10th May 2019
Institut d'Estudis Catalans,
Barcelona

5th MetNet International Annual Meeting 2019

Targeting Metabolism to Develop New Therapies

Abstract Book

Scientific Organizers:
Paula Mera
Ignasi Barba
Rosalía Rodríguez

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5th MetNet International Annual Meeting 2019

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10th May 2019

Societat Catalana de BIOLOGIA

Sala Prat de la Riba | Institut d'Estudis Catalans
Carrer del Carme 47, Barcelona

Registration
http://scb.iec.cat/

- **09:00-09:10** **Welcome Act**

SESSION 1 | Cell Metabolism in Neurodegenerative Disease

- 09:10-09:40. **Franck Oury.** INSERM, Paris, France
  Brain autophagy mediates the effects of youthful systemic factors on cognition and aging
- 09:40-10:10. **Albert Quintana.** UAB, Barcelona, Spain
  Dissecting Neuronal Susceptibility to Mitochondrial Disease
- 10:10-10:50. **Flash Talks.** Tamara Barcos-Rodríguez, Oriol Busquets, Cristina Miralpeix, Ingrid González-Casacuberta, Sara Ramírez
- **10:50-11:20** **Coffee Break**

SESSION 2 | Monitoring Metabolism *in vivo*

- 11:20-11:50. **Oscar Millet.** CIC bioGUNE, Derio, Spain
  Liver Metabolomics
- 11:50-12:20. **Marta Cascante.** UB, Barcelona, Spain
  Overcoming acquired drug resistance driven by metabolic reprogramming
- 12:20-12:50. **Kevin Brindle.** University of Cambridge, Cambridge, UK
  Molecular Imaging in Cancer
- 12:50-13:20. **Flash Talks.** Ana Paula Candiota, Camila Lema, Mariona Gallego-Mena
- **13:20-15:00** **Lunch**

SESSION 3 | Metabolic Dysfunction in Obesity and Cardiovascular Disease

- 15:00-15:30. **Rubén Nogueiras.** USC, Santiago de Compostela, Spain
  The p53 family as a new player in metabolism
- 15:30-16:00. **Antonio Rodríguez-Sinovas.** VHIR, Barcelona, Spain
  Targeting succinate dehydrogenase against myocardial infarction
- 16:00-16:30. **Verónica Jiménez.** UAB, Barcelona, Spain
  FGF21 gene therapy as treatment for obesity and insulin resistance
- 16:30-17:00. **Flash Talks.** Roberta Haddad-Tóvolli, Joan Sabadell-Basallote, Ana Magdalena Velázquez, María Isabel Hernández-Álvarez

- **17:00-17:10** **Closing Act**
- **17:10-19:00** **Networking**

Organizers
Ignasi Barba (VHIR)
Rosalía Rodríguez (UIC)
Paula Mera (UB)

Targeting Metabolism to Develop New Therapies

10th May 2019

Sala Prat de la Riba, Institut d'Estudis Catalans

Carrer del Carme, 47, Barcelona

Type 2 diabetes, obesity and related complications are serious metabolic diseases reaching epidemic proportions. Particularly, obesity affects over 17 % of adult population in Europe and it is one of the most important predisposing factors for insulin resistance, cardiovascular disorders, non-alcoholic fatty liver and even cancer and neurodegeneration.

The aim of this meeting is to bring together leading experts in the field with post-doctoral and doctoral students, to create an inspiring and open environment to explore potential targets and signaling pathways underlying abnormal metabolism of the cells and therefore to fight against these life-threatening health problems.

We thank all the participants, speakers and sponsors for their contribution and look forward to a fantastic meeting!

Sincerely,

Scientific Organizers:

Paula Mera

Ignasi Barba

Rosalía Rodríguez

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Societat Catalana de BIOLOGIA

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THANK YOU!!

PROGRAM

9:00-9:10	**WELCOME ACT** Paula Mera, UB Rosalía Rodríguez, UIC Ignasi Barba, VHIR
SESSION 1	**CELL METABOLISM IN NEURODEGENERATIVE DISEASES** *Chair:* Paula Mera, UB
9:10-9:40	**Franck Oury** (INSERM, Paris, France). "Brain autophagy mediates the effects of youthful systemic factors on cognition and aging"
9:40-10:10	**Albert Quintana** (UAB, Barcelona, Spain). "Dissecting neuronal susceptibility to mitochondrial disease"
10:10-10:50	**Flash talks** (selected from abstracts) *Tamara Barcos-Rodríguez (IDIBAPS); Oriol Busquets (UB); Ingrid González-Casacuberta (IDIBAPS); Cristina Miralpeix (UIC); Sara Ramírez (IDIBAPS)*

10:50-11:20 Coffee Break

SESSION 2	**MONITORING METABOLISM *IN VIVO*** *Chair:* Ignasi Barba, VHIR
11:20-11:50	**Oscar Millet** (CIC BioGUNE, Derio, Spain). "Liver Metabolomics".
11:50-12:20	**Marta Cascante** (UB, Barcelona, Spain). "Overcoming acquired drug resistance driven by metabolic reprogramming"
12:20-12:50	**Kevin Brindle** (University of Cambridge, UK). "Molecular imaging in cancer"
12:50-13:20	**Flash talks** (selected from abstracts) *Ana P.Candiota (UAB); Camila Lema (VHIR); Mariona Gallego-Mena (IDIBAPS)*

13:20-15:00 Lunch

SESSION 3	**METABOLIC DYSFUNCTION IN OBESITY AND CARDIOVASCULAR DISEASE** *Chair:* Rosalía Rodríguez, UIC
15:00-15:30	**Rubén Nogueiras** (USC, Santiago de Compostela, Spain). "The p53 family as a new player in metabolism".
15:30-16:00	**Antonio Rodríguez-Sinovas** (VHIR, Barcelona, Spain). "Targeting succinate dehydrogenase against myocardial infarction"
16:00-16:30	**Verónica Jiménez** (UAB-CBATEG, Barcelona, Spain). "FGF21 gene therapy as treatment for obesity and insulin resistance"
16:30-17:00	**Flash talks** (selected from abstracts) *Sara Ramírez (IDIBAPS); Joan Sabadell-Basallote (IISPV); Ana Magdalena Velázquez (UB); María Isabel Hernández (IRB)*
17:00-19:00	**Closing act – cocktail - networking**

SCB's assistants:

Mariàngels Gallego and Maite Sánchez

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Brain autophagy mediates the effects of youthful systemic factors on cognition and aging

Speaker 02

Franck Oury, PhD
Institut Necker Enfants-Malades (INEM) – INSERM U1151 – Université Paris V
Paris, France

Notes:

Dissecting neuronal susceptibility to mitochondrial disease

Speaker 03

Albert Quintana, PhD
Institut de Neurociències.
Dept. Biologia Cel·lular, Fisiologia i Immunologia
Universitat Autònoma de Barcelona
Barcelona, Spain

Notes:

Liver Metabolomics

Speaker 04

Oscar Millet, PhD
CIC bioGUNE, Bizkaia Science and Technology Park
Derio, Bizkaia, Spain.

