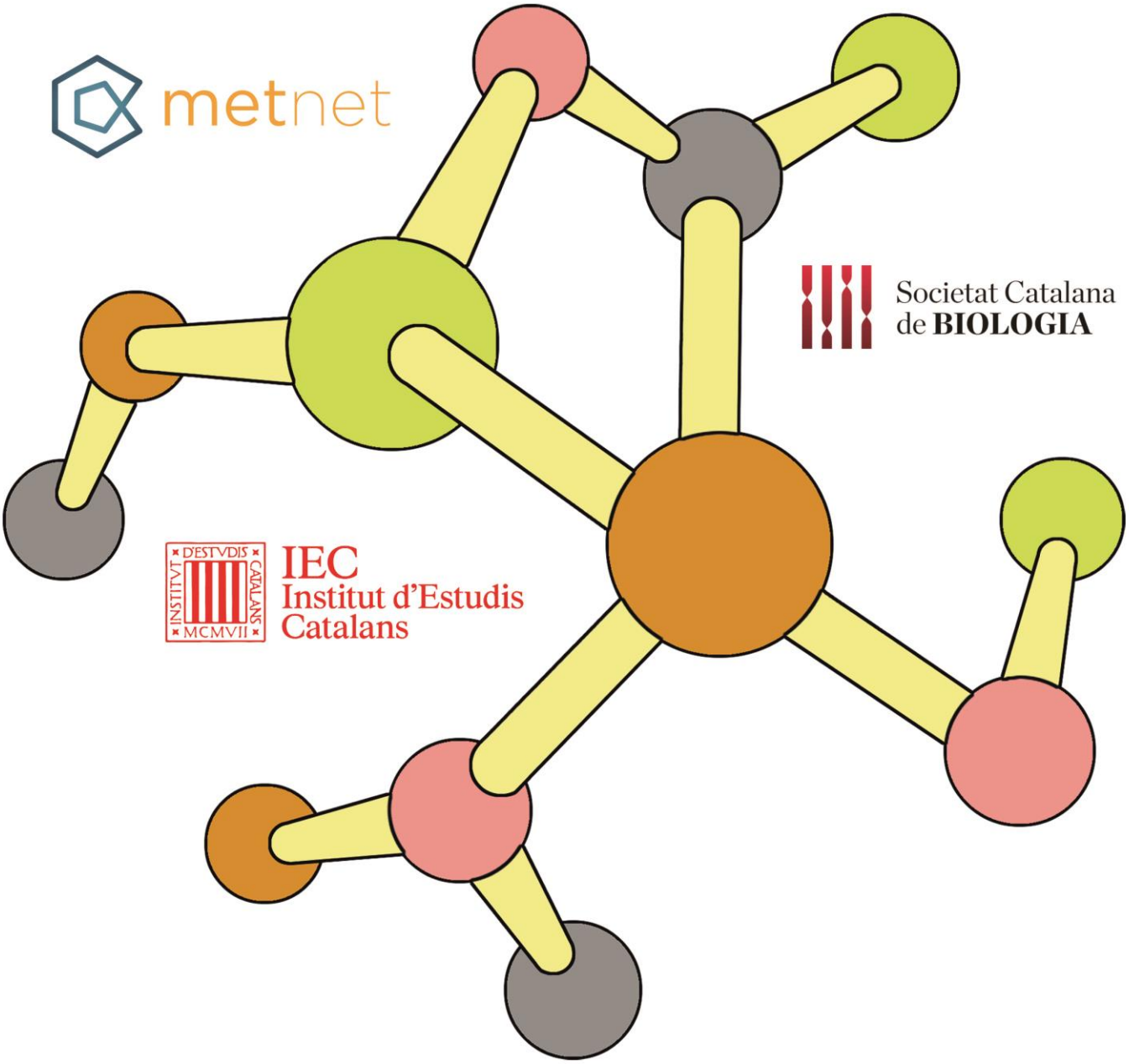
Societat Catalana de BIOLOGIA

IEC Institut d'Estudis Catalans



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Discover the latest advances in obesity, diabetes and metabolic disease research

***Institut d´Estudis Catalans*, Barcelona | 29-30 October 2026**

Scientific Highlights

***Session 1**:* Adipose Tissue-Islet Communication: Mechanisms and Therapeutic Insights
***Session 2**:* Adipose Tissue Crosstalk Across the Lifespan and Sexual Dimorphism
***Session 3**:* Advanced Methodologies in Metabolic Research
***Session 4**:* Novel Therapies for Metabolic and Rare Diseases
***Session 5**:* Fuel Flux and Organ Dialogues: Brown Adipose Tissue, Liver and Pancreas
***Session 6**:* Lifestyle, Environment and Metabolic Health

Confirmed Speakers

• Susanne Mandrup • Fredrik Karpe • Fawaz Alzaid • Kathrin Landgraf • Jorge Ivan Álvarez • Cristina Fornaguera • Ángela Valverde • Marta Giralt • Javier Botella • David Sebastián • Estela Lorza Gil • Lorenzo Pasquali • Aleksandar Matic

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Selected abstracts → Short Talks
Accepted posters → Flash Poster Presentations
Coffee breaks, lunch, and networking activities included with registration

DEADLINES:

ABSTRACT DEADLINE | 21 October 2026

REGISTRATION DEADLINE | 23 October 2026

Scan now and check out our panel of speakers and full programme, register and submit your abstracts!

Organizing Committee: Rebeca Fernández (IDIBAPS, CIBERDEM), Joel Montané (Blanquerna, URL), Marijana Todorčević (UB, CIBEROBN)

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DAY 1 | 29th October 2026

8:15-9:00 **Registration**
9:00-9:15 **Welcome**

Session 1| Adipose tissue-islet communication: mechanisms and therapeutic insights | *Chair: Rebeca Fernández*

9:15 - 9:45 **Estela Lorza Gil**, Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at University of Tübingen (IDM), Germany.
Tittle: Molecular crosstalk between pancreatic adipose tissue and islets

9:45- 10:15 **Fawaz Alzaid**, INSERM Institut Necker, Paris, France-Dasman Diabetes Institute, Kuwait City, Kuwait.
Tittle: Energetic and transcriptional control of inflammation in diabetes

10:15-10:45 **Short talks selected from abstracts**

10:45–11:15 **Coffee Break + Sponsor stands**

The EMBO Keynote Lecture

11:15–12:00 - EMBO Keynote speaker: **Susanne Mandrup**, University of Southern Denmark, Denmark
Tittle: Adipose tissue plasticity at single cell resolution

Session 2 | Adipose tissue crosstalk across the lifespan and sexual dimorphism | *Chair: Marijana Todorčević*

12:00-12:30 **Fredrik Karpe**, University of Oxford, United Kingdom
Tittle: Metabolic fuel trafficking as signals for tissue metabolic homeostasis

12:30-13:00 **Kathrin Landgraf**, University of Leipzig, Germany
Tittle: Drivers of adipose tissue dysfunction during the development of obesity in children

13:00-13:30 ***Short talks selected from abstracts***

13:30–15:00 **Finger food lunch + Sponsor stands**

14:00: V**isit to the Royal Academy of Medicine of Catalonia** (Optional; prior registration required)

Session 3 | Advanced methodologies in metabolic research | *Chair: Chair: Joel Montané*

15:00–16:20 - Industry & Technology Session
Advanced Imaging Solutions: Spatial Biology and Multiplexing (Leica microsystems)
AI Tools in Biomedical Research: From prediction to prevention: lessons from 20 years of digital phenotyping and AI in translational medicine (Aleksandar Matic, Koa Health)
Omics & Bioinformatics: Inflammation-induced chromatin remodeling reveals beta cell vulnerabilities (Lorenzo Pasquali, Universitat Pompeu Fabra, Spain)
3D Biology 2.0: Innovación, aplicaciones y casos prácticos con Seahorse, xCELLigence eSight y Cytation (Agilent)

16:20-17:20 **Short and flash talks selected from abstracts**

17:20-19:30 **Wine & cheese networking reception**

DAY 2 | 30th October 2026

Session 4 | Novel therapies for metabolic and rare diseases | *Chair: Marijana Todorčević*

09:00–09:30 **Jorge Ivan Álvarez,** University of Pennsylvania, USA
Tittle: Neurovascular Metabolic Susceptibility in a Human Genetic Model of Psychiatric Disease

9:30–10:00 **Cristina Fornaguera,** IQS, Universitat Ramon Llull**,** Spain
Tittle: Engineering RNA Nanomedicines for Rare Vascular-Metabolic Diseases

10:00–11:00 **Short and flash talks selected from abstracts**

11:00–11:30 **Coffee & refreshments** + **Workshops** + **Sponsor stands**

Session 5| Fuel flux and organ dialogues: brown adipose tissue, liver and pancreas | *Chair: Rebeca Fernandez*

11:30-12:00 **Ángela Martinez-Valverde,** Instituto de Investigaciones Biomedicas Sols-Morreale (Centro Mixto CSIC-UAM) and CIBERDEM (ISCIII), Madrid, Spain.
Title: Inter-organ crosstalk in metabolic reprogramming induced by incretin multiagonism to tackle obesity and co-morbidities

12:00-12:30 **Marta Giralt Oms**, Universitat de Barcelona, Spain. *Novogene Technology Olink*
Tittle: Brown adipose tissue: a source of metabolic regulators.

12:30: 13:30 **Short and flash talks selected from abstracts**

13:30–15:00 **Finger food lunch + Sponsor stands**

14:00: **Visit to the Royal Academy of Medicine of Catalonia** (Optional; prior registration required)

Session 6| Lifestyle, environment and metabolic health | *Chair: Joel Montané*

15:00–15:30 **Javier Botella**, University of Lausanne, Switzerland
Tittle: Mitochondrial regulation in human skeletal muscle: the role of exercise intensity

15:30–16:00 **David Sebastián,** Universitat de Barcelona, Spain
Tittle: Temporal mapping of mitochondrial fitness in obesity-related metabolic disease

16:00-17:00 **Short and flash talks selected from abstracts**

17:15-17:30 **Concluding remarks and prizes**

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Invited Speakers:

Angela Valverde
Instituto de Investigaciones Biomédicas Sols-Morreale-CIBERDEM
Spain

Estela Lorza Gil
Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich
University of Tübingen
Germany

Fawaz Alzaid
INSERM Institut Necker
France

Susanne Mandrup,
University of Southern Denmark

Fredrik Karpe
University of Oxford
United Kingdom

Kathrin Landgraf,
University of Leipzig
Germany

Jorge Ivan Álvarez
University of Pennsylvania
USA

Cristina Fornaguera
IQS, Universitat Ramon Llull
Spain

Marta Giralt
Universitat de Barcelona
Spain

Javier Botella
University of Lausanne
Switzerland

David Sebastián
Universitat de Barcelona
Spain

Aleksandar Matić
Koa Health

Lorenzo Pasquali
Universitat Pompeu Fabra
Spain

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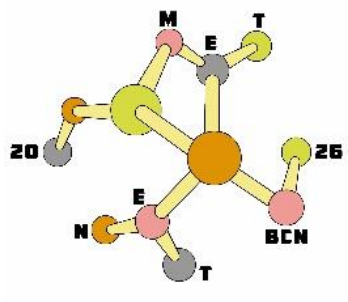

The EMBO Keynote Lecture
Prof. Susanne Mandrup, University of Southern Denmark, Denmark

"Adipose tissue plasticity at single cell resolution"

Prof. **Susanne Mandrup** is Professor at the Department of Biochemistry and Molecular Biology at the University of Southern Denmark, where she directs the Center of Excellence in **Functional Genomics and Tissue Plasticity (ATLAS)** and the Center for Adipocyte Signaling (ADIPOSIGN). She obtained her PhD in Biochemistry in 1992 and trained as a postdoctoral fellow with Prof. M. Daniel Lane at Johns Hopkins University before returning to Odense, where she became full Professor in 2008. Prof. Mandrup's group has made major contributions to the genome-wide understanding of the **crosstalk between transcriptional regulation and metabolism** in **adipocyte differentiation and function**. Combining **sequencing-based functional genomics** with detailed molecular analyses in mouse models, human biopsies and cell cultures, her lab now studies the **in vivo plasticity of adipose tissue cells**. She is an elected member of **EMBO**, Academia Europaea and the Royal Danish Academy of Sciences and Letters.

DEADLINES:

REGISTRATION DEADLINE| (without) | October 23rd 2026

ABSTRACT DEADLINE | 2nd October 2026

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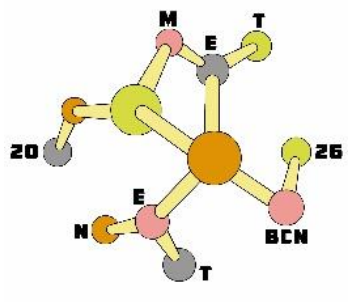

Dr. **Estela Lorza Gil** is a scientist and Group Leader of the Islet Facility at Helmholtz Munich and the University Hospital Tübingen. Her research focuses on pancreatic islet biology and the **interactions between adipose tissue and insulin-producing β-cells**, with the aim of understanding the mechanisms that drive type 2 diabetes. Using human **tissue biobanks** and innovative **organoid models**, she investigates how pancreatic fat influences islet function and metabolic disease.

Dr. **Fawaz Alzaid** is an INSERM researcher and co-group leader of the IMMEDIAB team (Immunity and Metabolism of Diabetes) at Institut Necker. His research focuses on metabolism and the transcriptional regulation of **innate immunity in diabetes**, its complications and comorbidities. He received his PhD from King's College London in 2012, trained as a postdoctoral researcher in London and Paris and joined INSERM in 2017. He is also a senior scientist at the Dasman Diabetes Institute in Kuwait.

