

CIBERDEM ANNUAL MEETING 2016

May 11th-13th
Hotel Campus
Campus UAB
Cerdanyola del Vallès
Barcelona

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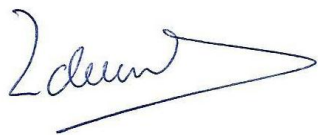
WELCOME LETTER

Dear CIBERDEM investigators,

It is my pleasure to welcome you to our Annual Meeting. The program of the meeting has been structured aiming to stimulate the interaction among CIBERDEM investigators and to reflect the quality and diversity of our research activity. This would have not been possible without your enthusiastic participation contributing a large number of abstract that we very much appreciate.

The opening lecture, in memory of Dr. Anna Maria Gomez Foix, will be the occasion to remember and honor the scientist and the friend. On the next two days, the meeting has been organized in three blocks of scientific sessions based on our three research programs. Each block includes an initial State of the Art lecture followed by several oral presentations from the different research groups. Poster sessions are fundamental to stimulate the interaction among investigators, an essential aspect of the meeting, and we have tried to arrange them in a somewhat more relaxed ambience. We will also have a session to update our knowledge on CIBERDEM Platforms, their organization, achievements and services. Finally, we will cover two relevant questions particularly important for the future of CIBERDEM research: funding opportunities, that will be discussed in a pre-meeting workshop addressed specifically to CIBERDEM post-docs, and technology transference.

I hope that you will find the meeting rewarding both scientifically and personally, that it fulfills your expectations, and I wish you a pleasant stay in the natural environment of the UAB Campus.

A handwritten signature in black ink, appearing to read 'Eduard', followed by a long, horizontal, slightly curved line that tapers to a point on the right side.

Eduard Montanya
Scientific Director, CIBERDEM

Wednesday, May 11th

15:30 REGISTRATION

16:00-17:30 WORKSHOP “Public funding opportunities for young CIBERDEM investigators”

Deborah Burks (Centro de Investigación Príncipe Felipe)
Angel Nadal (Universidad Miguel Hernández)
Joan J. Vendrell (Fundación Instituto de Investigación Sanitaria Pere Virgili)

Addressed to CIBERDEM Post-Docs

18:30 OPENING SESSION

Eduard Montanya, Scientific Director, CIBERDEM

18:45-19:30 OPENING LECTURE. In Memoriam Dr. Anna Maria Gomez Foix

Insights into muscle metabolism from cellular and animal models: Anna Maria Gómez-Foix,
1995-2015

Loranne Agius (Institutes of Cellular Medicine and Ageing and Health, Medical School, Newcastle
University)

Chair: Joan Guinovart (IRB, Barcelona)

19:30-21:00 POSTER AND REFRESHMENTS

19:30 CIBERDEM PIs MEETING

21:00 DINNER

Thursday, May 12th

9:00 PROGRAM 1 SESSION

*Chair: Angela Martinez Valverde (Consejo Superior de Investigaciones Científicas, Madrid)
Ricardo Rodríguez Ricardo Rodríguez (Pere Virgili Health Research Institute, Reus)*

9:00-9:30 State of the Art lecture

Diabetes: a lipid metabolism disease. New insights from metabolomic studies

LLuís Masana (Pere Virgili Health Research Institute, Reus)

9:30-11:00 Oral Presentation session 1

9:30 OP1. PCSK9 circulating levels and CETP plasma activity are associated independently of lipid lowering therapies

Josefa Girona¹, Daiana Ibarretxe¹, Núria Plana¹, Sandra Guaita¹, Núria Amigó², Mercedes Heras¹, and Luis Masana¹
¹ Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, "Sant Joan" University Hospital, Universitat Rovira i Virgili, IISPV, Reus, CIBERDEM

9:45 OP2. A novel GLP-1/Glucagon receptor dual agonist improves non-alcoholic steatohepatitis and liver regeneration in mice

MP Valdecantos, V Pardo, E Fernández-Millán, C García-Monzón, AI Arroba, L Ruiz, A González-Rodríguez, F Escrivá, C Álvarez, C Barbas, A Konkar, J Grimsby, CM Rondinone, AM Valverde
Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM), Madrid

10:00 OP3. Mitochondrial biogenesis induced by calorie restriction in white adipose tissue is mediated by PGC-1s co-activators: influence on glucose homeostasis

L. Cervela¹, M. de Marco¹, R. Pardo¹, P. Gama², P. García-Rovés², R. Simó¹, J. A. Villena¹
¹ Vall d'Hebron Institut de Recerca, Barcelona

10:15 OP4. Topical administration of somatostatin prevents retinal neurodegeneration in a spontaneous model of type 2 diabetes

Lidia Corraliza, Patricia Bogdanov, Cristina Solà-Adell, Cristina Hernández, Rafael Simó
Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, Barcelona, CIBERDEM

10:30 OP5. The cannabinoid sensitive receptors GPR55, GPR18 and CB1 might modulate inflammatory response in type 2 diabetes

S.Y. Romero-Zerbo, V. Espinosa-Jimenez, N. Cobo, B. Gauthier, F.J. Tinahones, G. Rojo, F.J. Bermudez-Silva
IBIMA-Hospital Regional de Málaga