Notes:

Overcoming acquired drug resistance driven by metabolic reprogramming

Speaker 05

Marta Cascante, PhD
Departament de Bioquímica i Biomedicina Molecular
Integrative Systems Biology, Metabolomics and Cancer Group
Facultat de Biologia, Universitat de Barcelona
Barcelona, Spain

Notes:

Molecular imaging in cancer

Speaker 06

Kevin Brindle, PhD
Department of Biochemistry
University of Cambridge
Cambridge, United Kingdom

Notes:

The p53 family as a new player in metabolism

Speaker 07

Rubén Nogueiras, PhD
Center for Research in Molecular Medicine and Chronic Diseases (CiMUS) - Universidad de Santiago de Compostela
Santiago de Compostela, Spain

Notes:

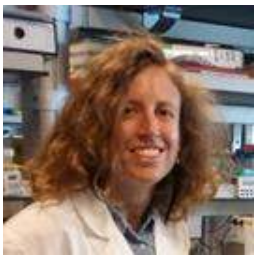
Targeting succinate dehydrogenase against myocardial infarction

Speaker 08

Antonio Rodríguez-Sinovas, PhD
Vall d'Hebron Institut de Recerca
Grup de Recerca en Malalties Cardiovasculars
Barcelona, Spain

Notes:

FGF21 gene therapy as treatment for obesity and insulin resistance



Speaker 09

Verónica Jiménez, PhD
Center of Animal Biotechnology and Gene Therapy (CBATEG),
Universitat Autònoma de Barcelona
Barcelona, Spain

Notes:

ABSTRACTS

(By alphabetical order of the presenter)

01

Doxorubicin induces metabolic and mitochondrial alterations at sub-lethal doses

Emili A. Arasa-Verge, David Garcia-Dorado, Ignasi Barba

Laboratori de Cardiologia Experimental, Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain. and CIBER-CV.

Doxorubicin is an anthracycline that has proved useful as antineoplastic drug in chemotherapy of hematological and solid tumors. However, this agent is known to induce severe cardiotoxicity that is only detectable by echocardiography once clinical manifestations have appeared. On the other hand, metabolic alterations usually precede clinical manifestations. Therefore, the main objective consists in the analysis and characterization of the metabolic pattern in order to identify potential early biomarkers of doxorubicin-induced cardiotoxicity.

Experiments were done on H9c2 cardiomyocyte-derived cell line at 80% confluence. Dose-response curves were used to determine doxorubicin sub lethal (IC<=10) and lethal (IC50) doses by resazurin staining that were found to be 0.1 and 1 μM respectively.

Cellular extracts (n=7 for each treatment) were analyzed by ^{1}H-NMR in order to characterize and quantify the metabolic pattern. In addition, 1-^{13}C-glucose (n=3 for each treatment) was employed to study the metabolic pathways involved in energy production. Mitochondrial function was evaluated using SeaHorse.

Cells treated with 0.1 μM doxorubicin showed no apparent effects on morphology while 1 uM doxorubicin induced cellular vacuolization.

Cells treated with 0.1 and 1 μM doxorubicin showed a reduction in the levels of total glutamate levels. Also, there is a reduction in the Glu 4 to Lac 3 ratio in experiments with 1-^{13}C labeled glucose (1.21, 0.72 and 0.28 for control, 0.1 and 1 μM Doxorubicin respectively) suggesting a reduction in mitochondrial function. These results were consistent with decreased oxygen consumption rates, 55 and 26% compared to control in presence of 0.1 and 1 μM doxorubicin respectively.

In conclusion, doxorubicin at the concentration found in the plasma of patients induced metabolic changes in H9c2 cells before cell viability was significantly reduced.

02

Defective glucose metabolism and mitochondrial dysfunction in iPSC-derived dopaminergic neurons from *LRRK2* Parkinson's disease patients: a potential linkage between diabetes mellitus type II and Parkinson's disease

Barcos-Rodríguez Tamara[1], Ferrer-Lorente Raquel[2], González-Casacuberta Ingrid[1], Juárez-Flores Diana Luz[1], Guitart-Mampel Mariona[1], Cantó-Santos Judith[1], Fernández-Santiago Rubén[3], Ezquerra Mario[3], Tobías Ester[1], Grau-Junyent Josep Maria[1], Martí Maria Josep[3], Cardellach Francesc[1], Raya Ángel[2], Morén Constanza[1] and Garrabou Glòria[1]

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Background: There are evidences of clinical and epidemiologic comorbidity between Parkinson disease (PD) and type II diabetes mellitus (T2DM), both being age-related chronic diseases. The existence of common molecular pathways, such as metabolic and mitochondrial dysfunction and accumulation of unfolded proteins, may contribute to this concomitance leading to common pathogenic outcomes as inflammation and oxidative stress. We hypothesized that glucose metabolism is altered in dopaminergic neurons (DAn) derived from induced pluripotent stem cells (iPSCs) from Parkinson's disease patients carrying leucine-rich repeat kinase 2 (*LRRK2*) mutations, potentially leading to either defective glucose entrance or subsequent glycolytic/mitochondrial imbalance, resulting in the generation of oxidative stress.

Aims: *i*) To explore glucose entrance into DAn-iPSCs through the measurement of phosphorylated insulin receptor substrate-1 (pIRS-1) activation through pIRS-1/β-actin and pIRS-1/total IRS-1 and *ii*) to study glycolytic vs. mitochondrial metabolism through the measurement of lactate levels in cell supernatants vs. OXPHOS protein expression and derived oxidative damage in DAn-iPSCs. Methods: The study design included DAn-iPSC from *LRRK2*-PD patients (n=2) and a group of healthy controls for comparison purposes (n=2). The cells were cultured in two different conditions: at basal conditions (B: 21.25 mM) and at high glucose concentration (HG: 50 mM), the latter resembling a pre-diabetogenic environment. We quantified protein levels of pIRS-1, total IRS-1 and subunits of each enzymatic complex of OXPHOS by means of Western Blot (all data normalized by β-actin); lactate levels were evaluated by epoc® Blood Analysis System and oxidized proteins were determined by means of OxyBlot Protein Oxidation Detection Kit.

Results: PD patients always presented decreased activation of pIRS-1 vs. controls, suggestive of defective glucose entrance into DAn-iPSC, both in basal (72.7% decrease of pIRS-1/β-actin and 68.5% decrease of the ratio pIRS-1/total IRS-1) and in high glucose conditions (75.9% decrease of pIRS-1/β-actin and 84% decrease of the ratio pIRS-1/total IRS-1). Additionally, PD patients presented trends towards biased glycolitic/oxidative metabolism compared to controls. Such

metabolic switch was evidencied by increased lactate production and altered OXPHOS protein expression both in basal and high glucose conditions that may explain altered oxidative stress.

Conclusions: *LRRK2*-PD patients seem to present less oxidative and more glycolytic profile, therefore promoting the anaerobic glycolysis, under diabetogenic conditions. Glucose uptake seems to be reduced in *LRRK2*-PD patients (as suggested by reduced levels of pIRS-1/total IRS-1 data), which in turn is processed in a more anaerobic (as suggested by higher lactate levels) and less oxidative pathway (as suggested by altered OXPHOS subunits). Defective glucose entrance in DAn-iPSCs from *LRRK2*-PD patients and switch towards anaerobic glycolisis and oxidative stress production reinforces insulin resistance as a pathologic condition that enworses PD prognosis and explains comorbidity between T2DM and PD.

Funding: Interciber PIE1400061

03

Lack of Metabolic, Mitochondrial, Renal And Hepatic Toxicity Of Enfuvirtide And Raltegravir Antiretroviral Administration: Randomized Crossover In Healthy Volunteers

Sergio Barroso, Constanza Morén, Àlex González-Segura, Judith Canto-Santos, Tamara Barcos-Rodriguez, Neus Riba, Joan A Arnaiz, Marcela Manriquez, Gemina Santana, José L Blanco, María Larousse, Montse Loncà, Elisa de Lazzari, Jaume Llopis, Josep Mallolas, Oscar Miró, Xavier Carné, José M Gatell, Glòria Garrabou*, Esteban Martínez*.