Dr. **Fredrik Karpe** is Professor of Metabolic Medicine at the University of Oxford and leads the Metabolic research Group (MRG). His research focuses on **human adipose tissue function, body fat distribution and the metabolic consequences of obesity**. Using genetic, cellular and whole-body approaches, he investigates how adipose tissue influences the risk of type 2 diabetes and cardiovascular disease, with particular interest in the metabolic effects of different fat depots. He also established the Oxford Biobank, a unique resource for human metabolic research.

DEADLINES:

REGISTRATION DEADLINE| (without) | October 23rd 2026

ABSTRACT DEADLINE | 1st October 2026

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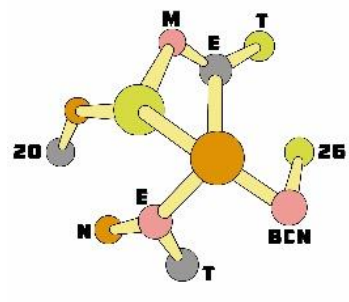

Dr. Kathrin Landgraf is a senior scientist and principal investigator at the **University of Leipzig** and the **Center for Pediatric Research** in Germany. Her research focuses on the mechanisms driving **childhood obesity**, adipose tissue dysfunction, and the early development of metabolic diseases. Her work combines translational and experimental approaches to identify novel regulators of obesity and cardiometabolic risk in children. Dr. Landgraf has received several prestigious awards, recognizing her significant contributions to pediatric metabolic research.

Dr. Lorenzo Pasquali is a principal investigator at the **Department of Medicine and Life Sciences of the Universitat Pompeu Fabra (UPF), Barcelona, Spain**, where he leads the **Endocrine Regulatory Genomics Laboratory**. His research combines genomics, molecular biology, and computational approaches to understand how genetic and epigenetic mechanisms regulate pancreatic β-cell function and contribute to diabetes. His work has provided important insights into the regulatory networks underlying both type 1 and type 2 diabetes.

Dr. Aleksandar Matić is Senior Vice President of Research & Development at Koa Health, where he leads the development of innovative AI- and data-driven approaches to understand and improve human health. Trained as a scientist with expertise spanning digital health, artificial intelligence, and computational modeling, his work focuses on uncovering complex interactions between biological, behavioral, and environmental factors that shape health outcomes, making him a leading voice in the application of data science to precision medicine and systems health.

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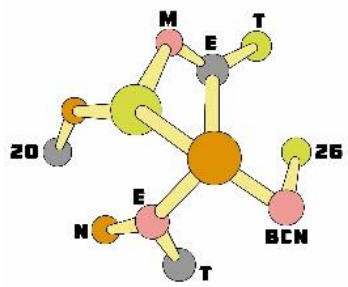

Dr. Cristina Fornaguera is a professor at the **Department of Bioengineering of IQS School of Engineering (Universitat Ramon Llull**), Barcelona, Spain, where she leads the Nano Therapies Lab (NanoTher Lab). Her research focuses on the design and validation of polymeric nanomedicines for the delivery of nucleic acids and other therapeutic agents across physiological barriers. Her work includes the development of mRNA vaccine platforms with improved targeting of antigen-presenting cells, with applications in infectious diseases and cancer immunotherapy.

Dr. Jorge Ivan Álvarez is an associate professor in the **Department of Pathobiology at the University of Pennsylvania**, Philadelphia, USA..His research investigates how the brain's barriers, the blood-brain and blood-meningeal barriers, regulate interactions between the immune and neurovascular systems. His work aims to uncover the mechanisms that drive neuroinflammatory diseases such as multiple sclerosis, as well as neuropsychiatric disorders, including those associated with 22q11.2 deletion syndrome

Dr. Javier Botella is a researcher at the **University of Lausanne**, Switzerland, specializing in exercise physiology and skeletal muscle metabolism. His research investigates how different types and intensities of exercise remodel mitochondria in human skeletal muscle, including the molecular stress responses, transcriptional regulation, and autophagy processes that underpin training adaptations. His work aims to inform more effective, individualized exercise prescription for health

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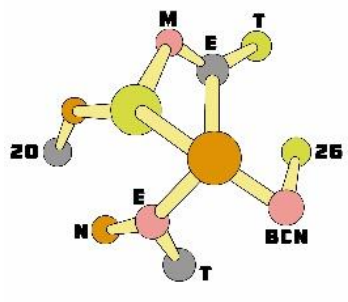

Dr. **Angela Martinez-Valverde** is a Senior Scientist at the Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM-CSIC/UAM) and a member of CIBERDEM, Spain. Her work focuses on the **molecular basis of obesity and its comorbidities**, exploring innovative therapeutic approaches for metabolic diseases, including novel treatments for obesity, diabetes, and **liver disease**. Her contributions have made her an internationally recognized researcher in the field of metabolic health.

Dr. **Marta Giralt**, is Professor of Biochemistry and Molecular Biomedicine at the University of Barcelona, Principal Investigator at the Institute of Biomedicine of the University of Barcelona (IBUB), and a member of CIBEROBN. Her research focuses on the **molecular regulation of adipose tissue function**, energy metabolism, obesity, and related metabolic diseases. An internationally recognized expert in metabolic biology, she has made major contributions to our understanding of brown adipose tissue and metabolic regulation.

Dr. **David Sebastián**, is an Associate Professor at the University of Barcelona, and group leader at the Institute of Biomedicine of the University of Barcelona (IBUB). His research focuses on **mitochondrial biology**, autophagy, and cellular metabolism, with particular emphasis on understanding how mitochondrial dysfunction contributes to obesity, diabetes, aging, and other metabolic diseases. His laboratory combines cellular and animal models to investigate the molecular pathways linking mitochondrial function to health and disease.

DEADLINES:

REGISTRATION DEADLINE| (without) | October 23rd 2026

ABSTRACT DEADLINE | 1st October 2026

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SPATIAL TRANSCRIPTOMICS REVEALS COORDINATED, CROSS-CELL-TYPE ADIPOSE TISSUE REMODELING IN A MOUSE MODEL OF CHILDHOOD OBESITY

Flavio Palmieri [1,2,*], Marcela Parra-Vargas [3,4], Daniela Grases[5], Mario Cuesta-Argos[6], Ana Bataller[4], Marta Ramon-Krauel[4], Carles Lerin[4], Eduard Porta[5], Andrea Soria-Gondek[6] , Josep C. Jiménez Chillarón [3,4]

1 Department of Systems Engineering, Automation and Industrial Computing (ESAII), Barcelona East School of Engineering (EEBE), Universitat Politècnica de Catalunya - BarcelonaTech (UPC)

2 B2SLab - Bioinformatics and Biomedical Signals Laboratory, Institut de Recerca i Innovació en Salut (IRIS), Universitat Politècnica de Catalunya - BarcelonaTech (UPC)

3 Biophysics Unit, Department of Physiological Sciences School of Medicine, University of Barcelona, L'Hospitalet de Llobregat, Spain

4 Institut de Recerca Sant Joan de Déu - Barcelona Children's Hospital, Esplugues del Llobregat, Spain

5. Josep Carreras Leukaemia Research Institute, Barcelona, Spain

6. Pediatric Surgery Service, Germans Trias i Pujol Hospital, Barcelona, Spain

* Corresponding author flavio.palmieri@upc.edu

Adipose tissue remodeling in obesity involves coordinated changes across multiple cell lineages, but its spatial organization remains poorly resolved at single-spot resolution, particularly in childhood-onset obesity. We applied spatial transcriptomics (10x Visium) to inguinal adipose tissue from a mouse model of childhood obesity induced by litter size reduction (n=3 control, n=4 obese). Cell-type annotation was validated across multiple independent computational strategies and a curated single-cell reference.

Unsupervised spatial niche detection revealed a ~7-fold expansion of an adipose stem/progenitor cell (ASPC)- and macrophage-enriched niche in obese tissue (PERMANOVA p=0.034), at the expense of a mixed adipocyte/vascular niche. This compositional shift was corroborated by three independent analyses: (1) pseudobulk differential expression showed inflammatory/antigen-presentation activation alongside loss of resident macrophage and mast cell markers; (2) cell-type-resolved differential expression revealed coordinated MHC-II upregulation extending beyond immune cells to adipocytes, endothelial cells and pericytes, together with widespread downregulation of haptoglobin, angiotensinogen, complement and adipsin; (3) spatial statistics showed increased ASPC/macrophage proximity to perivascular cells, alongside reduced spatial clustering of ASPC signal.

Notably, mature adipocytes specifically lost cell-cycle gene expression in obese tissue, suggesting adipose expansion proceeds through hypertrophic rather than hyperplastic mechanisms in this niche.

Together, these results support a model of obesity-driven, perivascularly-organized stromal-immune recruitment as a coordinated, cross-cell-type tissue response, generating testable hypotheses for future validation.

DECODING THE CHAMELEONIC IDENTITY OF THE SUBSET OF NEURONS CO-EXPRESSING ANTAGONISTIC NEUROPEPTIDES POMC AND AGRP

Mariana Ferreira[1], Maria Milà-Guasch[1], Clara Bullich-Vilarrubias[1], Carma Delicado[1], Pau García-Ramón[1], Júlia Fos-Domènech[1], Alicia G. Gómez-Valadés[1], Luís Osorio[1], Francisco Díaz-Castro[1], Roberta Haddad-Tóvolli[1,*] and Marc Claret[1, 2, 3,*]

[1] Neuronal Control of Metabolism (NeuCoMe) Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain.
[2] CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain.
[3] School of Medicine, Universitat de Barcelona, 08036 Barcelona, Spain.
*Correspondence: haddad@recerca.clinic.cat; mclaret@recerca.clinic.cat

The hypothalamic arcuate nucleus regulates energy homeostasis through distinct neuronal populations, including anorexigenic POMC and orexigenic AgRP, traditionally known for their coordinated yet opposing roles. Recent evidence challenges this view, suggesting POMC-neuron heterogeneity. An in-house POMC-neuron-specific RNA sequencing analysis revealed five POMC neuronal subpopulations, including one cluster co-expressing both POMC and AgRP genes (POMC:AgRP).
To explore its role in energy balance, we used a novel viral strategy, tTARGIT, to specifically ablate POMC:AgRP neurons in adult male and female mice, and assessed feeding and glucose metabolism.
POMC:AgRP neuron ablation in males reduced ad libitum food intake. This phenotype was accompanied by glucose intolerance, despite preserved insulin and glucagon sensitivity and gluconeogenesis. Also, elevated basal insulin levels and blunted insulin response to exogenous glucose were observed. Glucagon secretion was similarly unresponsive to a glucose challenge, suggesting POMC:AgRP participation in a brain-to-pancreas pathway coordinating endocrine responses to glucose. In contrast, females showed minimal or no metabolic phenotype, suggesting sex-dependent compensation or resilience, although larger cohorts are required.
Our results provide evidence for a functionally relevant neuronal population expressing POMC and AgRP neuropeptides, reinforcing POMC neuron heterogeneity. Rather than fitting in the classical POMC/AgRP dichotomy, POMC:AgRP neurons may exert context-dependent effects on feeding and glucose metabolism. Their "chameleonic" role may reveal an ability to integrate distinct metabolic signals and coordinate central and peripheral responses, possibly revealing neural mechanisms to fine-tune peripheral homeostasis.

BLOOD PRESSURE REDUCTION AND SEX-DEPENDENT METABOLIC RESPONSES TO AN AGRI-FOOD BY-PRODUCT-DERIVED PROTEIN HYDROLYSATE: ASSOCIATIONS WITH GUT MICROBIOTA

Fernando Aniento-Marcote[1]*, Diego Alavarsa-Cascales[1], Francisca I. Bravo[1], Cristina Torres-Fuentes[1]

[1] Biochemistry and Biotechnology Department, Universitat Rovira I Virgili, Marcel.li Domingo 1, 43007, Tarragona, Spain

*Corresponding author: fernando.aniento@urv.cat

Gut microbiota (GM) modulation and bioactive peptides from protein hydrolysates (PHs) have emerged as promising strategies against metabolic syndrome (MetS). Besides targeting MetS-related pathways, bioactive peptides may influence GM by serving as nitrogen sources and exerting antimicrobial effects. Their production from agri-food by-products supports circular economy approaches.

We previously developed a plant agri-food by-product-derived PH with antihypertensive effects in spontaneously hypertensive rats (SHR), which disappeared following antibiotic-induced GM depletion, suggesting GM involvement.

Here, we evaluated its antihypertensive and metabolic effects after prolonged administration in a diet-induced MetS model and assessed sex-dependent responses. Male and female Wistar rats received a cafeteria (CAF) diet for 12 weeks and were supplemented with vehicle or PH (55 mg/kg/day) during the final 4 weeks. Food intake, body weight, and systolic blood pressure (SBP) were monitored weekly; oral lipid and glucose tolerance tests (OLTT, OGTT) were performed during weeks 2 and 3, respectively.

CAF feeding increased SBP to hypertensive levels, whereas PH significantly reduced SBP in both sexes. Metabolic responses were sex-dependent: in females, PH reduced adiposity index and plasma leptin and tended to lower postprandial triglycerides, whereas in males it improved glucose tolerance. Both alpha and beta diversity differed between CAF-VH and CAF-PH females, indicating PH-induced changes in GM diversity and composition. Among PH-treated rats, males showed higher relative *Escherichia* abundance than females, which positively correlated with plasma leptin and OLTT AUC.

These findings support PH as a sustainable strategy for improving blood pressure and metabolic health in MetS, highlighting sex-dependent responses and GM involvement.

Acknowledgements

This work was funded by PID2023-151563OB-I00 project from MCIN/AEI/10.13039/501100011033 I00 and associated predoctoral FPI fellowship (PRE2023 151563OB I00).

Neonatal overfeeding programs sex-specific WAT–liver lipid fluxes and metabolic outcomes

Ivonne Palacios-Marin[1,2,3] Cristina Barosa[4], Francesc Ribas-Aulinas[2], Sara van Beurden[1], Julia Fos-Doménech[1], Ana Lopez-Bataller[2], John G Jones[4], Josep C Jimenez-Chillarón[2,5], Laura Herrero[1,3]

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Introduction: Childhood obesity is a major public health challenge because it mostly persists into adulthood, amplifying the lifetime risk of metabolic disease. The white adipose tissue (WAT)–liver axis plays a central role in metabolic homeostasis by coordinating lipid storage, mobilization, and hepatic lipid handling. Although sex differences in metabolic risk are well recognized, whether childhood obesity programs WAT–liver crosstalk in a sex-specific manner remains poorly understood.

Aims: To investigate how neonatal overfeeding programs sex-specific short- and long-term metabolic adaptations across the WAT–liver axis. To determine whether an adult "second hit" with a high-fat diet (HFD) exacerbates or reshapes this interorgan phenotype.

Methods: Neonatal obesity was induced in mice by adjusting litter size at birth to 8 pups per dam for controls (C) or 4 pups per dam for the overfed small-litter group (SL). Mice were weaned onto a standard chow diet (SCD), and at 3-months old half of the cohort received a 45% high-fat diet (HFD). Longitudinal phenotyping, including metabolic testing, was performed from birth to adulthood. WAT and liver characterization, including *in vivo* lipid fluxes in subcutaneous (scWAT) and visceral (vWAT) depots and liver, were assessed at 2-weeks and 7-months of age using stable isotope tracing in male and female mice.

Results: Litter-size reduction increased food intake in SL mice during the lactation period, resulting in higher body weight (BW) from day 7 onwards. By 2-wks of age, SL mice of both sexes exhibited increased scWAT and vWAT adiposity, adipocyte hypertrophy that was more pronounced in females, and hyperinsulinemia. Interestingly, neonate SL males, but not females, developed early hepatic steatosis, suggesting early sex-specific partitioning of excess lipids

between WAT and liver. Despite SCD-feeding from weaning, increased adiposity persisted in SL males and females throughout adulthood. However, a marked sexual dimorphism emerged with age: adult SL females normalized their metabolic profile and adipocyte size, whereas SL males retained adipocyte hypertrophy and developed glucose intolerance and insulin resistance. Hepatic steatosis also worsened with age in SL males but remained absent in SL females throughout adulthood. HFD feeding further increased BW and adiposity in SL-HFD mice, particularly in females, while exacerbating hepatic steatosis, and metabolic impairment in males. *In vivo* lipid flux analyses revealed an early sexual dimorphism across the WAT–liver axis. At 2-weeks, SL males exhibited increased WAT lipid uptake, whereas SL females displayed a broader WAT response, with additional increases in *de novo* lipogenesis (DNL) and fatty acid (FA) desaturation. Hepatic flux remodeling was more selective: SL males tended toward increased DNL and reduced FA desaturation, while SL females exhibited reduced FA desaturation alone. By adulthood, lipid fluxes revealed persistent sex-divergent programming across the WAT–liver axis. Under SCD, SL females maintained WAT and hepatic flux profiles comparable to controls, whereas SL males showed selective WAT lipid remodeling and marked hepatic dysregulation, with a hepatic profile consistent with decreased endogenous FA synthesis and increased uptake and esterification of circulating FA. HFD exacerbated these alterations in males, while changes emerged in females only under HFD and remained more selective and less pronounced.

Conclusions: Our results show that neonatal overfeeding programs the WAT–liver axis lipid fluxes in a sex-dependent manner. Despite persistent obesity in both sexes, females recovered metabolic homeostasis and remained protected from hepatic steatosis, preserving greater metabolic flexibility even after an adult HFD challenge. In contrast, males developed progressive metabolic dysfunction and hepatic steatosis that were further exacerbated by HFD. These divergent outcomes paralleled sex-specific WAT and hepatic lipid flux profiles, identifying differential lipid partitioning across the WAT–liver axis as a potential driver of female resilience versus male vulnerability to early-life overnutrition.

Choline Transporter-Like 1 (CTL1/SLC44A1) modulates lipid metabolism and membrane-associated cellular processes

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The choline transporter-like protein CTL1, encoded by SLC44A1, has traditionally been described as an intermediate-affinity, sodium-independent choline transporter involved in phosphatidylcholine synthesis. However, recent evidence suggests that CTL1 is not a choline transporter per se, although its precise biological role remains unclear. The association of SLC44A1 alterations with neurological diseases, skeletal muscle dysfunction and cancer highlights its potential relevance in human health. Previous studies have linked CTL1 deficiency to lipid metabolism alterations, suggesting a role in lipid homeostasis.

To further investigate CTL1 function, we generated HEK293 and HCT116 cell lines stably overexpressing CTL1, using GFP-expressing cells as controls. Lipidomic analyses revealed alterations triglyceride metabolism, supporting the involvement of CTL1 in lipid homeostasis and membrane dynamics. Furthermore, CTL1 overexpression induced cell-type-dependent changes in cellular behaviour. In HCT116 cells, it reduced proliferation, migration and colony formation under both adherent and suspension conditions. Conversely, in HEK293 cells, CTL1 overexpression enhanced adhesion to laminin, with no detectable effects on proliferation or migration.

Overall, our findings support a role for CTL1 in lipid metabolism regulation and reveal its influence on cellular processes related to proliferation, migration, adhesion and clonogenicity. Although the mechanisms linking lipid alterations to these cellular phenotypes remain unknown, these results provide new insights into CTL1 function and its potential contribution to neurological diseases and cancer.

Keywords: SLC44A1, CTL1, lipid metabolism, lipid rafts, membrane dynamics, cell migration, neurodegeneration, cancer

Characterization of a new lncRNA in white adipose tissue of children with obesity

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Introduction: According to the WHO's 2024 report, 160 million children and adolescents (ages 5–19) were living with obesity. Spain is the leading country in Europe in childhood obesity. Importantly, 80% of the children with obesity will remain with obesity until the adulthood facing risks of chronic diseases. Effective treatment strategies are urgently needed to improve public health. Long non-coding RNAs (lncRNAs) have emerged as key regulators of metabolic homeostasis. However, little is known about the role of lncRNAs in children's adipose tissue.

Objective: Our aim is to investigate a new lncRNA (CYTOR) differentially expressed in the subcutaneous adipose tissue (scWAT) of children with and without obesity.

Methods: Transcriptomic profiling of pediatric adipose tissue (KIDSADIPOSEQ) identified a target lncRNA associated with childhood obesity, which was validated by qPCR in pediatric scWAT. The primary transcript of this lncRNA in human adipose tissue was predicted using the GTEx database, followed by a homology analysis to compare the primary transcripts between human and mouse. To investigate the mechanism of CYTOR (Cytor) in adipose tissue at the molecular level, an *in vivo* model was established using C57BL/6J mice fed a high-fat diet (HFD) for 4 weeks, and *in vitro* models were employed using human SGBS and mice 3T3-L1 preadipocytes. Nuclear and cytoplasmic fractions of both cell types were isolated using an NER kit for subsequent analysis.

Results: Our previous KIDSADIPOSEQ transcriptomic analysis identified significant upregulation of CYTOR in the scWAT, with this finding further validated by in the pediatric scWAT dataset. Its primary transcript was computationally predicted using GTEx in human adipose tissue and murine ortholog analysis, revealing 73.88% sequence identity between the two. Given the high sequence

identity revealed by homology comparison, we further validated the overexpression of CYTOR in both scWAT and visceral adipose tissue (vWAT) *in vivo* childhood mouse model. *In vitro*, CYTOR expression was strongly upregulated time-dependently during 3T3-L1 adipocyte differentiation. To assess subcellular localization, we examined the nuclear/cytoplasmic distribution of CYTOR (Cytor) in SGBS and 3T3-L1 cells, and found it predominantly localized in the cytoplasm.

Conclusion: Our results indicate that the lncRNA CYTOR is a functional mediator of metabolic adaptation in obesity. In future, targeted knockdown of CYTOR will be used to validate these predictions and explore the underlying molecular mechanisms.

Acknowledgment: This study was supported by grants PID2023-148783OB-I00 to LH funded by MICIU/AEI/10.13039/501100011033 and by FEDER/UE; CB06/03/0001 to LH funded by CIBEROBN; 2021SGR00367 to LH funded by the Government of Catalonia, and a doctoral fellowship from China Scholarship Council (CSC, 2024) to SL.

Chronic NRN1 administration protects against Diet-Induced Obesity

Obesity and associated metabolic disorders represent a major global healthcare challenge. Neuritin 1 (NRN1) has emerged as a novel candidate in metabolic regulation. This study evaluated the therapeutic efficacy, safety profile, and metabolic mechanisms of chronic NRN1 administration in a high-fat diet (HFD)-induced obesity mouse model. Chronic NRN1 administration protects against high-fat diet-induced obesity by increasing energy expenditure and enhancing thermogenic activity in brown and subcutaneous adipose tissues without triggering muscle degradation or systemic organ damage. These findings position NRN1 as a promising therapeutic target for obesity and metabolic diseases.

Keywords: Neuritin1, Obesity, Thermogenesis, Energy expenditure

OBESITY-DRIVEN ALTERATIONS IN THE BIODISTRIBUTION OF POLYMERIC NANOMEDICINES

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Obesity alters body composition, plasma protein binding, hepatic and glomerular function, and immune status, potentially affecting biodistribution of nanomedicines. However, most nanomedicines are developed using lean models, leaving a translational gap. Here, we investigated the biodistribution of fluorescent 30–50 nm polymeric micelles (PMs) in lean and obese mice. PMs were synthesized from poly(ethylene glycol)-b-poly(L-aspartamide) block copolymers bearing aldehyde side chains. Two micelles were prepared: non-crosslinked PMs and crosslinked PMs stabilized by hydrazone core-crosslinking. Both micelles were IV-administered to C57BL/6J mice fed a high-fat diet (60% kcal) for 6 w, and biodistribution was measured at 2 and 24 h post-injection. HFD-mice showed nearly 2× the weight gain of controls with elevated blood cholesterol and AST/ALT ratios. Crosslinked PMs exhibited prolonged circulation, with up to 7× greater retention in blood at 24 h, due to enhanced stability against dilution in biological fluids. They were predominantly cleared by the liver. In contrast, non-crosslinked PMs were mainly eliminated through the kidneys, potentially following dissociation into unimers small enough to pass through glomerular filtration. At 24 h, lean mice showed greater PM accumulation in subcutaneous adipose tissue than HFD-mice, potentially reflecting differences in tissue vascularization. In conclusion, core crosslinking influenced PM circulation and clearance, while metabolic phenotype was associated with differences in adipose tissue accumulation. These findings show the need to develop nanomedicines that not only remain stable in circulation but also achieve robust and predictable biodistribution across different metabolic phenotypes to improve their translational potential.

Mitochondrial Alterations and Impaired Mitophagy in the Development of Insulin Resistance: Insights from Muscle Models

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Autophagy is a process responsible for degrading and recycling damaged organelles and protein aggregates. When this process is altered, the accumulation of cellular waste triggers oxidative stress, chronic inflammation, and organelle dysfunction. **Mitophagy**, the selective autophagic clearance of damaged mitochondria, can be compromised in obesity and diabetes (T2D). However, the mechanism underlying these mitochondrial alterations remains poorly understood. A central hypothesis is that dysregulation of the process leads to the accumulation of damaged mitochondria, and this impairment exacerbates mitochondrial damage and insulin resistance (IR). To investigate this further, we utilized both *in vitro* and *in vivo* models. To mimic lipid overload,- observed in an obesogenic environment- **C2C12 myotubes** were treated with palmitate to induce IR and mitochondrial stress. In this model, palmitate reduced mitophagic flux, mitochondrial function, and lysosomal function, correlating with IR. For *in vivo* studies, male **Mito-QC mice** (C57BL/6J background) were fed either a control or high-fat diet (HFD) for 8 weeks. HFD-fed mice developed a clear IR profile. Furthermore, data showed that HFD markedly reduced lysosomal proteolytic efficiency, evidenced by decreased cathepsin and alkaline phosphatase activity. These findings suggest that the mitophagy is impaired confirming the observed in literature however the restoration of some parts of the process are not enough to recover the insulin resistance state. More efforts are required for a better understanding of T2D pathogenesis and will direct future research towards new target therapies.

PROGRANULIN, IDENTIFIED BY A MULTI-OMICS APPROACH, IS A BATOKINE THAT NEGATIVELY CORRELATES WITH BROWN FAT ACTIVITY IN HUMANS

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ABSTRACT

Background and aim: Brown adipose tissue (BAT) presence and activity correlate with cardiometabolic health in rodent models and humans. BAT is a site of energy expenditure that consumes glucose and lipids to sustain non-shivering thermogenesis. Additionally, BAT secretes regulatory molecules (batokines) that help govern systemic metabolism and are central to its protective effects. However, the BAT secretome remains largely uncharacterized, particularly for batokines with endocrine actions and their role in humans.

Methods: We combined plasma proteomics with multi-tissue transcriptomics in C57BL/6 mice at thermoneutrality (30°C) or exposed to cold (4°C, 24 h) to identify novel batokine candidates. We characterized a progranulin (Pgrn) BAT-specific knockout mouse model and Pgrn-invalidated brown adipocytes in culture. Besides, we assessed PGRN circulating levels in infants and correlated them with thermography-based parameters of BAT activity.

Results: The concordant analysis of mouse multi-omics revealed Pgrn as a batokine candidate exclusively upregulated by cold in BAT. Targeted invalidation of Pgrn in BAT resulted in enhanced local thermogenesis, morphological changes, and a remodeled gene expression pattern of BAT. Brown adipocytes lacking Pgrn replicated enhanced activity at the gene, protein, and functional levels. In humans, circulating PGRN levels increased during the first year of life and were negatively correlated with BAT activity at 12 months of age.