Internal Medicine Department - Hospital Clínic of Barcelona (HCB) and CIBERER (ISCIII). Muscle Research and Mitochondrial Function Laboratory, Cellex-IDIBAPS, Faculty of Medicine and Health Sciences - University of Barcelona

Context: Classical antiretroviral agents may acutely impact on metabolic, mitochondrial, renal and hepatic function in HIV-infected and uninfected persons. Fusion and integrase inhibitors are supposed to be safer, but have been scarcely investigated. To avoid any interference with HIV or other antiretrovirals, we assessed markers of these toxicities in healthy adult volunteers treated with Enfuvirtide (T20) or Raltegravir (RAL).

Methods: Twenty-six healthy participants were randomized to T20/90mg vs. placebo (n=12) or RAL/400mg vs. placebo (n=14) every 12h in two 7-day periods separated by a 4-week washout period. Major end-points were changes in lipid profile (total cholesterol, highdensity-lipoprotein (HDL)-cholesterol, low-density-lipoprotein (LDL)-cholesterol, triglycerides), insulin resistance (glucose) and mitochondrial toxicity (mitochondrial DNA content –mtDNA– in peripheral blood mononuclear cells). Renal and hepatic toxicity (creatinine, alanine transaminase (AST), alanine aminotransferase (ALT), bilirubin and total plasma proteins) and overall safety were also analysed. Effect of period, treatment, and basal measures were evaluated for each end-point.

Results: Neither T20-administration nor RAL-administration yielded to any statistic significant change in the markers of metabolic, mitochondrial, renal or hepatic toxicity assessed. No symptoms indicative of drug toxicity were neither found in any subject.

Conclusions: In absence of HIV infection, or concomitant treatment, short-term exposure to T20 or RAL in healthy adult volunteers did not lead to any indicative changes in toxicity markers thus presuming the safe profile of both drugs.

04

Adipose-derived survivin as molecular link between obesity and cancer

Ester Benaiges, Victoria Ceperuelo-Mallafré, Míriam Ejarque, Elsa Maymó, Ana Madeira, Joan Vendrell, Sonia Fernandez-Veledo

Diabetis i Malalties Metabòliques Associades, de l'Institut d'Investigacions Sanitàries Pere Virgili de Tarragona.

Background and aims: Obesity-related inflammation, abnormal production of adipokines and more recently adipose-derived stem cells (ASCs) are candidate pathogenic links between obesity and cancer. Interestingly, the anti-apoptotic effects of some adipokines have been highlighted as a mechanism of cell survival promoting cancer progression. In this context, our group has recently shown that survivin, a member of the inhibitor of apoptosis family which is considered a biomarker of tumor progression, is upregulated in obesity both at local and systemic levels. We hypothesized that high levels of ASC-derived survivin in obese tumor microenvironment might affect macrophage and tumor cell phenotype promoting cancer progression.

Materials and methods: ASCs were isolated from visceral adipose tissue of lean and obese subjects. THP-1 cells were used as a model for human monocytes/macrophages and HepG2 as a human liver cancer cell. Survivin gain-of-function and conditioned media studies were performed. Gene and protein expression were assessed by qPCR and WB. Secretion, proliferation, apoptosis, migration and invasion analysis were assayed.

Results: Conditioned medium from obese ASCs promotes a tumor associated macrophage (TAM) phenotype in THP-1 cells and exacerbates tumorigenic traits of hepatic progenitor cells and cancer cells. Accordingly, survivin overexpression in macrophages also polarizes them to a TAM phenotype. Remarkably, conditioned medium from survivin-overexpressed macrophages affect tumor progression, inducing migration and invasion and raising angiogenesis, inflammation and invasiveness capacity of cancer cells.

Conclusion: Our results point to survivin as a new molecular player in the communication between ASCs, macrophages and tumor cells.

05

Male and Female Rats Fattened on Cafeteria Diet Show Differences from Two Caloric Restriction Patterns

Bosch de Basea L[1], Romero M[1,2,3], Esteve M[1,2,3], Grasa M[1,2,3]

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The effects of different dietetic interventions after diet-induced obesity were assessed in male and female Wistar rats. At the beginning of the experiment, Wistar rats were divided into two groups for each gender: Control group (CG; standard diet available ad libitum, n=6) and Cafeteria group (KG, diet composed by biscuits, bacon, carrot, pâté, milk mixed with 22,5% of white sugar and 15% cacao, n=24). After 12 weeks, CG and 6 KG rats were sacrificed and the remaining KG were divided into three groups for each gender: standard diet ad libitum (R ad libitum; n=6), 30% restricted standard diet (R 30%; n=6), and two days at week 75% restricted diet (R 75%; ad libitum every day except Monday and Friday when caloric content was 75% reduced, n=6). After 3 weeks, all the rats were sacrificed. During the whole experiment KG diet was daily changed around 6 pm in order to maintain the circadian rhythm of food intake. Body weight and food intake were assessed every week. Tissues were weighted and stored. Furthermore, blood metabolites and hormones were measured. The 75% calorie restriction group failed to cause a difference in intake with respect to the control group at the end of the treatment as they compensated for the restriction on days fed ad libitum. As expected, body weight and food intake were increased in KG. After dietetic interventions, body weight in males just decreased significantly in R30%. However, body weight in females decreased significantly in all the groups. Glucose, NEFA, cholesterol, and lactate remain unaltered as a consequence of K diet or later diets. However, TAG and lactate levels in K males increased in K diet and were normalized by all the dietetic interventions. Besides, adipose tissue in males only returned to the control weight in the 30% restriction group, unlike the brown adipose tissue (BAT) which was also normalized in the standard diet ad libitum. Meanwhile, female's adipose tissue decreased in ad libitum, 30% and 75% restriction groups. These data demonstrate that female rats are more sensitive to caloric restriction and diet composition changes than those in male rats.

06

c-Jun N-terminal kinase 1: a Key to modulate brain metabolism

Busquets O [1,2,3,4], Ettcheto M [1,2,3,4], Verdaguer E [3,4,5], Auladell C [3,4,5], Folch J [$2,3], Camins A [$1,3,4]

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Type III Diabetes has been described as the process between peripheral metabolic alterations and the sporadic forms of Alzheimer's disease. This idea is founded in results from The Rotterdam Study or research by Dr. Hoyer or Dr. de la Monte. As of now, evidence suggests that an impairment in the functionality of the insulin receptor (IR) would be one of the reasons for the malfunction and degeneration of the neuronal network that, eventually, causes for the appearance of cognitive deficits. The cJUN N-terminal Kinases (JNK) are a subfamily of the Mitogen Activated Protein Kinases, a group of proteins that respond to many cellular stimuli. They are expressed from three different genes (Mapk8, Mapk9 and Mapk10) which produce up to 10 different products, that are later classified into three isoforms. Isoform 1 (JNK1) has been described to be a negative regulator of the activity of the IR.

By using whole body transgenic knock-out mice we evaluated the effects of the absence of JNK1. Also, some of the animals were fed a high-fat diet (HFD) enriched with palmitic acid in order to assess its effects on metabolism. Initial studies revealed that the lack of JNK1 caused for body weight reductions, along with high resistance to the effects of HFD. Also, there was increased insulin sensitivity and levels of multiple IR signalling pathway proteins. Behavioural novel object recognition test examinations also showed how in JNK1 knock-out mice, there was neuroprotection against diet-induced memory loss. Complementary analysis revealed no alterations in dendritic spine density and morphology or other related synaptic proteins. Wild-type animals fed with HFD did show a decrease. Furthermore, it was observed that lack of JNK1 causes for increased protein levels of the complexes responsible of the electron transport chain and antioxidant enzymes, while HFD causes for their reduction.

In conclusion, JNK1 absence proved to be beneficial when evaluating metabolic parameters and thus, its modulation could prove to be an interesting strategy for a novel pharmacological treatment of the sporadic forms of AD or other peripheral and central metabolic alterations.

Funding: This research was supported by funds from the Ministerio de Economía y Competitividad (SAF2017-84283-R) and the Biomedical Research Networking Centre in Neurodegenerative Diseases (CIBERNED) (CB06/05/2004).

07

One step beyond: using metabolism to assess therapy efficacy in preclinical glioblastoma

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*[1] Department of Biochemistry and Molecular Biology, Biosciences Faculty, UAB, Cerdanyola del Vallès, Spain. [2] Department of Animal Medicine and Animal Surgery, Veterinary Faculty, UAB, Cerdanyola del Vallès, Spain. [3] Department of Applied Mathematics, Liverpool John Moores University, Liverpool, UK. [4] Centro de Investigación Biomédica en Red (CIBER) – UAB, Cerdanyola del Vallès, Spain. *Email: AnaPaula.Candiota@uab.cat*

Introduction: Glioblastoma (GB) is the most common primary malignant brain tumour, with poor prognosis even after aggressive therapy (Temozolomide, TMZ, plus radiotherapy). Magnetic Resonance Spectroscopic Imaging (MRSI), coupled to pattern recognition, is being used for noninvasive assessment of therapy response to TMZ in preclinical GL261 GB [1, 2], proving also useful to evaluate other therapeutic approaches such as cyclophosphamide [3]. Volumetric, 3D-like MRSI was used in a longitudinal study with the GB preclinical model, and an oscillatory pattern of response to TMZ was detected with 6-7 days periodicity [1], which we hypothesize relates to the immune system cycle participation in response as described by others [4]. The purpose of the present study was to evaluate response a different schema of TMZ administration (every 6 days, as in [5]) and to characterize the cellular population contributing to the MRSI response pattern upon TMZ therapy.

Methods: GL261 GB tumours were induced in C57BL/6j mice (n=15) and TMZ administered every 6 days at 60 mg/kg (n=12) as in [6], while n=3 were controls. High-resolution T2w MRI and consecutive 14ms TE MRSI with 3-4 grids were acquired every 2 days [1]. A semi-supervised non-negative matrix factorization (NMF) methodology was applied for calculating nosologic maps [2] and the tumour responding index (TRI) [1]. Immunostainings for CD3 (T cells) and Iba-1 (microglia/macrophages) characterization were performed in 6 additional mice from [1] and analyzed with NDPView and Image J.

TRI oscillations (6.0±1.3 days) were confirmed in mice treated with the every-6-days TMZ administration (n=3), in agreement with 6.3±1.3 days reported in [1], which suggests cyclical spectral pattern changes along therapy time, while tumour volume remains essentially constant. MRSI acquired had good quality and the expected spectral features as in [1,2]. Significant differences ($p<0.05$) were found for CD3 (4.8±2.9 vs 3.3±2.5 positive cells/field) and Iba-1 (21.9±11.4 vs 16.8±9.7% of immunopositive areas), respectively, between the responding and non-responding areas predicted by the NMF. Responding zones achieved up to 42% Iba-1 immunopositivity, in contrast with a minimum of 1.4% in control/unresponsive.

Discussion/Conclusions: The 6-day TMZ administration schedule caused the TRI to oscillate at a frequency (ca. 6 days) agreeing with the mouse immune system cycle [7]. Accordingly, MRSI spectral changes could be reflecting immune system presence/activation/action against GL261 tumours. Immunohistochemistry showed higher number of T-lymphocytes and microglia/macrophages in zones classified by NMF as “responding”. Since macrophages can represent up to 30% within GB tumour mass [8], they could be partially contributing to spectral pattern changes. Indeed, the participation of the immune system in TMZ therapeutic effects has been described [4]. Results suggest that in this model, the MRSI response pattern could act as

an immune system biomarker, which could be useful for monitoring any therapeutic approach acting through immune system elicitation or boosting.

References

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08

BAT thermogenesis activation is linked to hypothalamic endocannabinoids in the development of diet-induced obesity in male and female mice

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The endocannabinoid (eCB) system plays a critical role in the central regulation of energy homeostasis and is linked to obesity development. However, the effect of acute and chronic high fat diet (HFD) administration on endocannabinoids levels and its relationship to thermogenesis and obesity development has not been studied. Our aim is to analyse the time-course of hypothalamic 2-arachidonoylglycerol (2-AG) and anandamide (AEA) levels in male and female mice during diet-induced obesity (DIO), and whether the differences observed correlate to changes in brown adipose tissue (BAT) thermogenesis, leptin response, body weight and circulating eCB.

Male and female C57BL/6J mice were fed a standard diet (SD) or HFD for 7, 14, 28, 60 and 90-days. Body weight, food intake, BAT thermogenesis and hypothalamic and plasmatic eCB were analysed on the time-course of the experiment.

High fat feeding induced a transient increase in hypothalamic eCB, overall at 7 days of feeding, followed by a decrease to basal levels at longer HFD exposure. This profile positively correlates with BAT thermogenesis activation, which also has a peak at 7 days of high fat diet, whereas it negatively correlates with leptinemia and plasmatic eCB. Body weight does not show a significant increase until 28 days or more. This profile was marked by a component of sexual dimorphism. The initial increase of hypothalamic eCB is produced by an increase expression of enzymes involved in the synthesis of 2-AG and AEA. Furthermore, peripheral administration of β3-adrenergic induced BAT thermogenesis and increased hypothalamic eCB. These results suggest that HFD activate BAT thermogenesis and this activation signals to the hypothalamus to synthesise eCB. Altogether, we postulate that there is a cause-consequence correlation between the BAT thermogenesis activation and hypothalamic eCB in the DIO model. This study could add insight into the understanding of hypothalamic regulation of obesity.

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09

Influence of maternal diet in the incidence of intrauterine growth restriction and cardiovascular remodelling

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Background – Intrauterine growth restriction (IUGR) is a common complication of pregnancy which affects 5-10% of newborns. It has been associated with a variety of adverse perinatal outcomes such as cardiovascular remodelling (CVR). IUGR increases the risks of intrauterine demise, neonatal morbidity, and neonatal death. Recently, some metabolic sensors highly influenced by diet as GDF15 have been suggested to have potential role as cardiomyokines. However, until date, no studies have evaluated their effect in neonatal development. **Aim** – To investigate the validity of novel metabolic cardiomyokine (GDF15) and the modulation of diet in the diagnosis and prevention of IUGR. **Methods** – A single-site, cross-sectional and observational study at the Maternal-Foetal Medicine Department of the Hospital Clinic of Barcelona (Spain) was conducted for two years. It included 20 IUGR pregnancies and 29 uncomplicated pregnancies with appropriate for gestational age newborns (CTL). Maternal serum was obtained at two occasions, at the first trimester and at delivery, to evaluate GDF15. In addition, cord blood was collected at delivery to measure levels of novel and established cardiomyokines (GDF15 and BNP). Clinical and diet data was also collected to evaluate IUGR and CVR association with maternal diet intake. **Results** – Compared with the CTL group, IUGR newborns had elevated BNP levels, validating the source of the studied sample; BNP levels were negatively correlated with birth percentile although not significantly (p-value 0.106). GDF15 was also significantly increased in IUGR newborns compared to controls (p-value 0.027). Additionally, IUGR mothers presented a significant increase of GDF15 levels at delivery compared to first trimester of gestation in front of non-significant differences in controls. From the dietetic point of view, IUGR consumed less monounsaturated fatty acids with statistical significant difference (p-value 0.045) and tend to consume less lipids, polyunsaturated fatty acids, vitamin E and olive oil. Birth percentile was positively and significantly correlated with total olive oil intake (p-value 0.029). Birth percentile was positively correlated with olive oil and lipids consumption, as well as polyunsaturated fatty acids consumption with placental weight, all of them not reaching statistical significance. **Conclusions** – According to differences among IUGR pregnant women (fatty acids, lipids, vitamin E and olive oil), Mediterranean diet and olive oil might be potential tools for prevention of this obstetric complication. Novel metabolic cardiomyokine GDF15 emerge as promising biomarker for IUGR, either at maternal and neonatal level. *Funding: PI15/00817 and PI18/00451 (ISCII-FEDER) and CIBERER.*