Conclusions: This study identifies PGRN as a batokine that negatively regulates BAT activity in mice and that shows inverse associations with human BAT function as well. Further investigations may determine its biomarker potential and role in modulating BAT activity, interorgan signaling, and overall metabolic health.

Funding. This research was supported by: State Agency of Research (AEI) of the Spanish Ministry of Science (MICIN/AEI/10.13039/50110 0011033 and FEDER, UE) grant PID2023-1467810-I00 (M.G. & F.V.); Spanish Ministry of Science, Innovation, and Universities "Ayudas para contratos de formación de doctores" grant FPU20/03364 (A.M-A.); Spanish Ministry of Science (MCIN/AEI/10.13039/501100011033 and European Union Next Generation EU/PRTR) "Ramon y Cajal" researcher grant RYC2022-037961-I (M. P.).

Phenotypic convergence, molecular divergence: adipocyte progenitor fingerprints in developmental and diet-induced obesity in mice

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Objective: We investigated whether developmental and diet-induced obesity, which reflect childhood and adult adiposity in humans, engage distinct molecular programs in adipocyte progenitor cells (APCs) despite leading to similar adipose tissue expansion.

Methods: APC transcriptomes were analyzed in inguinal (iWAT) and epididymal (eWAT) adipose depots from murine models of developmental obesity induced by litter size reduction (SL) and adult-onset obesity induced by high-fat diet (HFD).

Results: Both SL and HFD models induced comparable adipocyte hypertrophy without major alterations in canonical lipogenic or adipogenic genes. However, *Dlk1* and *Wisp2*, negative regulators of preadipocyte commitment, were consistently upregulated. Therefore, we analyzed transcription profile in APCs of both models. APC transcriptomic analyses revealed distinct molecular signatures: developmental obesity suppressed immune-related pathways in iWAT, whereas diet-induced obesity activated cholesterol metabolism, immune regulation, and extracellular matrix remodeling, particularly in eWAT, consistent with fibro-inflammatory progenitor signatures.

Conclusions: Developmental and diet-induced obesity are mechanistically distinct despite phenotypic convergence. The timing of obesity onset imprints depot-specific transcriptional programs in APCs that influence adipose tissue plasticity. These findings underscore the importance of diversifying preclinical models to better reflect obesity heterogeneity. Furthermore, our data supports that treating adult or childhood obesity will require different strategies, as their molecular underlying mechanisms are also different.

Altered autophagy contributes to brown adipose tissue dysfunction during aging

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Brown adipose tissue (BAT) function declines in aging and white adipose tissue reduced browning is associated with metabolic dysfunction. Autophagy is a fundamental homeostatic process that ensures cellular integrity by degrading and recycling damaged proteins and organelles. BAT transcriptomic profiling across aging revealed an upregulation of autophagy-related genes alongside a decline in thermogenic gene expression, consistent with enhanced macroautophagy in BAT of aged mice. Chaperone-mediated autophagy (CMA) is a selective form of autophagy that specifically targets proteins containing a KFERQ-like motif for degradation. Compared with macroautophagy, a transcript-based CMA score and LAMP2A protein levels, which is the key mediator of (CMA) were reduced in aged BAT. Pharmacological activation of CMA restored BAT thermogenic function in aged mice. Together with complementary in vitro studies in brown adipocytes, these findings suggest that increased macroautophagy and reduced CMA activity during aging may promote the accumulation of specific CMA substrates that negatively regulate BAT function, contributing to impaired thermogenic capacity in aged BAT.

LINKING STRESS AND METABOLISM: POMC NEURONS AND β-HYDROXYBUTYRATE IN RESILIENCE

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Stress resilience is a key protective factor against the development of stress-related psychiatric disorders, yet the neural circuits underlying adaptive responses to stress remain poorly understood. Proopiomelanocortin (POMC) neurons have emerged as candidate integrators of metabolic and stress-related signals, while growing evidence suggests that ketogenic signaling influences both neuronal and metabolic function. This project hypothesizes that POMC neurons act as a nexus between stress, metabolism and β-hydroxybutyrate (BHB)-mediated signaling, contributing to resilience-promoting mechanisms.

First, endogenous and exogenous BHB dynamics were characterized through kinetic and circadian analyses. BHB displayed circadian fluctuations, and exogenous administration reproduced levels associated with 24-hour fasting-induced ketosis. We then examined whether hypothalamic POMC neurons respond to social stress. Acute and chronic social stress robustly increased c-FOS expression in POMC neurons, with approximately 20% of POMC-positive cells activated following stress exposure. Notably, these effects occurred without changes in body weight or food intake, indicating that POMC neurons are recruited by social stress independently of overt metabolic alterations.

Building on these findings, this project investigates the role of BHB in stress adaptation. BHB regulation was characterized through kinetic and circadian analyses, and exogenous BHB administration was used to mimic 24-hour fasting-induced ketosis. Using behavioral, molecular, metabolic and neuroimaging approaches, this work aims to determine how ketogenic signaling modulates stress-responsive circuits and resilience-related phenotypes.

CHARACTERIZATION OF THE EXTRA-MITOCHONDRIAL ROLES OF MITOFUSIN 2 IN PEROXISOMAL FUNCTIONALITY AND MATURATION

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RESUM

Metabolic homeostasis in eukaryotic cells relies on the coordinated activity of mitochondria, the endoplasmic reticulum (ER), and peroxisomes. While the mechanisms underlying mitochondria-ER communication are well established, how peroxisomes are integrated into this network and how their growth and metabolic function are sustained remains poorly understood. Despite the hypothesis that the ER and mitochondria supply peroxisomes with membrane proteins via vesicles, the lack of a clearly defined fusion machinery has raised questions about this pathway's role under basal conditions. In this study, we identify Mitofusin 2 (Mfn2), previously considered a mitochondria-restricted fusion GTPase, as a mediator of peroxisome maturation. We show that Mfn2 localizes not only to mitochondria but also to peroxisomes, where it is required to maintain peroxisomal architecture, dynamics, and metabolic function. Loss of Mfn2 leads to a striking remodeling of the network severe metabolic dysfunction.

EXPOSOMIC INSIGHTS INTO EXERCISE IN YOUNG ADULTS: INTEGRATING METABOLIC PROFILING AND DIETARY STATUS

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Introduction. Eccentric resistance exercise performed with the Flywheel Russian Belt Deadlift (FRBD) has been associated with oxidative stress and skeletal muscle damage. An exposomics approach can provide a more complete picture of how young adults respond to acute eccentric exercise. Therefore, this study aimed to characterize urinary metabolomic alterations before exercise, immediately after FRBD, and 24 hours post-exercise.

Methods: Twenty-four young adults completed a FRBD protocol. Urine samples were collected at PRE, POST, and POST24. Untargeted metabolomic profiling was performed using RP-HPLC-QTOF-MS. Metabolomic features were aligned and analyzed in MetaboAnalyst 6.0 using one-way ANOVA (FDR correction) Dietary intake was assessed using a 3-day food record, and mean daily macronutrient intake was compared with Spanish dietary recommendations.

Results: Untargeted metabolomics showed 436 significantly altered mass spectrometry features (FDR < 0.05). Fisher's LSD post-hoc analysis revealed significant differences between PRE vs POST and POST vs POST24. Twenty metabolites were putatively annotated, including hypoxanthine which increased significantly at POST. Participants' diet averaged 2247.9Kcal/day, with 39.0% of energy from carbohydrates below Spanish recommendations, and 21.1% from protein and 38.7% from fat, higher than Spanish recommendations.

Conclusion. Exercise-induced changes in the urinary metabolome revealed the metabolic effects of FRBD, characterized by a transient increase in purine metabolism intermediates that fully resolved within 24 h. Integrating dietary data provides a broader exposomic understanding of exercise adaptation.

Short Talks

Flash Posters

ROLE OF MITOFUSIN 2 IN THE REGULATION OF MITOCHONDRIAL AND PEROXISOMAL DYNAMICS IN A CELLULAR MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) involves dopaminergic neuron loss driven by oxidative stress and mitochondrial dysfunction. Peroxisomes cooperate with mitochondria in reactive oxygen species handling and lipid metabolism, but their role in PD is largely unexplored. Mitofusin 2 (MFN2) regulates mitochondrial fusion and promotes mitochondria-peroxisome contacts, yet its role in each compartment under PD-like stress is undefined. We established a 6-hydroxydopamine (6-OHDA) model of PD in differentiated SH-SY5Y cells to dissect the compartment-specific role of MFN2 in organelle dynamics and survival. Differentiation with retinoic acid and BDNF was validated by NeuN and tyrosine hydroxylase staining. Differentiated cells were treated with 6-OHDA to induce oxidative damage, alone or after transfection with full-length MFN2 or with MFN2 targeted to mitochondria or peroxisomes, with pMito-RFP and Ub-GFP-SKL as markers. MFN2 was measured by Western blot and immunofluorescence, viability by MTT, and organelle morphology. Statistics: two-tailed Student's t-test or one-way ANOVA with Tukey post hoc. 6-OHDA reduced viability to 40-55% and MFN2 levels by ~50% ($p<0,05$), fragmenting the mitochondrial network (less area, more particles) and the peroxisomal population (smaller, more numerous). Full-length MFN2 rescued both compartments, raising viability to 70-84% ($p<0.05$). Mitochondria-targeted MFN2 restored mitochondrial morphology (74-86% viability; $p<0.05$) but not peroxisomes, whereas peroxisome-targeted MFN2 restored peroxisomes and improved viability (62-74%; $p<0.05$) without affecting mitochondria. Thus, MFN2 preserves mitochondrial and peroxisomal integrity through compartment-autonomous mechanisms, identifying peroxisomal dynamics as an overlooked contributor to neuronal resilience in PD.

DEVELOPMENT AND CHARACTERIZATION OF PHOTOSENSITIZER-FUNCTIONALIZED OM-PBAE NANOPARTICLES AS MULTIFUNCTIONAL PLATFORMS FOR MRNA DELIVERY

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Oligopeptide-modified poly(β-amino ester) (OM-pBAE) polymers are biodegradable platforms for mRNA delivery that can be functionalized with therapeutic components. This study aimed to formulate and characterize mRNA-loaded nanoparticles based on DBCO-functionalized OM-pBAEs modified with different photosensitizers through strain-promoted azide–alkyne cycloaddition (SPAAC). Nanoparticles incorporating aza-BODIPY, bacteriochlorin (Bchl), or pyropheophorbide-a (Pyro) were formulated with firefly luciferase (Fluc) mRNA. Physicochemical properties were characterized by dynamic light scattering and zeta potential measurements, and biological evaluation was performed in HeLa cells using BCA and luciferase assays.

Increasing the NP/mRNA ratio from 10:1 to 40:1 decreased Z-average from 357.1 to 217.6 nm and PDI from 0.3695 to 0.2278. Although 40:1 showed the most favorable profile, 25:1 was selected based on previously established conditions. Aza-BODIPY-PEG nanoparticles showed a Z-average of 274.5 nm (PDI 0.356), whereas Bchl-PEG showed a markedly higher Z-average of 1.36×10^4 nm (PDI 0.399). Pyro-PEG was excluded from *in vitro* experiments due to its unfavorable physicochemical profile. Estimated mRNA concentrations ranged from 84.4 to 208 µg/mL and were used for nominal doses of 0.4–1.0 µg mRNA/well. BCA measurements showed no clear dose-dependent reduction in cellular protein content; Bchl-PEG values were 320.98 ± 69.90 and 332.32 ± 55.58 µg/mL at 0.4 and 1.0 µg mRNA/well, respectively. Luciferase activity was low and variable, preventing reliable comparison of mRNA delivery.

Overall, these results support the feasibility of photosensitizer-functionalized OM-pBAE nanoparticles, while highlighting the need for improved reproducibility, mRNA quantification and biological replication.

Brain VDR in Energy Homeostasis: Insights into Its Regulation in Type 2 Diabetes

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Vitamin D (VD) deficiency is associated with increased diabetes incidence, but the role of its nuclear receptor (VDR) in Type 2 diabetes (T2D) remains unclear. We previously reported reduced Vdr expression in pancreatic islets from murine models of Type 1 and Type 2 diabetes, suggesting that VDR downregulation in β-cells may be a common feature of diabetes. Here, we investigated the role of VDR in T2D, focusing on energy homeostasis.

VDR expression was reduced in pancreatic islets from genetic T2D mouse models and mice fed a high-fat diet (HFD). In contrast, transgenic mice overexpressing VDR specifically in pancreatic β-cells (Tg-VDR) maintained high islet VDR levels despite HFD feeding. Remarkably, Tg-VDR mice were protected from HFD-induced body weight gain and hyperglycemia, displaying a metabolic phenotype comparable to wild-type mice fed a control diet. This protection was associated with increased energy expenditure rather than reduced food intake, suggesting a role for VDR in energy balance.

Given the role of the central nervous system (CNS) in energy homeostasis, we investigated VDR expression in brain regions involved in metabolic control. Unexpectedly, the RIP-driven transgene was ectopically expressed in hypothalamic regions of Tg-VDR mice, accompanied by increased sympathetic nervous system activity. These findings suggest that CNS VDR expression may contribute to increased energy expenditure and resistance to HFD-induced metabolic dysfunction.

Overall, our preliminary data identify a potential link between the VD/VDR axis, CNS-mediated energy expenditure, and metabolic homeostasis in T2D. Ongoing studies will determine how VDR expression is regulated in the diabetic brain and whether epigenetic mechanisms contribute to its metabolic effects, potentially uncovering novel therapeutic targets to restore VDR signalling and metabolic homeostasis.

SYNTHESIS AND CHARACTERISATION OF PEG-BASED INTERMEDIATES FOR CRBN-BASED PROTAC SYSTEMS

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Targeted protein degradation using proteolysis-targeting chimeras (PROTACs) is an emerging strategy that enables the selective elimination of disease-related proteins through recruitment of the ubiquitin–proteasome system. This work aimed to synthesise and characterise polyethylene glycol (PEG)-based intermediates incorporating thalidomide and 1,3,5-triazine fragments for the development of cereblon (CRBN)-based PROTAC systems, including their potential application in homoPROTAC strategies.

PEG linkers of different lengths were evaluated, and PEG–triazine and PEG–thalidomide derivatives were prepared mainly through aromatic nucleophilic substitution (SNAr) reactions, followed by Boc deprotection and final coupling steps. Products were isolated by chromatographic techniques and characterised primarily by ^{1}H- and ^{13}C-NMR spectroscopy, complemented by Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy when available.

Boc-protected PEG–triazine derivatives showed yields of 86%, 84% and 78% for 1PEG, 2PEG and 3PEG, respectively, whereas PEG–thalidomide derivatives gave more moderate yields of 48% for 1PEG and 28% for 2PEG. NMR analysis identified characteristic signals corresponding to the thalidomide fragments, triazine core and PEG linkers. Final 1PEG, 2PEG and 3PEG products showed spectroscopic features compatible with the simultaneous presence of these structural elements.

Overall, the results establish a modular synthetic strategy for the preparation of CRBN-oriented PROTAC intermediates with variable PEG linker lengths, providing a basis for the future development of homoPROTAC systems, further structural optimisation and biological evaluation.

EXERCISE AND VITAMIN D AS COMPLEMENTARY MODULATORS OF GLUCOSE HOMEOSTASIS IN TYPE 2 DIABETES: THE DIAVITEX CLINICAL TRIAL

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Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and progressive pancreatic β-cell dysfunction, leading to impaired glucose homeostasis. Skeletal muscle is a major site of insulin-mediated glucose disposal and a key target of both physical exercise and vitamin D action. The active form of vitamin D, calcitriol, binds to the vitamin D receptor (VDR), expressed in skeletal muscle and pancreatic cells, thereby modulating muscle function and insulin secretion. Importantly, physical exercise has been shown to increase VDR activation in skeletal muscle and circulating 25-hydroxyvitamin D levels, suggesting greater vitamin D availability. This provides a biological rationale for a potential synergistic effect of both interventions on metabolic regulation. However, evidence regarding their combined effects in T2DM remains limited and inconclusive.

DIAVITEX is a randomized, double-blind clinical trial designed to investigate the independent and combined effects of vitamin D treatment and a 16-week supervised aerobic and resistance exercise program in individuals with T2DM. The primary outcome is insulin resistance assessed by HOMA-IR, together with glucose, lipid, inflammatory, renal and anthropometric parameters. Physical activity is assessed using the International Physical Activity Questionnaire-Short Form (IPAQ-SF).

During study implementation, 12 healthy controls (mean age 59.17 ± 15.17 years; BMI 23.57 ± 1.90 kg/m²) and one participant with T2DM (55 years; BMI 32.0 kg/m²) were included in the baseline assessment. Mean energy expenditure was 1,069.25 ± 254.47 MET-min/week in healthy controls, compared with 972 MET-min/week in the participant with T2DM, with all participants classified as moderately physically active. Baseline blood analysis in the participant with T2DM showed controlled glucose levels (glucose: 92 mg/dL; glycated hemoglobin [HbA1c]: 5.5%), adequate vitamin D status (35.8 ng/mL), and elevated triglycerides (260 mg/dL). These preliminary findings establish the basis for the continued recruitment and implementation of DIAVITEX to evaluate the metabolic effects of exercise and vitamin D in T2DM.

Maternal supplementation with Sulforaphane and Silibinin attenuates Western Diet-induced metabolic alterations in female offspring

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Early-life nutritional exposures may shape susceptibility to obesity and metabolic disease. We investigated whether maternal supplementation with sulforaphane (SFN), silibinin (SYL), or their combination during gestation and lactation modulates offspring responses to an obesogenic challenge. Pregnant Wistar rats received placebo, SFN (10 mg/kg), SYL (100 mg/kg), or SFN+SYL orally each day until weaning. At weaning, male and female offspring were fed a standard diet (SD) or a western diet (WD; 45% energy from fat, 20% fructose and 12.5% sucrose) until postnatal day 91. Body weight, adiposity, glucose-insulin homeostasis, circulating markers, indirect calorimetry and hepatic endpoints were assessed by sex. WD did not markedly increase body weight, but increased adiposity and hepatic lipid accumulation. Maternal treatment effects were strongly sex-dependent. In females, SFN, SYL and SFN+SYL reduced visceral adiposity and the leptin-to-adiponectin ratio; fasting insulin and HOMA-IR were reduced by maternal SFN and SYL, and maternal treatments attenuated the insulin response during the OGTT while glucose handling was only modestly affected, consistent with improved insulin efficiency. In males, effects on adiposity and glucose-insulin homeostasis were weaker, with more modest responses to both post-weaning diet and maternal treatments. Overall, maternal SFN and SYL supplementation modulated metabolic programming in a sex-dependent manner, with the clearest protective effects in WD-fed females. Ongoing analyses, including Western blotting, liver histology and global DNA methylation profiling in adipose tissue, will further investigate the mechanisms underlying these sex-dependent metabolic responses. This work was supported by Horizon Europe project PAS GRAS ID 101080329.

MATERNAL BETAINE SUPPLEMENTATION DURING BREASTFEEDING MODIFIES THE INFANT GUT MICROBIOTA AND EXECUTIVE FUNCTION AT 4 YEARS

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Background: Infant gut microbiome development influences long-term health. We previously showed that higher breast milk betaine concentrations are associated with increased offspring *Akkermansia* abundance and lower adult obesity in mice. Betaine is an osmolyte and methionine-cycle metabolite present in the diet and breast milk.
Objective: To investigate whether maternal betaine supplementation during breastfeeding impacts milk composition, infant gut microbiota, growth, and neurodevelopment.
Methods: A double-blind, placebo-controlled trial with 47 lactating mothers with overweight or obesity randomized to betaine (400mg/day, n=25) or placebo (n=22) for 3 months postpartum was performed (ClinicalTrials.gov NCT04633044). We collected maternal plasma and milk, infant anthropometric data, and fecal samples at 1, 3, and 12 months. 28 infants underwent neurodevelopmental assessment at 4 years.
Results: Betaine supplementation increased maternal plasma and milk betaine, with similar infant growth. At 1 year, the betaine group showed reduced gut microbiota α-diversity and higher Bifidobacterium (29.7% vs. 18.4%, p=0.021) and *Akkermansia* (2.5% vs. 0.4%, p<0.001). At 4 years, the betaine-group showed better BRIEF-P Global Executive Composite scores than controls (adjusted-mean difference, -8.34; 95%CI, -14.32 to -2.37; p=0.008), as well as Inhibitory Self-Control (-6.8; 95%CI, -12.1 to -1.6; p=0.012) and Emergent Metacognition (-8.4; 95%CI, -15.6 to -1.1; p=0.025) scores.
Conclusions: Maternal betaine supplementation during breastfeeding was associated with long-term changes in infant gut microbiota and better global executive functioning at 4 years. These results support potential long-term effects of maternal nutritional interventions during lactation on child neurodevelopment.

SEX-SPECIFIC GUT MICROBIOME ALTERATIONS INDUCED BY FEEDING A CDAA DIET CORRELATE WITH FAECAL LITHOCHOLIC ACID (LCA) CONCENTRATION AND MARKERS OF LIVER INJURY IN SPRAGUE-DAWLEY RATS

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Aim: We have previously described sexual dimorphism in susceptibility to hepatic fibrosis associated with distinctive lipidomic, faecal bile acids (BA) and gene expression signatures in choline-deficient L-amino acid-defined diet (CDAA)-fed Sprague-Dawley (SD) rats. Here, we determined whether sex-specific differences also exist in the gut microbiota response to the CDAA diet.

Method: Male and female SD rats were distributed into 2 groups for each sex: 1) control group (n = 8) fed standard chow; 2) CDAA group, fed the CDAA diet (n = 8) for three months. Faecal samples were collected for microbiome analysis by using 16S rRNA sequencing.

Results: Feeding SD rats a CDAA diet reduced gut microbial diversity as reflected by changes in alpha-diversity indices (Shannon index, Faith's phylogenetic diversity and observed features). Our results revealed distinct gut microbial signatures associated with both CDAA feeding and sex. CDAA-fed females were mainly enriched in taxa belonging to the Clostridia/Oscillospirales lineage, whereas males exhibited a broader microbial profile including Akkermansiaceae, Bacteroidaceae and other families. *Alistipes*, *Peptococcus*, and *Ruminococcus* genera were more abundant in faecal samples from CDAA-fed female rats than from male, and were positively associated to the faecal abundance of several secondary BA, such as LCA. Moreover, *Peptoccocus* abundance was negatively correlated with markers of liver injury and fibrosis.

Conclusions: CDAA diet reproduces key microbiological and metabolic features of steatohepatitis-associated dysbiosis. Observed sex-specific alterations in the gut microbiota may contribute to the divergent secondary bile acid profile and liver injury responses observed between male and female rats after a CDAA intervention.

Grants: PID2023-146140OB-I00, funded by MICIU/AEI/10.13039/501100011033, FEDER-UE, Predoctoral FI-DUR 2025 Jianing Zhou-Wu, and 2021SGR-00345.

SEXUAL DIMORPHISM IN METABOLIC AND THERMOGENIC RESPONSE TO INTERMITTENT FASTING IN A MURINE MODEL OF DIET-INDUCED OBESITY

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Abstract

Diet-induced obesity disrupts energy homeostasis and promotes metabolic dysfunction, while biological sex may influence both the development of obesity and the metabolic response to nutritional interventions. Intermittent fasting (IF) has emerged as a nutritional strategy capable of improving metabolic regulation and energy balance; however, sex-specific responses to IF remain insufficiently understood. This study aimed to determine whether IF induces differential metabolic and thermogenic adaptations in male and female mice with diet-induced obesity. Male and female C57BL/6 mice were fed a high-fat diet (HFD; 60% kcal from fat) for 12 weeks. A subgroup underwent an IF protocol consisting of two non-consecutive 24-h fasting periods per week. Body weight, fasting glucose and glucose tolerance (GTT) were assessed, while thermogenic activation was estimated by interscapular temperature using infrared thermography. IF improved metabolic parameters in both sexes, including fasting glucose and glucose tolerance ($p<0.05$). However, sex-specific adaptations were observed: females were more resistant to HFD-induced weight gain, whereas males exhibited greater thermogenic activation, reflected by increased interscapular temperature. These findings demonstrate that biological sex influences metabolic and thermogenic adaptations to IF in diet-induced obesity and support the importance of considering sex as a biological variable when evaluating nutritional strategies targeting metabolic health.

Post-weaning obesogenic diets induce early metabolic alterations in a sex-dependent manner

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Background: Childhood obesity is associated with early metabolic dysfunction, highlighting the need for translational models reproducing systemic and tissue-specific alterations. Post-weaning diet-induced murine models are useful, but their biochemical and adipose tissue transcriptional profiles remain incompletely characterized.

Aim: To characterize a post-weaning childhood obesity murine model induced by high-fat and high-sucrose diets, assessing systemic metabolic and white adipose tissue transcriptional profiles.

Methods: Post-weaning female and male C57BL/6 mice at week 3 of age were fed with control, 60% high-fat (HFD), or 45% high-fat sucrose (HFSD) diets for 4 weeks. Body weight and food intake were monitored, and body composition was analyzed by MRI. Metabolic parameters were analyzed in serum samples and gonadal white adipose tissue (gWAT) was used to assess relative gene expression.

Results: Obesogenic diets increased adiposity in both sexes, despite modest changes in overall body weight, with the strongest effects generally observed in HFD-fed mice. HFD impaired glucose homeostasis, increasing fasting glucose and HOMA-IR in both sexes, while circulating insulin increased primarily in males. Lipid homeostasis was also altered in a sex- and diet-dependent manner: HFD increased circulating cholesterol in both sexes and triglycerides in females. In gWAT, HFD decreased adiponectin expression and increased Ccl2 expression in males, suggesting early adipose tissue dysfunction and inflammatory activation.

Conclusion: Our post-weaning childhood obesity murine model shows increased adiposity and early metabolic alterations, with a more pronounced phenotype in the HFD group. Glucose and lipid homeostasis, together with gWAT gene expression, were differently affected depending on sex and diet.

STUDY OF MITOCHONDRIAL QUALITY DURING MUSCLE SENESCENCE: GENERATION AND DESCRIPTION OF AN IN VITRO MODEL

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Senescence of muscle stem cells (MuSCs) is viewed as an important contributor to age-related muscle dysfunction and sarcopenia. During aging, MuSCs gradually lose their capacity for differentiation and regeneration, accompanied with a decrease in mitochondrial function and autophagic flux, suggesting impaired maintenance of mitochondrial quality. However, the mechanisms underlying such age-related defect and whether loss of mitochondrial quality is a cause or a consequence of muscle cell senescence remain incompletely understood.

In this project, we aim to assess whether loss of mitochondrial quality is a driver of muscle cell senescence and the molecular mechanisms involved. For that, a senescent C2C12 muscle cell model was generated through D-galactose treatment, and mitochondrial quality will be evaluated at different time points during the establishment of senescence. The senescent status was evaluated through assessing the expression of age-related proteins like p16 and p53, as well as the β-galactosidase staining. Further analyses revealed an association between cellular senescence and impaired mitochondrial function, particularly a reduction in ATP-linked mitochondrial respiration level. Additional assessments of mitochondrial status also indicated compromised mitochondrial function in the senescent C2C12 model, consistent with previous findings.