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Neuronal differentiation from neural crest stem cells of LRRK2 Parkinson's disease patients

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Background: In vivo access of dopaminergic neurons (DaN), the definite target tissue of Parkinson's disease (PD), is not possible. Despite the great amount of cell models to study this pathology, there are important limitations from most of them. DaN derived from induced pluripotent stem cells (iPSCs) requires dedifferentiation and redifferentiation processes with high economic and temporal costs as well as low differentiation efficiency rates. On the other hand, genetic PD associated to leucine-rich repeat kinase 2 (LRRK2) mutations has been related to mitochondrial dysfunction in different cell models, but never assessed in the present model of study. **Aims:** i) to develop neuronal differentiation from neural crest stem cells (NCSC) from PD patients carrying LRRK2 mutation and ii) to characterize mitochondrial respiration in NCSC adipose stem cells. **Methods:** Explants of adipose tissue biopsies from the forearm of a control and LRRK2G2019S-PD patient were cleansed, cut in 1-2 mm2 and cultured in DMEM supplemented with pyruvate, fetal bovine serum, growth factors and antibiotics to promote growth of NCSCs. Adipose stem cells specific markers CD73, CD90 and CD105 were confirmed by flow cytometry. DaN differentiation was induced using neurobasal medium supplemented with B27, epidermal and fibroblast growth factors. The following measurements were assessed in NCSCs and DaN in parallel: Neuronal markers MAP2, BIII tubulin and tyrosine hydroxilase were determined by western blot. Proliferation rates were analyzed by hemocytometer counting. Mitochondrial function was assessed in NCSCs by high resolution respirometry. **Results:** We observed positive signals for specific markers of both adipose stem cells and neuronal cells including dopaminergic differentiation, through tyrosine hydroxilase. As expected, growth rates were lower under neuronal differentiation. Finally, preliminary results suggest decreased respiratory rates in NCSC from LRRK2-PD patients with respect to the control. **Conclusions:** Neuronal differentiation from adipose stem cells exhibit neuronal markers and lower growth rates, as expected. Lower rates of overall mitochondrial respiration in NCSC from LRRK2-PD patients may account for mitochondrial suboptimal function in the model, as observed in the disease and other cell models. Altogether, our findings suggest that DaN/NCSC may represent an adequate model of study for PD, allowing for in vivo assessment.

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Metabolic reprogramming in skin-derived fibroblasts from Parkinson's Disease patients that carry PRKN mutations suggests the activation of the mitochondrial integrated stress response (ISRmt)

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Background: Mutations in PRKN cause early-onset Parkinson's disease (PRKN-PD), widely associated to mitochondrial dysfunction. In primary mitochondrial diseases, the presence of mitochondrial lesion activates cell-specific stress responses as part of the mitochondrial integrated stress response (ISRmt). The ISRmt comprises a nuclear transcriptomic and metabolic reprogramming driven by the activation of mTORC1 as a protective response in front of mitochondrial dysfunction. Eventually, metabolite and redox signaling including the 1C metabolism (de novo serine synthesis, folate metabolism, methyl cycle, transsulfuration and glutathione biosynthesis pathways) and pyridine nucleotide pools are modified to provide the affected cells with molecules to fight the mitochondrial lesion. The ISRmt has been reported in primary mitochondrial diseases and has been suggested to be relevant in pathologies with secondary mitochondrial dysfunction such as PD. We have previously reported upregulation of genes involved in the ISRmt in fibroblasts from PRKN-PD patients. **Objectives:** To explore if the deregulated transcriptomic pathways related to mitochondrial dysfunction-associated ISRmt in PRKN-PD skin-derived fibroblasts translate to alterations in the stress response pathways at molecular and functional level. **Methods:** We analyzed the aminoacidic profile, transsulfuration and glutathione biosynthesis pathways (including glutathione levels as well as glutathione reductase (GR) and peroxidase (GPx) enzymatic activities), pyridine nucleotide pools (NAD and NADP) and phosphorylated vs. total SP6 (mTORC1 substrate) in PRKN-PD (n=5) and control (n=7) fibroblasts by HPLC, Western Blot and spectrophotometry. **Results:** We found a moderate increase of overall amino acids levels in PRKN-PD compared to control fibroblasts. Specifically, significant increased levels of aspartate, aspargine, methionine and lysine were obtained. We observed significantly increased CTH protein levels in PRKN-PD fibroblasts. We observed upward trends in GR and GPx enzymatic activities and a significant increase in the ratio between the reduced and the oxidized forms of glutathione (GSH/GSSG) in PRKN-PD fibroblasts. Not significant trends to increased NADPH levels were found in PRKN-PD fibroblasts. Significant increased of p-SP6/SP6 ratio was found in PRKN-PD compared to control fibroblasts. These results suggest the enhancement of aminoacid synthesis and transsulfuration pathway, the latter leading to increased glutathione synthesis. Activation of these pathways could be regulated by activation of mTORC1. **Conclusions:** Our findings suggest that the activation of the

ISRmt observed in PRKN-PD fibroblasts at transcriptomic level translate to a metabolic reprograming in front of a potential mitochondrial dysfunction in these cells. mTORC1 activation identified in PRKN-PD fibroblasts may act as a key upstream regulator of the ISRmt. Our findings suggest that the activation of the ISRmt in front of mitochondrial dysfunction may occur in both primary mitochondrial and secondary mitochondrial diseases such as in PRKN-PD, opening up new areas of research to address therapeutic targets to modify the course of the disease.

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Mechanisms underlying changes in food preference during pregnancy: implications for fetal programming and obesity development

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Pregnancy entails remarkable whole-body biological adaptations. Extensive evidence in humans shows significant alterations in taste perception and nutrient preference throughout pregnancy, resulting in frequent food cravings with a recurrent search for high-caloric, high-palatable foods that can affect overall metabolism in both mothers and the offspring. However, the underlying mechanisms and neurocircuits implicated in taste perception and preference changes during pregnancy are still unknown. To investigate this, we use the C57Bl/6 mouse as an experimental model. Females exposed to a two-bottle taste preference paradigm show 25% increase in sweet sensitivity from the second week of pregnancy onwards. This increase in sensitivity is also observed when pregnant mice are exposed to a food-choice paradigm. In correlation to human observations, pregnant mice crave for high-palatable food, increasing binge eating behavior by 35%. Interestingly, all the behavioral differences observed arise during the second week of pregnancy, when 17β-estradiol and progesterone hormonal levels increase, and normalize after the weaning of the offspring. Indeed, pregnancy hormonal levels are directly correlated to the increase in sweet taste preference. fMRI studies show pregnancy-specific network functionality changes in brain regions responsible for reward and motor responses, including the limbic system, and the dorsal striatum, as well as in areas related to gustatory functions; the insular and piriform cortex, and the VPM thalamus. The behavioral and the functional changes in hedonic-related brain areas seen in pregnant mice suggests a link between pregnancy steroids surge and dopamine-induced motivational food behavior. Gene expression analysis and immunofluorescence studies confirm dopamine signaling alterations in both the VTA and the striatum that happen exclusively during pregnancy. In addition, the offspring of females with free or intermittent access to high-fat high-sucrose diet during pregnancy show altered glucose homeostasis and obesity predisposition. Our preliminary data suggests that pregnancy modifies the neuroconnectome of critical brain regions implicated in gustatory and motivational behavior, altering maternal dietary preferences and habits favoring the consumption of high-dense and –palatable food. These abnormal dietary patterns induced by pregnancy, in the prevailing western life-style, may underlie serious detrimental metabolic outcomes in both mothers and offspring leading to obesity.

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Deficient ER-mitochondrial phosphatidylserine transfer causes liver disease

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Non-alcoholic fatty liver is the most common liver disease worldwide. Here, we show that the mitochondrial protein mitofusin 2 (Mfn2) protects against liver disease. Reduced Mfn2 expression was detected in liver biopsies from patients with non-alcoholic steatohepatitis (NASH). Moreover, reduced Mfn2 levels were detected in mouse models of steatosis or NASH, and its re-expression in a NASH mouse model ameliorated the disease. Liver-specific ablation of Mfn2 in mice provoked inflammation, triglyceride accumulation, fibrosis and liver cancer. We demonstrate that Mfn2 binds phosphatidylserine (PS) and can specifically extract PS into membrane domains, favoring PS transfer to mitochondria, and mitochondrial phosphatidylethanolamine (PE) synthesis. Consequently, hepatic Mfn2 deficiency reduces PS transfer and phospholipid synthesis, leading to endoplasmic reticulum (ER) stress and the development of a NASH-like phenotype and liver cancer. Ablation of Mfn2 in liver reveals that disruption of ER-mitochondrial PS transfer is a new mechanism involved in the development of liver disease.

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GBA mutation influences mitophagy in 3D neurosphere models

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Glucocerebrosidase (*GBA*) mutations are the most important risk genetic factor for the development of Parkinson disease (PD). *GBA* encodes the lysosomal enzyme glucocerebrosidase (GCase). Loss of GCase activity in cellular and animal models have implicated lysosomal and mitochondrial dysfunction in PD disease pathogenesis, although the exact mechanisms remain unclear. We hypothesize that *GBA* mutations impair mitochondria quality control.

We have characterized mitochondrial content, carbonyl cyanide-m-chlorophenyl-hydrazine (CCCP)- induced mitophagy and macroautophagy flux in a 3D-neurosphere-model derived from neural crest stem cells containing heterozygous and homozygous $_{N370S}$*GBA* mutations.

Our findings indicate that mitochondrial accumulation occurs in mutant $_{N370S}$*GBA* neurospheres under basal conditions, and clearance of depolarised mitochondria is impaired following CCCP treatment. A significant increment in TFEB-mRNA levels, the master regulator of lysosomal and autophagy genes, may explain an unaltered macroautophagy flux in $_{N370S}$*GBA* neurospheres. PGC1α-mRNA levels were also significantly increased following CCCP treatment in heterozygote, but not homozygote neurospheres, and might contribute to the increased mitochondrial content seen in cells with this genotype.

Mitochondrial impairment occurs early in the development of GCase-deficient neurons. Furthermore, impaired turnover of depolarised mitochondria is not only a result of impaired fusion of autophagosomes with lysosomes, but at an earlier stage of mitophagy induction.

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Metabolic differences in the spectra obtained from 1H-NMR between physical and pharmacological stress test in a population at risk of cardiovascular events.

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Introduction: Cardiac Stress tests in combination with myocardial perfusion studies with radioisotopes are a key tool for the diagnosis of cardiovascular disease. Pharmacological stress for the diagnosis of ischemic heart disease is indicated in patients with incapacity for physical exercise or when it does not reach the minimum threshold. Clinical interpretation is similar for both stress and pharmacological stress however; populations are different and prediction power is lower for pharmacological testing. So, pharmacological testing does not completely reproduce dynamic changes that occur with physical activity and with this, useful information could be lost when evaluating the burden of ischemic heart diseases.

Objective: Evaluate any possible metabolic differences associated to physical stress and pharmacological stress tests in a population at risk of cardiovascular events

Methods: Serum samples from 126 consecutive patients were obtained before stress test and at the time of maximum intensity. According to the results of the tests, the cases and controls were defined as positive and negative for ischemia respectively (physical stress: 39 cases and 44 controls; pharmacological stress: 20 cases and 23 controls). 1H NMR spectra (CPMG 32 ms) were obtained at 9.4 Tesla for each sample and data was imported into SIMCA v 14.0 for pattern recognition. Unsupervised (PCA) classification and supervised discriminant analysis (OPLS-DA) were performed, models were considered significant when CV-ANOVA<0.05. Variables of interest were obtained from S-plots. Patient´s information was registered in a database.

Results: 76.2% of the population were men. Average age was 63.8 years (±10.1) in the group of physical stress patients likewise average age was 73.7 years (±8.8) in the pharmacological stress group which is consistent with previously published cohorts. There were no differences in the metabolic profile in samples obtained before the stress test. However, we were able differentiate between physical and pharmacological stress groups (CV ANOVA p=1.68e-18). These differences were due to the increase in lactate during the maximum effort reached in the physical stress group. Interestingly, the pharmacological stress samples obtained at the peak of activity clustered with physical stress samples obtained before performing the test.

Conclusion: Physical stress testing induces changes in the metabolic profile that are not reproduced in pharmacological testing. This evidence could contribute to reconsidering the clinical interpretation of the results and could lead to better diagnosis.

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Understanding dark/light metabolic switches in the retina

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Mounting studies from several laboratories point to deficiencies in energy metabolism as an underlying mechanism of pathology in the progression of inherited blindness. Reprogramming the metabolome is emerging as one promising therapeutic path to preserve cone function in different forms of retinitis pigmentosa. Understanding glucose metabolism in photoreceptor cells is therefore becoming of the utmost importance. We have recently undertaken a comprehensive phosphoproteomic analysis of the dark/light retina, that revealed a robust physiological regulation of intermediate enzymes in glucose metabolism. We have identified major phosphorylation checkpoints in the glucose metabolic pathway, that may serve to shunt metabolites and redirect fluxes to the pentose phosphate pathway and one-carbon metabolism and/or fatty acid synthesis depending on the bioenergetic demands in the dark and light states. Measurements of glucose uptake in dark/light retinas showed no changes in rate of entry, while light caused a 20% reduction in glucose flux at the fourth step of glycolysis, and a 40% reduction in oxygen consumption rate measured by high resolution respirometry. In conclusion, light triggers rapid metabolic switches that are likely central to photoreceptor function and viability in the widely varying illumination conditions of the natural world.