Together, these results establish an in *vitro* model for studying muscle cell senescence and provide evidence for a causal role of mitochondrial dysfunction in its development. In addition, this model provides a useful platform for further investigating the mechanisms involved and the screening of potential compounds for the treatment of sarcopenia.

When the Diseased Liver Affects the Heart: MASLD and the Modulating Role of Chronic Exercise

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the leading cause of chronic liver disease and is closely associated with cardiovascular disease, the main cause of mortality in these patients. However, the mechanisms linking hepatic dysfunction to cardiac damage, especially in the absence of obesity, remain poorly understood, nor is it known whether exercise can simultaneously reverse both alterations.

The aim of this study was to demonstrate whether MASLD, in the absence of obesity, alters heart structure and function, and to evaluate whether chronic exercise can reverse these effects. To this end, male mice with a liver-specific Mitofusin 2 deficiency (Mfn2-LKO) were used, a model that develops MASLD without obesity. For four weeks, the mice followed a treadmill exercise protocol, and cardiac function was assessed before and after exercise using echocardiography.

Mfn2-LKO mice showed a reduced running capacity compared to wild-type (WT) mice, indicating potential systemic functional impairment. Echocardiographic analysis to assess cardiac function revealed that Mfn2-LKO mice present an increased end-diastolic left ventricular anterior wall thickness and end-diastolic posterior wall thickness, indicating the presence of ventricular hypertrophy. Furthermore, Mfn2-LKO mice exhibited decreased end-systolic posterior wall thickness values, as well as a lower ejection fraction and fractional shortening, indicating the presence of systolic dysfunction.

We can conclude that hepatic dysfunction in a MASLD model can induce structural and functional alterations in the heart, even in the absence of obesity.

Exploring the Role of Mfn1 Ablation in Modulating Lipid Droplet Metabolism

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Mitochondria are dynamic organelles essential for energy production, with their activity and morphology regulated by fusion and fission processes. Mitofusin 1 (MFN1) is a key protein involved in outer membrane fusion; however, its broad functions in cellular metabolism are not well understood. The aim of this study is to investigate the metabolic and mitochondrial roles of MFN1 in vitro using MFN1 knockout and wild-type mouse embryonic fibroblasts (KO1) and in vivo using liver-specific MFN1 knockout mice. Microscopic analysis of liver tissues and MEF cells revealed that the lack of MFN1 increased the volume of lipid droplet. Consistently, triglyceride levels were increased in MFN1-deficient conditions. Under basal conditions, increased [^{14}C]-palmitate incorporation was observed in KO1 MEFs and KO1 primary hepatocytes, although mitochondrial fatty acid beta-oxidation remained unchanged. Under fasted conditions, MFN1-deficient cells showed a greater increase in lipid droplet number and volume, displayed a twofold rise in triglyceride levels compared with basal condition. Despite reduced Dgat2 expression, the incorporation of radiolabeled palmitate was increased in MFN1-deficient MEFs and hepatocytes. Notably, beta-oxidation was reduced in MFN1 deficient cells under fasting conditions.

High-resolution Oroboros revealed improved mitochondrial activity in the fed state, but under fasted conditions, a significant functional decline was observed in MFN1-deficient

mice. Furthermore, DNP-MRSI revealed an increased lactate to alanine ratio in the liver of KO mice during fasting, consistent with impaired mitochondrial pyruvate utilization. This ratio remained unchanged in the fed state.

These data indicate that MFN1 plays essential role in regulating lipid metabolism and mitochondrial activity in a nutrient-dependent manner.

Distinct Structural and Connectivity Changes in the Maternal Brain Underlie the Development of Gestational Food Cravings

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Pregnancy is a critical period of readjustments in the female body to support fetal development and prepare for motherhood. These adaptations occur along with structural and functional brain remodeling, driven by hormonal fluctuations that may rewire neural circuits and promote neuroplasticity in the maternal brain. These neuronal adaptations may underlie changes in feeding, caregiving, and social behaviours during gestation and lactation. However, these behavioral changes and patterns of brain remodeling show significant interindividual variability, and have not been addressed so far.
Here, we provide a functional study integrating mouse and human datasets to investigate whether individual differences in maternal brain activity and connectivity are associated with food craving episodes, a common and poorly understood feeding behaviour reported by over 50% of pregnant women. Using whole-brain mapping of Fos, a marker of neural activation, we identified 68 brain regions with specific activation patterns in pregnant mice exposed to our food craving paradigm. This allowed us to define the mouse food craving circuitry integrating gustatory, olfactory, visual, and somatosensory regions with limbic, salience, cognitive, and reward networks. In humans, the maternal brain showed morphometric and functional connectivity differences in individuals who experience food cravings. The integration of both datasets supports a conserved hierarchical organization of brain networks underlying complex behaviours such as food cravings, reinforcing our findings' translational relevance. These results identify a previously undescribed neural circuitry underlying maternal food cravings conserved across species, and provide new insights on how pregnancy-induced plasticity shapes behaviour.

IDENTIFICATION AND CHARACTERIZATION OF NEURONS CO-EXPRESSING PROOPIOMELANOCORTIN (POMC) AND AGOUTI-RELATED PROTEIN (AGRP) IN ADULT MICE

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There is evidence that neurons co-expressing the antagonistic Proopiomelanocortin (POMC) and Agouti-related Protein (AgRP) genes exist during murine embryonic development. However, their presence and molecular characterization in adults remain unexplored. This study aims to evaluate the presence of POMC:AgRP hybrid neurons in adulthood and determine their transcriptional and functional identity.

Using an integrated scRNAseq reference atlas of the murine hypothalamus (Hypomap), canonical POMC neurons (Pomc>0 & Agrp=0), canonical AgRP neurons (Pomc=0 & Agrp>0) and hybrid neurons (Pomc>0 & Agrp>0) were selected from adult male mice fed a standard chow diet. The transcriptomic profile of hybrid neurons was compared against the canonical populations applying independent differential gene expression and GO enrichment analysis. A supervised learning model assessed whether hybrid neurons constitute a distinct neuronal identity or a transitional state. The congruence between upstream input-sensing and downstream signaling pathways was also examined, alongside sex differences and an analogous human population.

Our analysis demonstrates the presence of hybrid neurons in adulthood, in both mice and humans, without evidence of sex-dimorphism. Hybrid neurons were predominantly enriched in genes encoding ribosomal machinery and local synaptic translation. They clustered into two groups: approximately one half displayed AgRP neuron-like signatures whereas the other half exhibited POMC neuron-like signatures, with concordant input and output signaling pathways.

These findings indicate that hybrid neurons do not constitute a distinct neuronal identity, but align closely with a single canonical lineage, while displaying a transcriptional program consistent with enhanced protein synthesis.

TRANSCRIPTOMIC ANALYSIS REVEALS SEX-SPECIFIC METABOLIC REPROGRAMMING AND DIFFERENTIAL HEPATIC RESPONSES TO A HIGH FAT HIGH FRUCTOSE HIGH CHOLESTEROL DIET IN SPRAGUE-DAWLEY RATS

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Background and Aims: Previous findings demonstrated a sexual dimorphism in the response of Sprague-Dawley (SD) rats to a high fat, high fructose, high cholesterol (HFHFrHC) diet. To further investigate the molecular basis underlying these sex-dependent differences, hepatic transcriptomic profiling was performed

Method: RNAseq was performed in liver samples from male and female SD rats fed either a chow (CT) or a HFHFrHC diet. Differentially expressed genes (DEGs) were identified, followed by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), gene set enrichment (GSEA), and protein-protein interaction (PPI) network analyses.

Results: 700 DEGs in males (301 up- and 399 down-regulated) and 551 in females (212 up- and 339 down-regulated) were identified. Comparison of diet-responsive gene sets identified 503 male-specific, 354 female-specific, and 197 shared genes. In males, the diet-responsive genes were predominantly associated with arachidonic acid and linoleic acid metabolism, glutathione related redox processes, and ferroptosis. PPI analysis identified *Scd*, *Acaca*, *Elovl6* and *Acsm* family genes as prominent network nodes, supporting a transcriptional program centered on fatty acid (FA) metabolic remodeling and oxidative stress. In females, DEGs were mainly enriched in cholesterol/sterol biosynthesis and the mevalonate pathway, together with AMPK, insulin and bile acid related signaling. PPI analysis highlighted *Srebf1*, *Hmgcr*, *Hmgcs1*, *Sqle*, *Mvk*, *Mvd* and *Dhcr24* as key network nodes, indicating coordinated remodeling of the cholesterol biosynthetic axis.

Conclusion: RNAseq analysis showed distinct sex-specific transcriptional responses, characterized predominantly by FA/redox remodeling in male and cholesterol/sterol remodeling in female HFHFrHC fed rats.

EXPLORING SKELETAL MUSCLE–LIVER CROSSTALK IN OBESITY

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Obesity affects both skeletal muscle (SkM) and the liver, two metabolically active organs involved in systemic energy homeostasis. Alterations in SkM quantity and function may accompany metabolic dysfunction-associated steatotic liver disease, suggesting interrelated metabolic disturbances. In a cohort of 39 women with obesity, we explored the SkM–liver axis by assessing associations between muscle quantity and function and non-invasive markers of hepatic steatosis and fibrosis.

SkM phenotype was assessed by SkM mass as percentage of body weight (%SkMM), handgrip strength (HGS), sit-to-stand test (SST), Timed Up and Go (TUG), and gait speed (GS). Liver phenotype was characterized by hepatic steatosis index (HSI) and fibrosis-4 index (FIB-4). Participants were stratified according to HGS, presence of hepatic steatosis, and HSI relative to the cohort mean. Group comparisons and Pearson correlation analyses were performed.

Participants had a mean age of 45.6±10.4 years and BMI of 41.4±5.2 kg/m². Women with HSI above the cohort mean had lower %SkMM than those with lower HSI (26.53% vs 29.46%, p=0.0005), with no significant differences in HGS, SST, TUG, or GS. Conversely, HGS categories did not differ in HSI or FIB-4, and imaging-detected hepatic steatosis was not associated with muscle mass, strength, or physical performance.

These exploratory findings suggest an association between a higher steatosis index and lower relative SkM mass, without evidence of concomitant impairment in muscle strength or physical performance. However, this association should be interpreted cautiously, as both relative SkM mass and steatosis indices may be influenced by adiposity. Larger studies accounting for age and detailed body composition are warranted.

URINARY VOLATILOMICS IDENTIFIES COMPOUNDS WITH DISCRIMINATORY POTENTIAL IN PHENYLKETONURIA

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Urinary volatile organic compounds (VOCs) may capture metabolic and nutritional alterations associated with phenylketonuria (PKU). This study aims to investigate the urinary volatilome of adults with PKU and HPA using an HS-SPME-GC-MS approach compared with healthy controls.

This study included 36 adults with PKU, 14 individuals with hyperphenylalaninemia and 32 healthy controls (Consortium PKU.CAT). Urinary VOCs were analyzed by HS-SPME-GC-MS. VOC identification was performed using authentic standards when available and the NIST mass spectral library.

Fifty-nine urinary VOCs were identified, including ketones, aldehydes, furans and alcohols. Fifteen differed significantly among groups after ANOVA, Fisher's LSD post hoc test and false discovery rate correction (FDR < 0.05). Receiver operating characteristic curve analyses revealed that four compounds showed an AUC > 0.85 for discriminating PKU from controls (3,4-dimethylbenzaldehyde, cyclopentanone, benzeneacetaldehyde and benzaldehyde). Patients with HPA exhibited an intermediate volatilomic profile between controls and PKU, with 3,4-dimethylbenzaldehyde showing the highest discriminatory ability between HPA and controls.

The urinary volatilome differed across adults with PKU, HPA and controls, revealing VOCs with relevant discriminatory potential. These findings suggest that urinary volatilomics may complement metabolic characterization in PKU and identify potential biomarkers linked to disease or dietary management although further validation is required.

Financial support: María de Maeztu Unit of Excellence Grant CEX2021-001234-M, INSA-UB joINT rEseaRch ACTions INTERACT24 and grant PID2024-156020OB-I00 funded by MICIU/AEI/10.13039/501100011033 and, by FEDER, UE. The authors acknowledge Consortium PKU.CAT.

Alterations in Mitochondrial Dynamics and Mitophagy in White Adipose Tissue in Childhood Obesity

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Background: Childhood obesity has substantial health and economic consequences. In mice and humans, obesity is associated with marked metabolic remodeling of white adipose tissue (WAT), mitochondrial dysfunction, and altered mitochondrial dynamics and mitophagy. Whether these changes also occur in WAT in childhood obesity, and whether they vary by depot or sex, remains unclear.

Aim: To examine the expression of proteins involved in mitochondrial dynamics and mitophagy in visceral and subcutaneous WAT from a mouse model of childhood obesity and from children, and to explore associations with obesity-related complications.

Methods: We used a diet-induced model of early-life overnutrition and measured mitochondrial protein levels by Western blotting in visceral (vWAT) and subcutaneous (sWAT) depots of male mice. Primary human preadipocytes from both depots of three children (two boys and one girl) were differentiated into adipocytes. Lipid droplet accumulation and levels of BNIP3 (mitophagy), MFN2 and OPA1 (fusion), and oxidative phosphorylation (OXPHOS) complexes were compared between sexes and depots. Protein levels were normalized to TIMM44.

Results: Preliminary analyses showed depot-specific differences in male overweight mice. Compared with controls, Bnip3, Mfn2 and Opa1 levels were higher in vWAT, whereas Pink1, Parkin and Opa1 levels were lower in sWAT. At the same time point in the human cell model, adipocytes from the girl accumulated more lipid droplets than those from the boys. Within each child, lipid droplet accumulation was greater in subcutaneous than in visceral adipocytes. In this small sample, BNIP3 levels were relatively constant across depots and donors. Levels of MFN2, OPA1 and OXPHOS complexes II, III and IV were lower in the girl's adipocytes than in those from the boys, while complex V (ATP synthase) levels were unchanged.

Conclusion: These preliminary findings suggest that obesity-related changes in proteins involved in mitochondrial dynamics and mitophagy differ by adipose depot in mice. In the human cell model, fusion proteins and respiratory chain components differed between adipocytes from the girl and the boys, whereas BNIP3 levels remained stable. Larger cohorts are needed to confirm these observations and clarify the roles of depot and sex in childhood obesity.

BILIRUBIN SIGNALS A DECREASE IN MITOCHONDRIAL PROTEIN TRANSLATION AND SUPERCOMPLEX ASSEMBLY

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The exact role of bilirubin in the development of metabolic diseases is still unclear. Bilirubin can scavenge lipophilic reactive oxygen species and is produced from the direct product of heme catabolism biliverdin (Baranano et al., PNAS 2002). Bilirubin synthesis occurs in the cytosol of all cells and is catalysed by biliverdin reductase A (BLVRA). Previous studies showed that increased bilirubin production, fueled by an elevation of biliverdin transported from the mitochondria into the cytosol, caused beta cell dysfunction and hepatic insulin resistance (Shum M, et al ., Science Translational Medicine 2021; Molecular Metabolism 2022). However, the exact mechanism by which bilirubin decreases mitochondrial oxidative phosphorylation (OXPHOS) and the consequences of inducing bilirubin synthesis inside the mitochondria on metabolic diseases remain undefined. Thus, our aims are: i) Determine the mechanism by which decreased bilirubin production elevates mitochondrial function in BLVRA KO HepG2 cells and ii) to determine the effects of locating BLVRA inside the mitochondrial matrix, to effectively induce a primary increase in bilirubin synthesis inside the mitochondria.

HEPATIC METABOLIC DISORDER ASSOCIATED WITH OVARIAN-UTERINE DYSFUNCTION

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Recent findings highlight the significant role of the mitochondrial fusion protein Mfn2 in liver metabolism, particularly its involvement in metabolic dysfunction-associated steatohepatitis (MASH).

Mfn2 binds phosphatidylserine (PS) and promotes its transfer from the endoplasmic reticulum (ER) to mitochondria, where it is used for the synthesis of phosphatidylethanolamine (Hernández-Alvarez et al., 2019). Loss of Mfn2 function disrupts this transfer, leading to impaired liver metabolism, oxidative stress, and mitochondrial dysfunction, all of which contribute to the progression of liver diseases. Our group has demonstrated that male Mfn2 liver knockout (L-KO) mice exhibit defective phospholipid transport between the ER and mitochondria, which is linked to a reduction in key enzymes involved in PS synthesis in mitochondria-associated membranes (MAMs). This deficiency results in altered lipid metabolism, ultimately leading to steatohepatitis.

There is a sexual dimorphism according to Mfn2 action in liver. We observed that ablation of Mfn2 in the liver of female mice is related to dysregulated estrus cycle, which can affect the proper development of various tissues, including the ovaries and uterus, where significant morphological disruptions are observed.

These findings suggest that Mfn2 deficiency in females interacts in a complex manner with hormonal regulation. Impaired Mfn2 function may impact lipid availability, thereby affecting hormonal synthesis and potentially influencing estrogen-related metabolic pathways.

CHARACTERIZING URINARY (POLY)PHENOL METABOLIC PROFILES ACROSS CLINICAL PHENOTYPES AND AGE GROUPS IN PHENYLKETONURIA

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Dietary management of phenylketonuria (PKU) involves restricting natural protein intake, which may alter the consumption of plant-derived (poly)phenols and their subsequent metabolism by the host and gut microbiota. However, urinary (poly)phenol metabolic profiles remain poorly characterized in PKU. This study aimed to investigate differences in urinary (poly)phenol metabolites across clinical phenotypes and age groups using targeted metabolomics.

This case-control study included 173 pediatric and adult participants grouped as healthy controls, hyperphenylalaninemia (HPA), BH4-responsive PKU, and classical PKU. Urinary metabolites were analyzed by targeted UPLC-ESI-QqQ-MS/MS. Of 289 identified metabolites, 230 were included in statistical analyses. Multivariate and univariate analyses assessed differences across the eight phenotype-by-age groups.

Metabolic profiles varied by phenotype and age, with 108 metabolites differing significantly across groups. These included flavan-3-ols, phenyl-γ-valerolactones, lignans, and phenylacetic, phenylpropanoic and benzoic acid derivatives. Whole-grain-related markers were lower in PKU and inversely associated with the proportion of energy from protein substitutes. Flavan-3-ol-derived metabolites were higher in children than adults among controls, HPA and BH4-responsive PKU, but not in classical PKU.

These findings highlight phenotype- and age-related variation in urinary (poly)phenol profiles, potentially linked to dietary management and gut microbial metabolism. This approach may contribute to nutritional exposomics research in PKU.

This research acknowledges the financial support provided by INSA joINT rEseaRch ACTions INTERACT 2024 (2025-2027) and from the grant PID2024-156020OB-I00 funded by MICIU/AEI/10.13039/501100011033 and, by FEDER, UE. BB-M thanks AGAUR-Generalitat de Catalunya (2022 FI_B 01012) and Montcelimar UB fellowship. INSA-UB recognized as a María de Maeztu Unit of Excellence, Grant CEX2021-001234-M, funded by MICIU/AEI/10.13039/501100011033. The authors acknowledge Consortium PKU.cat.

MECP2 duplication remodels mitochondrial architecture while preserving basal metabolic function

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MECP2 duplication syndrome (MDS) is a rare X-linked neurodevelopmental disorder that predominantly affects males and is characterised by severe developmental delay, seizures and recurrent respiratory infections. Despite its substantial clinical impact, the underlying cellular mechanisms remain incompletely understood.

We investigated whether increased *MECP2* dosage alters mitochondrial organisation and central carbon metabolism. Using primary fibroblasts derived from patients and healthy controls, we combined three-dimensional mitochondrial imaging, protein analysis, high-resolution respirometry and stable isotope tracing with [U-$^{13}C_6$]glucose and [U-$^{13}C_5$]glutamine.

MDS fibroblasts exhibited a markedly remodelled mitochondrial network, characterised by smaller mitochondria, reduced surface area and fewer branches. These changes were accompanied by reduced DRP1 expression, whereas MFN2, OPA1 and total mitochondrial content remained unchanged.

Despite these structural alterations, no significant differences were detected in basal, ATP-linked or maximal respiration, or in spare respiratory capacity. Similarly, ^{13}C-tracing analysis revealed no significant differences in glucose- or glutamine-derived carbon incorporation into glycolytic intermediates or tricarboxylic acid (TCA) cycle metabolites.

Together, these findings indicate that *MECP2* duplication is associated with substantial mitochondrial remodelling without a detectable impairment of basal respiratory function or central carbon metabolism under the conditions tested. This dissociation between mitochondrial architecture and metabolic function raises the possibility that cellular adaptations maintain basal metabolic homeostasis despite structural alterations. Future studies will investigate whether metabolic stress reveals functional vulnerabilities that are not apparent under basal conditions.

CREATINE SUPPLEMENTATION ATTENUATES HEPATIC STEATOSIS AND METABOLIC DYSFUNCTION IN A MOUSE MODEL OF DIET-INDUCED DIABETES

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Metabolic dysfunction-associated steatotic liver disease (MASLD) frequently coexists with type 2 diabetes and impaired physical performance. We investigated whether creatine supplementation attenuates hepatic and metabolic alterations in a high-fat diet (HFD)/streptozotocin (STZ) mouse model. Twenty-one middle-aged C57BL/6 mice were allocated to control diet, HFD/STZ, or HFD/STZ supplemented with creatine (n=7/group). Creatine was administered orally (100 mg/kg/day) for four weeks. Hepatic histology, circulating transaminases, body composition, glucose and insulin tolerance, and treadmill performance were evaluated. Compared with untreated HFD/STZ mice, creatine-supplemented animals showed lower hepatic steatosis scores, reduced circulating AST and ALT activities, and lower fat mass. Creatine also attenuated the deterioration in maximal treadmill running speed and improved the response to insulin tolerance testing. However, glucose tolerance was not normalized, and no clear improvements were observed in grip strength, suspension time, or circulating triglycerides. These findings suggest that short-term creatine supplementation mitigates hepatic steatosis and selected metabolic and functional disturbances in middle-aged mice exposed to HFD/STZ. Further studies are needed to establish the mechanisms underlying these tissue-specific effects.

Exploring the Role of Mfn1 Ablation in Modulating Lipid Droplet Metabolism

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Mitochondria are dynamic organelles essential for energy production, with their activity and morphology regulated by fusion and fission processes. Mitofusin 1 (MFN1) is a key protein involved in outer membrane fusion; however, its broad functions in cellular metabolism are not well understood. The aim of this study is to investigate the metabolic and mitochondrial roles of MFN1 in vitro using MFN1 knockout and wild-type mouse embryonic fibroblasts (KO1) and in vivo using liver-specific MFN1 knockout mice. Microscopic analysis of liver tissues and MEF cells revealed that the lack of MFN1 increased the volume of lipid droplet. Consistently, triglyceride levels were increased in MFN1-deficient conditions. Under basal conditions, increased [^{14}C]-palmitate incorporation was observed in KO1 MEFs and KO1 primary hepatocytes, although mitochondrial fatty acid beta-oxidation remained unchanged. Under fasted conditions, MFN1-deficient cells showed a greater increase in lipid droplet number and volume, displayed a twofold rise in triglyceride levels compared with basal condition. Despite reduced Dgat2 expression, the incorporation of radiolabeled palmitate was increased in MFN1-deficient MEFs and hepatocytes. Notably, beta-oxidation was reduced in MFN1 deficient cells under fasting conditions.

High-resolution Oroboros revealed improved mitochondrial activity in the fed state, but under fasted conditions, a significant functional decline was observed in MFN1-deficient

mice. Furthermore, DNP-MRSI revealed an increased lactate to alanine ratio in the liver of KO mice during fasting, consistent with impaired mitochondrial pyruvate utilization. This ratio remained unchanged in the fed state.

These data indicate that MFN1 plays essential role in regulating lipid metabolism and mitochondrial activity in a nutrient-dependent manner.

Epigenetic and Transcriptional Drivers of Mitochondrial and Metabolic Dysfunction in Intrauterine Growth Restriction

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Background

Intrauterine Growth Restriction (IUGR) is an obstetric condition that affects 10% of newborns and is characterized by impaired fetal growth and placental insufficiency. Mitochondrial and metabolic alterations have been described at a functional level; however, upstream regulators and etiology remain unknown. Our objective was to identify novel epigenomic and transcriptomic signatures through multi-omic integration to explain the metabolic rearrangement.

Methods

We implemented a multi-omic pipeline analyzing RNA-seq and genome-wide DNA methylation data from a cohort of IUGR and control mother-newborn pairs (n=14 per group). RNA-seq and DNA methylation were conducted on maternal peripheral blood, neonatal cord blood and/or placental tissue. Finally, individual analysis on both omic layers was analyzed and integrated to identify key regulatory players.

Results

RNA-seq data revealed the downregulation of glucose, lipid, and protein metabolic fluxes in both maternal and newborn IUGR groups, accompanied by an upregulation of the carnitine shuttle and *omega-3* metabolism. In parallel, we identified altered methylation profiles in regulators of mitochondrial function across maternal, newborn, and placental tissues (*NRF1, MTERF1,* and *MFN1*), alongside tissue-specific mediators like *COQ3* hypermethylation in the placenta. Integrative multi-omic analysis identified 96 and 121 epigenetically regulated genes in mothers and newborns, respectively, including *RTN1* and *MROH6*, both associated with mitochondrial function. Finally, integrative gene set enrichment analysis revealed coordinated pathway alterations in maternal "Oxidative Phosphorylation" and "Mitochondrial Gene Expression", and neonatal "Adipogenesis" and "Placental Development".

Conclusions

This multi-omic approach identified convergent transcriptomic and epigenetic dysregulation affecting mitochondrial function and metabolic programming, revealing tissue-specific molecular signatures associated with IUGR.

Funding: PI18/00451, PI21/00935, PI24/00428, FI22/00142 (ISCIII-FSE+), and 2025/STEP00231

Evaluation of kynurenine/tryptophan metabolite signature in Inclusion Body Myositis patients' urine samples

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Fundings: This study was supported by Instituto de Salud Carlos III (ISCIII-FEDER, grants PI21/00935, FI22/00142 and PI24/00428) and co-funded by the European Union. The group is accredited by the Catalonian Government as SGR 01423.

Background: Inclusion Body Myositis (IBM) is a progressive inflammatory myopathy characterized by muscle fiber degeneration, mitochondrial alterations and $CD8^+$ T-cell infiltration, leading to muscle weakness and atrophy. Despite being the most common inflammatory myopathy in older adults, IBM remains difficult to diagnose and monitor due to its slow progression, clinical overlap with other disorders and the need to obtain information from invasive muscle biopsy. Identifying non-invasive biomarkers could improve diagnosis, disease monitoring and therapeutic assessment. In a previous exploratory study (n = 6), urinary metabolites related to nucleic acid metabolism showed potential as biomarkers. Here, we validated these findings in a larger cohort (n = 14) and explored additional interconnected metabolic pathways.

Methods: Purine, pyrimidine, and amino acid profiles were analysed in 14 IBM patients and 12 healthy controls urine samples. Eighty-four metabolites were first quantified by UHPLC-MS/MS and evaluated by sensitivity, specificity, AUC-ROC, and Youden's J index, identifying eleven discriminant compounds that were secondarily quantified using more precise LC-MS/MS QTRAP 6500 (SCIEX) system. Metabolite levels were normalized to creatinine levels and correlated with disease severity and progression. Transcriptomic data from IBM patients' skeletal muscle was used to assess the potential source of this metabolic dysregulation.