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Carnitine palmitoyltransferase 1C as the first negative regulator of the endocannabinoids hydrolase ABHD6

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Brain-specific carnitine palmitoyltransferase 1 (CPT1) C is an endoplasmic membrane protein that has minimal CPT1 catalytic activity but it is able to bind malonyl-CoA, a major regulator of the energetic status of the cell. CPT1C is involved in hypothalamic energy balance, synaptic plasticity and cancer (see Casals, N; 2016 for review). However, the exact mechanism by which CPT1C exerts its functions is poorly known. A proteomic study of WT and CPT1C deficient mice brains come up with a new putative partner of CPT1C, the α/β-hydrolase domain containing 6 (ABHD6), a serine hydrolase of the endocannabinoid system that catalyses the main endocannabinoid, 2-arachydonoyl glycerol (2-AG). Although ABHD6 is not the principal hydrolase of 2-AG, it has been demonstrated to be important for the regulation of several functions such as metabolic flexibility, inflammation, insulin secretion, adipose tissue browning and brown fat activation, development of epilepsies and cancer. In the present study, we have developed a high sensitive fluorescent hydrolase activity assay based on the ABHD6 ability to hydrolyze the 4-methylumbelliferyl-heptanoate (4-MUH) substrate. This method allows studying ABHD6 activity in HEK293T cells and, for the first time, detecting ABHD6 activity in hypothalamic and hippocampal homogenates. ABHD6 specific activity has been proved by its selective inhibitor WWL70. Moreover, we have confirmed the interaction of CPT1C with ABHD6 by both FRET analysis and immunoprecipitation. We have found that this interaction is crucial for the regulation of ABHD6 activity, since CPT1C inhibits more than 50% of ABHD6 activity, and this regulation depends on malonyl-CoA detection by CPT1C. Currently, we are focus on studying this effect on CB1 receptor downstream signaling. Altogether, we have identified CPT1C as the first negative regulator of ABHD6 activity, which brings new insights into the understanding of this enigmatic hydrolase of the endocannabinoid system.

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High PI3K activity in EC promotes lipids utilisation and protects from obesity

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In response to nutritional variation, white adipose tissue (WAT) undergoes a physiological remodelling that involves qualitative and quantitative changes in resident cells and is coordinated with angiogenesis. In a condition of chronic over nutrition WAT expansion is associated to insufficient vascularisation which in turn leads to local hypoxia, inflammation and adipocytes death (hallmark of obesity). Currently, enhanced WAT angiogenesis is believed to be a promising intervention to ameliorate obesity associated metabolic dysfunctions. However, we still lack understanding of the cell intrinsic function of endothelial cells (EC) in WAT remodelling. Here we take advantage of our mouse model of PTEN (a dual lipid/protein phosphatase that negatively regulate the activity of PI3K) deletion in EC to promote vessel growth, in a cell autonomous manner. To this end, we crossed Ptenflox/flox mice with PdgfbiCreERT2 transgenic mice that express a tamoxifen-inducible Cre recombinase in EC; 4-hydroxytamoxifen was administered in vivo at postnatal day 1 (P1) and P2 to activate Cre expression.

Increased EC proliferation, induced by enhanced PI3K activity, promotes vascular hyperplasia exclusively in WAT and leads to a progressive reduction of fat mass not associated with increased thermogenesis, WAT browning or defective nutrient absorption. Improved adipose vascularisation prevents from high fat diet induced WAT hypertrophy, limits body weight gain and ameliorate glucose tolerance. Increased PI3K activity in EC induces a metabolic switch towards an oxidative metabolism and makes EC addicted to lipids. In vivo inhibition of □-oxidation is sufficient to revert vascular hyperplasia thereby preventing excessive WAT depletion. Taken together our results suggest that the level of PI3K activity in EC defines metabolic fuel preference and determines the differential ability of EC to proliferate in a lipid enriched environment. Specifically high PI3K activity in EC promotes lipids mobilisation and usage; thus preventing unhealthy WAT expansion and consequently the onset of obesity related comorbidity.

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Increased localization of APP-C99 in mitochondria associated ER membranes causes mitochondrial dysfunction in Alzheimer disease

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In the amyloidogenic pathway associated with Alzheimer disease (AD), the amyloid precursor protein (APP) is cleaved by β-secretase to generate a 99-aa C-terminal fragment (C99) that is then cleaved by γ-secretase to generate the β-amyloid (Aβ) found in senile plaques. In previous reports, we and others have shown that γ-secretase activity is enriched in mitochondria-associated endoplasmic reticulum (ER) membranes (MAM), and that ER-mitochondrial connectivity and MAM function are upregulated in AD. We now show that C99 is localized to MAM, where it is normally processed rapidly. In AD, however C99 accumulates above normal levels in MAM regions, resulting in increased sphingolipid turnover and an altered lipid composition of both MAM and mitochondrial membranes. In turn, this change in mitochondrial membrane composition interferes with the proper assembly and activity of mitochondrial respiratory supercomplexes, thereby likely explaining the bioenergetic defects characteristic of AD.

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Oncostatin M is elevated in patients with insulin resistance, induces inflammation in human adipocytes and this effect is partially revert by experimental immunoneutralization

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Background: Oncostatin M (OSM) is a cytokine that has been described to play a key role in inflammation. However, its contribution to the inflammatory state during obesity is not fully understood. Here, we aim to evaluate the levels of OSM in patients with obesity and altered glucose homeostasis, the effects of OSM on human adipocytes, and also whether OSM immunoneutralization could revert metabolic impairments caused by a high-fat diet (HFD) in mice.

Subjects: 28 patients with severe obesity and 4 individuals with normal weight were included in this study. Additionally, obese patients were stratified according into two groups by glycemia status: obese normoglycemic and obese hyperglycemic. Subcutaneous and visceral white adipose tissue were obtained to examine OSM gene expression. Human adipocytes were used to evaluate the effect of OSM in the inflammatory response. Finally, HFD-fed C57BL/6J mice were injected with anti-OSM antibody to evaluate its effects.

Results: OSM was elevated in subcutaneous but not in visceral fat from patients with obesity and hyperglycemia, and correlated with insulin levels, HOMA-IR, and inflammatory markers. OSM completely inhibited adipogenesis and induced inflammation in human mature adipocytes. Finally, OSM receptor KO mice showed increased GLUT4 mRNA levels, and OSM immunoneutralization resulted in a reduction of glucose levels and CCL2 expression in mice fed a HFD.

Conclusions: OSM may contribute to the inflammatory state during obesity, and could be implicated in the development of insulin resistance. Therefore, its pharmacological inhibition in conditions of obesity could be a potential approach to reduce symptoms of type 2 diabetes.

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POMC neuron-derived neurosteroids: a potential link between central insulin resistance and cognition

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Extensive evidence demonstrates an overlap between metabolic diseases, such as obesity and type 2 diabetes (T2D), and cognitive pathologies. Interestingly, both insulin resistance (IR) and mitochondrial dysfunction are common features of the aforementioned disorders. Mitochondria play numerous essential cellular functions, including steroid biosynthesis, which are well-known molecules implicated in cognition. Our hypothesis proposes that alterations in brain-derived steroids (neurosteroids) underlie the cognitive decline in the context of central IR and metabolic conditions. To initially address this hypothesis, we fed C57Bl/6 mice with western diet (WD) for four days (4d), an insufficient time-frame to cause overweight or overt metabolic complications. Nevertheless, this short-term schedule caused a significant attenuation in insulin signaling and a decrease in mitochondrial respiratory capacity in the arcuate nucleus of the hypothalamus (ARC) of 4d-WD mice. No changes were observed in the hippocampus or cortex. Mice on 4d-WD exhibited cognitive impairments, as evaluated by the Barnes maze (BMT) and the novel object recognition test (NORT). Remarkably, these alterations were accompanied by a ~50% reduction in the ARC levels of the steroid-precursor pregnenolone.

Given that the ARC was particularly susceptible to the deleterious effects of WD, we focused on this brain region. POMC neurons represent a major neuronal population of the ARC implicated in energy balance and metabolic control. To assess the potential role of POMC neuron-derived neurosteroids in central IR and cognitive function, we generated mice lacking the mitochondrial cholesterol transporter STARD1 exclusively in POMC neurons (POMCStarD1KO), thus preventing neurosteroid synthesis. As expected, we observed a ~50% reduction in pregnenolone concentration in the ARC. POMCStarD1KO mice displayed normal body weight and glucose metabolism under both standard or long-term WD. However, we observed attenuated insulin signaling specifically in the ARC of mutant animals. Interestingly, POMCStarD1KO mice performed poorly in a NORT, suggesting that reduced levels of POMC neuron-derived neurosteroids is sufficient to produce cognitive impairments.