Results: Kynurenine and hydroxykynurenine showed the best profiles in terms of sensitivity, specificity and AUC-ROC (0.64/1/0.82; 0.73/1/0.87). In this pathway, 5 kynurenine-related metabolites were increased in IBM (fold change 1.6–4). Among them, quinolinic acid displayed the best performance (0.8/1/0.93), especially when combined with 3-methylhistidine, indoleacetic acid, and carnitine (0.9/1/0.94; 0.9/1/0.94; 0.9/1/0.92). Additionally, Creatine, L-kynurenine, and 5-hydroxytryptophan correlated with disease severity and/or progression ($p < 0.05$). Genes involved in the production of kynurenine-related metabolites showed a tendency to being increased in IBM muscle compared to control muscle.

Conclusions: Urinary metabolites, particularly from the kynurenine pathway, show strong potential as non-invasive biomarkers for IBM, correlating with disease severity and progression. These findings enhance our understanding of IBM and might complement current diagnostic and monitoring tools.

TfR1 DEFICIENCY BEYOND COMBINED IMMUNODEFICIENCY. PIVOTAL ROLE OF MITOCHONDRIAL FUNCTION AND IRON-SULFUR CLUSTER BIOGENESIS IN DISEASE PATHOGENESIS

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TFRC encodes transferrin receptor (TfR1), a critical protein for cellular iron uptake. To date, biallelic mutations in *TFRC* have only been associated to patients with combined immunodeficiency (CID). We report three patients from two unrelated families with novel *TFRC* mutations that do not exhibit CID but instead present with hypertrophic cardiomyopathy and severe microcytic anemia, respectively. Our aim was to investigate whether mitochondrial function and Fe-S cluster biogenesis are altered as a consequence of TfR1 dysfunction and whether these novel clinical phenotypes share common pathogenic mechanisms with those observed in CID patients.

The effect of *TFRC* variants was functionally analyzed in several tissues and in HAP1 knock-in cell (KI) models, including both the novel variants and the prevalent CID-associated mutation. Functional studies demonstrated that *TFRC* mutations induce mitochondrial dysfunction by targeting key aspects of mitochondrial physiology. All variants impaired transferrin uptake and disrupted iron homeostasis, resulting in defective Fe-S cluster biogenesis, impaired protein lipoylation, OXPHOS deficiency, and reduced cellular respiration. Furthermore, *TFRC* variants cause variable degree of apoptotic cell death, which correlated with patients' clinical severity. Nicotinamide riboside supplementation partially rescued oxygen consumption rate and improved cell survival across all KI models.

In conclusion, our data provides substantial advance in the knowledge of TfR1 deficiency in humans, underscoring the pivotal role of Fe-S cluster biogenesis and mitochondrial function in disease pathogenesis, and suggests the likelihood of additional phenotypes beyond CID, regardless of the associated genotype.

Funding: PI19/01310 and PI22/00856, funded by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union-European Regional Development Fund. AGAUR 2021: SGR01423.

Mitochondrial and metabolic reprogramming in idiopathic inflammatory myopathies: muscle-specific signatures across disease subtypes

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Background

Idiopathic Inflammatory Myopathies (IIM) are heterogeneous autoimmune muscle diseases characterized by chronic inflammation and muscle weakness. Although immune activation is central, mitochondrial and metabolic alterations have been suggested to contribute to pathogenesis. We investigated mitochondrial and metabolic transcriptional remodeling across IIM subtypes in skeletal muscle and peripheral blood representing immune populations.

Methods

Muscle and peripheral blood samples from patients with dermatomyositis, antisynthetase syndrome, immune-mediated necrotizing myopathy, inclusion body myositis (N=10 per group), and controls (N=6) were analyzed by RNA-seq. Differential expression was analyzed using DESeq2. Pathway alterations were evaluated using Gene Set Enrichment Analysis (GSEA) and single-sample GSEA (ssGSEA). Mitochondrial transcriptional programs were further examined using the MitoCarta 3.0.

Results

Muscle transcriptomes revealed enrichment of inflammatory and interferon signaling pathways across IIM subtypes, confirming shared immune activation. Metabolic pathway analysis identified mitochondrial and lipid metabolism alterations, including oxidative phosphorylation and fatty acid metabolism. Although present across IIM groups, their magnitude and pathway involvement differed among subtypes. Dermatomyositis displayed stronger suppression of oxidative phosphorylation, whereas IMNM and ASyS showed distinct alterations in glycolytic and lipid metabolic pathways. MitoCarta analysis revealed coordinated dysregulation of mitochondrial transcription in inflamed muscle. At the individual level, ssGSEA demonstrated substantial metabolic heterogeneity within subtypes. In contrast, peripheral blood transcriptomes showed only modest metabolic alterations, indicating that mitochondrial remodeling is muscle-specific.

Conclusions

Our findings reveal widespread mitochondrial and metabolic transcriptional remodeling across IIM subtypes, particularly in skeletal muscle. These findings support further investigation of mitochondrial and metablolic pathways as potential therapeutic targets.

Funding: Supported by FIS-PI24/00428 (ISCIII, FEDER, European Union), SGR-01423, FI22/00142 (ISCIII–NextGenerationEU), and CSC (No.202408210121).

IMMUNOMETABOLIC AND NEUROBIOLOGICAL SIGNATURES OF FIBROMYALGIA AND THEIR RESPONSE TO BASIC BODY AWARENESS THERAPY: A TRANSLATIONAL PILOT STUDY

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BACKGROUND: Fibromyalgia (FM) is a multisystem disorder with chronic pain, affective and body-awareness disturbances, but its metabolic and neurobiological basis remains incompletely defined. We investigated associations between patient-reported outcomes (PROMs) and circulating biomarkers with explored changes following Basic Body Awareness Therapy (BBAT).

METHODS: Ten women with FM and ten matched healthy controls underwent assessment of pain (VAS), disease impact (FIQR), anxiety/depression (HADS, BDI) and body awareness/dissociation (Body Connection Scale). Circulating inflammatory (IL-1β, IL-6, IL-10, TNF-α), neurotrophic/neuronal (BDNF, NfL), metabolic/mitochondrial-related (FGF21, GDF15, humanin, spermidine, dopamine) biomarkers and total antioxidant capacity were assessed. Seven FM participants completed BBAT.

RESULTS: FM participants showed higher IL-6 ($p=0.002$), NfL ($p=0.006$) and BDNF ($p=0.034$), while FGF21 ($p=0.059$) and IL-1β ($p=0.082$) showed trends toward increased levels between groups. In FM, inflammatory markers were intercorrelated; humanin inversely correlated with pain ($\varrho=-0.740$) and BDNF with FIQR ($\varrho=-0.669$). After BBAT intervention, VAS decreased 34.8% ($p=0.002$) and HADS-A 25.9% ($p=0.039$). FGF21 showed the largest longitudinal effect ($r=0.57$) and the only significant ANOVA signal ($p=0.049$; $\eta^2=0.199$), with lower concentrations at T2, although pairwise comparisons were not significant. Changes in IL-6 correlated with pain ($\varrho=0.764$, $p=0.046$), and BDNF with FIQR ($\varrho=0.786$, $p=0.036$).

CONCLUSIONS: FM displayed an integrated inflammatory, neuronal, neurotrophic and metabolic/mitochondrial biomarker profile associated with clinical burden. Preliminary BBAT findings indicate clinical improvement accompanied by changes in metabolic/mitochondrial signalling, highlighting FGF21 as a candidate treatment-responsive biomarker.

Funding: Partially supported by Cátedra ASISA-UdL 2025

NOVEL FUNCTIONAL VALIDATION STRATEGY TO ELUCIDATE THE PATHOGENICITY OF GENETIC VARIANTS IN INHERITED METABOLIC DISORDERS USING CRISPR/Cas9 AND FLOW CITOMETRY

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Functional validation of variants of uncertain significance (VUS) is a major bottleneck in the diagnosis of inherited metabolic disorders (IMDs). To face this problem, we propose a novel and time-efficient CRISPR/Cas9-based strategy using fluorescence-activated cell sorting (FACS) and next-generation sequencing (NGS) without need of generating clonal cell lines.

We performed a proof-of-concept by introducing 4 benign and 2 pathogenic TMEM70 variants, together with 3 VUS reported in ClinVar into HAP1 cells using CRISPR/Cas9. Pools containing mixtures of edited and unedited cells were sorted by FACS according to ATP5A expression levels, a biomarker associated with TMEM70 deficiency. NGS analysis of sorted populations using specific informatic algorithms showed that all benign variants were uniformly distributed across the different cell fractions, whereas the 2 pathogenic variants were significantly overrepresented (4 to 8-fold) in populations with lower ATP5A expression. The 3 tested VUS showed a distribution comparable to that of benign variants, supporting their functional reclassification as benign. The reliability of this new strategy was further validated in knock-in clonal cell lines for each variant, using biochemical methods routinely applied in the diagnosis of TMEM70 deficiency.

This workflow enables the functional discrimination of pathogenic and benign variants and also provides evidence for VUS reclassification. The new strategy modifies the conventional diagnostic workflow, allowing functional testing in genetically modified pooled cells instead of using clonal cell lines. This approach significantly reduces the time required for variant validation. Although this proof-of-concept focuses on IMDs, it could potentially be extended to other genetic disorders.

Funding: Project PI22/00856, funded by ISCIII and co-funded by the European Union. AGAUR 2021: SGR01423.

THERAPEUTIC RESCUE OF BIOENERGETIC DEFECTS IN KEARNS-SAYRE SYNDROME PATIENT-DERIVED FIBROBLASTS

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Kearns-Sayre Syndrome (KSS) is a rare mitochondrial disease caused by single large-scale mitochondrial DNA deletions and impaired bioenergetics. Emerging mitochondria-targeted therapeutic strategies remain largely unexplored in KSS. This study evaluated pharmacological candidates in a validated KSS patient-derived fibroblast model.

Ten compounds targeting oxidative phosphorylation, antioxidant capacity, and mitochondrial biogenesis were selected from the literature. Extracellular lactate levels were used as an initial readout to identify pharmacological hits for downstream analyses, including GDF15 quantification, mitochondrial respiration, ATP production, mitochondrial protein profiling and transcriptomic characterization.

Rapamycin (RAP), bezafibrate (BEZ) and resveratrol (RSV) significantly reduced lactate levels (-19%, -14%, and -21% respectively), whereas the remaining candidates showed no effect. RAP reduced GDF15 levels (-30%, $p<0.01$) but did not induce detectable changes in mitochondrial bioenergetics or gene expression. BEZ maintained GDF15 levels while promoting the most bioenergetic improvement, significantly increasing basal mitochondrial respiration (+30%, $p<0.05$), mitochondrial ATP production (+34%, $p<0.05$), and mitochondrial mass (+18% TOM20, $p<0.05$), accompanied by moderate transcriptomic changes. In contrast, RSV significantly increased GDF15 levels (+1194%) and induced modest improvements in spare respiratory capacity (+51%, p=NS), together with increased total ATP production (+27%, p=NS), while eliciting the most extensive transcriptomic response, consistent with the activation of adaptive stress-response and metabolic remodelling pathways.

These findings reveal distinct pharmacological response profiles in KSS fibroblasts. BEZ primarily enhances mitochondrial bioenergetic capacity, whereas RSV promotes a broader adaptive transcriptional program associated with cellular metabolic adaptation. Together, these results support the further evaluation of mechanistically distinct therapeutic strategies for KSS.

Funding: Fundación Mutua Madrileña (AP183672023), ER24P3AC7221 (CIBERER-ISCIII), FI22/00142 (ISCIII-NextGenerationEU), SGR-01423, FI24/00498, 2025-STEP-00231.

INTEGRATIVE EPIGENOMIC AND TRANSCRIPTOMIC ANALYSES REVEAL DISEASE-ASSOCIATED REGULATORY SIGNATURES IN KEARNS–SAYRE SYNDROME

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Introduction: Kearns–Sayre syndrome (KSS) is a rare mitochondrial disorder characterized by single large-scale mitochondrial DNA (mtDNA) deletions that impair oxidative phosphorylation. Although the primary mtDNA defect is well established, the mechanisms contributing to downstream cellular dysfunction and phenotypic variability remain poorly understood. We performed a comprehensive multi-omics integration analysis in fibroblasts from KSS patients to identify novel molecular contributors.

Methods: In this cross-sectional multicenter study, fibroblasts from 12 KSS patients and 11 age- and sex-matched controls were analyzed. Genome-wide DNA methylation profiling was conducted using the Infinium MethylationEPIC v2.0 array, and transcriptomic profiling was performed by RNA sequencing (Illumina PE150). Differential methylation (limma) and differential expression (DESeq2) analyses were performed, followed by integrative epigenomic–transcriptomic analysis using the ELMER algorithm.

Results: KSS fibroblasts exhibited a distinct DNA methylation signature, with 2,447 differentially methylated positions (802 hypomethylated and 1,645 hypermethylated CpGs) mainly affecting distal regulatory regions and enriched for Polycomb-associated repressive chromatin marks. RNA sequencing identified 26 differentially expressed genes, including the mitochondrial-related genes *RHOT2* and *TSTD1*. Integrative epigenomic-transcriptomic analysis identified 156 significant negative CpG-gene correlations and revealed epigenetically deregulated transcriptional networks, including genes with reported roles in mitochondrial pathways.

Conclusions: These findings suggest that epigenetic dysregulation may contribute to KSS pathogenesis beyond primary mtDNA deletions, highlighting altered nuclear–mitochondrial crosstalk and identifying new candidate molecular players and regulatory networks.

Funding: Fundación Mutua Madrileña (AP183672023), ER24P3AC7221 (CIBERER-ISCIII), FI22/00142 (ISCIII-NextGenerationEU), SGR-01423, FI24/00498, 2025-STEP-00231.