Collectively, our studies unveil a potential axis linking hypothalamic IR, mitochondrial malfunction, cognitive decline and reduced neurosteroid synthesis. Further research is needed to precisely determine the role of POMC neurons and the molecular mechanisms underlying the suggested metabolic-cognitive connection.

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Secretome of adipose-derived stem cells is altered in type 2 diabetic patients

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The adipose tissue and its resident stem cells are altered in metabolic disorders. These cells, so called adipose-derived stem cells (ASCs), possess a central role in maintaining adipose tissue homeostasis by regulating adipocyte turnover. Moreover, ASCs are gaining popularity as a cellular-based tool for clinical applications due to their immunomodulatory and wound healing capabilities. These functions are partially mediated by secreted factors involved in paracrine signaling routes which in type 2 diabetic (T2D) patients could be compromised. Here, we use an untargeted proteomics approach by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to identify and quantify the secreted factors by cultured ASCs isolated form the subcutaneous adipose tissue of obese (control) and T2D donors. Out of 231 quantified proteins by LC-MS/MS, 52 factors were found differentially secreted by T2D-ASCs. Among them, the secretion of proteins associated with wound healing and protease inhibitors are downregulated, whereas factors implicated in immune system processes, extracellular matrix organization and endoplasmic reticulum stress are found to be more secreted in T2D-ASCs. These results suggest that a comprehensive analysis of secreted proteins by untargeted proteomics could be appropriate for the study of the altered behavior of stem cells under pathological conditions, to find new biomarkers as indicators of ASC functionality and to assess the suitability of using ASC-related products for therapy depending on the donor metabolic conditions.

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Hepatic steatosis in female rats induced by high-fat high-fructose diet: effects of caffeine and green coffee extract

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Background: Non-alcoholic fatty liver disease (NAFLD) has a multifactorial etiology, including environmental factors such as the excessive consumption of fat-enriched diets and beverages sweetened with simple sugars such as fructose. To study NAFLD underlying mechanisms and to identify new potential pharmacological targets, we are using a model in which female rats were fed a high-fat diet, as an exogenous source of fatty acids, supplemented with 10% fructose in drinking water, to induce de novo hepatic lipogenesis.

Methods: Eight-week-old female Sprague-Dawley rats were randomly distributed into 4 groups (n=12): control (CT; standard rodent chow), fructose plus high fat diet (FHFD; high fat diet [46.9% kcal as saturated fat from cocoa butter] and 10% w/v fructose in drinking water), FHFD plus caffeine (CAF; 5 mg/Kg/day) and FHFD plus green coffee extract (EXT; 250 mg/Kg/day, providing 5mg/Kg/day of caffeine). The CT and FHFD rats were fed with the respective diets for three months. The CAF and EXT groups received the FHFD diet for the first two months, and then the diets were supplemented with CAF or EXT for the last month of the protocol. Biochemical and zoometric parameters were determined, an oral glucose tolerance test was performed at the end of the experiment, and gene/protein expression in hepatic tissue is currently being measured.

Results and discussion: Our aim was to obtain a model of hepatic steatosis where the origin of lipids deposited in the liver was partly exogenous (provided by the high-fat diet) and partly derived from de novo lipogenesis (induced by liquid fructose supplementation). Despite rats supplemented with FHFD consumed almost twice more calories than controls, their body weight at the end of treatment was not increased compared to CT. However, significant hepatic triglyceride accumulation, increased liver weight, hypertriglyceridemia and an abnormal glucose tolerance test were observed in FHFD rats. Neither CAF nor EXT significantly affected these parameters related to non-treated FHFD rats. Currently, we are performing western blot analysis to determine if three main nodes related to energy metabolism have been affected in this model (mechanistic target of rapamycin [mTOR], autophagy, and endoplasmic reticulum stress), and whether treatment with CAF or EXT modulated these alterations. Our preliminary results show an increase in phosphorylated IRE1 in FHFD rats, which is further enhanced by CAF treatment. Moreover, CAF increased ULK-1 phosphorylation, an autophagic marker. Given the crosstalk between these pathways, we will explore whether CAF effects on autophagy are related to the activation of the IRE1 branch of the endoplasmic reticulum stress.


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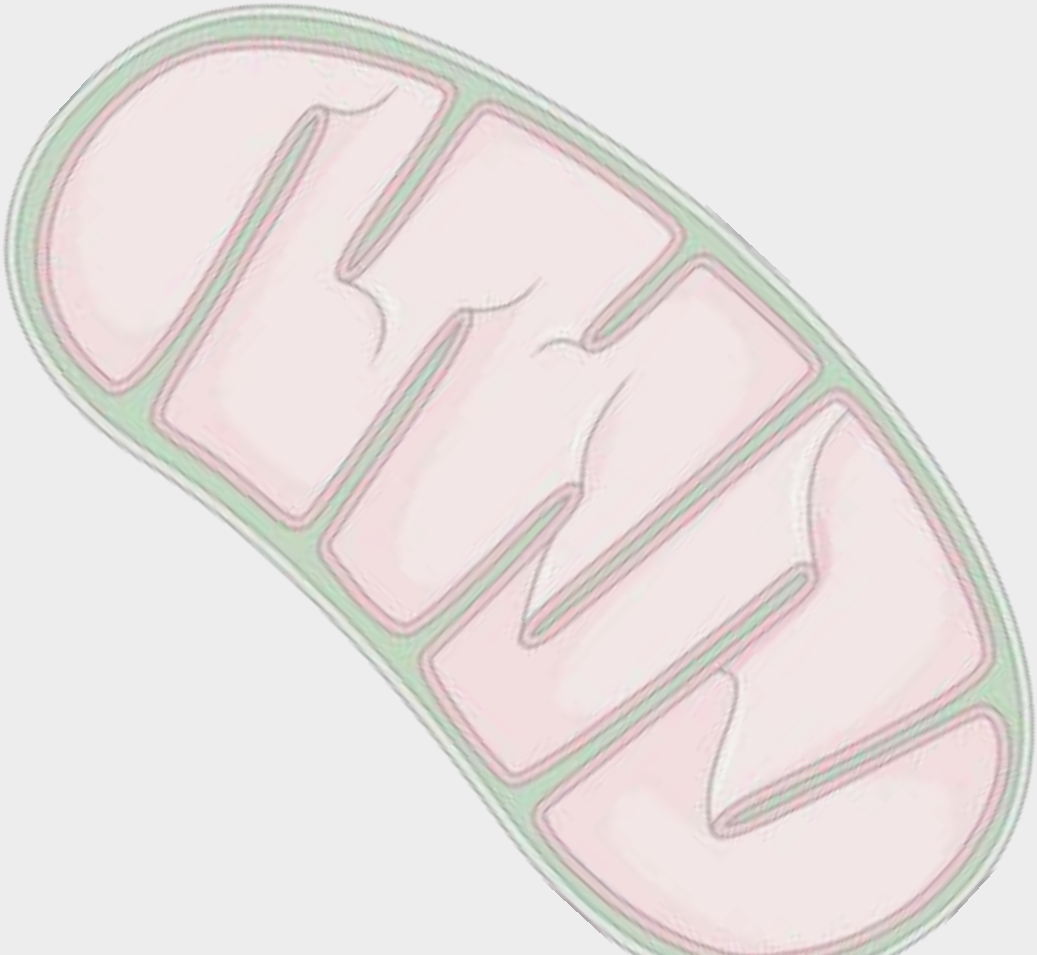
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