10:45 OP6. Low-frequency and rare variants in type 2 diabetes mellitus by exome-sequencing
Galan-Chilet I¹, Pérez D¹, Martínez-Barquero V¹, Martínez-Hervas S², Real JT², Ascaso JF², Pérez-Soriano C¹, Marín P¹, García AB^{1,3}, Chaves FJ¹

¹ Genetic Diagnosis and Genotyping Unit, Biomedical Research Institute Hospital Clinic of Valencia (INCLIVA)

11:00-11:30 Coffee break

11:30-12:45 Oral Presentation session 2

11:30 OP7. The autoimmune disease candidate gene dexi modulates virus-induced pancreatic beta cell inflammation and death

Izortze Santin¹, Reinaldo Dos Santos², Laura Marroquí², Amaia Jauregi-Miguel³, Decio L. Eizirik² and Luis Castaño¹.
¹ *Endocrinology and Diabetes Research Group, BioCruces Health Research Institute, Barakaldo, CIBERDEM*

11:45 OP8. Fatty acids regulate TNF α levels released from adipocyte by changes in the methylation status of the TNF α promoter

Eva García-Escobar¹, Elehazara Martín-Rubio¹, Natalia Colomo¹, Juan M Gomez-Zumaquero², Sergio Valdés¹, Sara García-Serrano¹, Ana Lago-Sampedro¹, Gemma Rojo-Martinez¹
¹ *UGC Endocrinología y Nutrición, Instituto de Investigación Biomédica de Málaga (IBIMA)*

12:00 OP9. Glycemic control and antidiabetic treatment trends in patients with type 2 diabetes cared for in primary care during 2007-2013 in catalonia

Manel Mata Cases^{1,2,3}, Josep Franch Nadal^{2,3,4}, Jordi Real², Dídac Mauricio^{2,4,5}
¹ *Centre d'Atenció Primària La Mina, Sant Adrià de Besòs (Barcelona). Gerència d'Àmbit d'Atenció Primària Barcelona Ciutat, Institut Català de la Salut,* ² *Grup DAP_Cat (Grup de Recerca en Diabetis en Atenció Primària a Catalunya) de la Unitat de Suport a la Recerca de Barcelona de l' Institut Universitari d'Investigació en Atenció Primària Jordi Gol (IDIAP-Jordi Gol),* ³ *CIBERDEM*

12:15 OP10. ApoA-I mimetic administration, but not increase in ApoA-I-containing HDL levels, reduces tumour growth in mice with inherited breast cancer

Lidia Cedó^{1,2}, David Santos^{1,2}, Annabel García-León¹, Lucía Baila-Rueda³, Josep Maria Carbó⁴, Antonio Zorzano⁵, Annabel F. Valledor⁴, Enrique Lerma^{1,6}, Fernando Civeira³, Srinivasa T. Reddy⁷, Joan Carles Escolà-Gil^{1,2,8}, Francisco Blanco-Vaca^{1,2,6,8}
¹ *Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Institut d'Investigacions Biomèdiques (IIB) Sant Pau, Barcelona,* ² *CIBERDEM*

12:30 OP11. Genetic and pharmacological HDL-raising strategies partly rescue macrophage-specific reverse cholesterol transport in diabetic mice

Teresa L. Errico^{1,2}, Lídia Cedó^{1,2}, David Santos^{1,2}, Joan C. Escolà-Gil^{1,2}, Francisco Blanco-Vaca^{1,2}, Josep Julve^{1,2}
¹ *Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau. Institut d'Investigacions Biomèdiques Sant Pau, Barcelona,* ² *CIBERDEM*

13:00-13:30 LECTURE: The relevance of technology transference in biomedical research and its management in CIBER

Luzma García Piqueres (Transferencia Tecnológica, CIBER, Madrid)

Chair: Eduard Montanya, Scientific Director CIBERDEM

13.30-15:30 LUNCH AND POSTERS

15:30 PROGRAM 2 SESSION

Chair: *Franz Martin Bermudo (Universidad Pablo de Olavide, Sevilla)*
Noèlia Tellez (IDIBELL, Barcelona)

15:30-16:00 State of the Art lecture

Non-coding genome function in pancreatic islets and diabetes

Jorge Ferrer (IDIBAPS, Barcelona)

16:00-17:30 Oral Presentation session 3

16:00 OP12 Identification of new mechanisms involved in the postnatal regeneration of endocrine pancreas: role of the autophagy in beta-cells and its relationship with IGF-2

Fernández-Millán E¹, Álvarez-Cilleros D², de Toro-Martín J³, Lizárraga-Mollinedo E^{1,4}, Escrivá F^{1,2}, Álvarez C^{1,2}
¹*Facultad de Farmacia, UCM, Madrid, CIBERDEM*

16:15 OP13. AAV-mediated specific overexpression of IGF1 in the pancreas prevents and counteracts type 1 diabetes

Cristina Mallo, Estefania Casana, Veronica Jimenez, Alba Casellas, Claudia Jambrina, Victor Sacristan, Meritxell Morró, Laia Vilà, Xavier León and Fatima Bosch

Center of Animal Biotechnology and Gene Therapy and Department of Biochemistry and Molecular Biology, School of Veterinary Medicine, Universitat Autònoma de Barcelona, Bellaterra, CIBERDEM

16:30 OP14. Cellular patterning of insulin/IGF1 signalling by insulin receptor substrate 2 gene expression

Manzano Núñez F., Acosta Umanzor C., Leal Tassias A., Burks DJ., Noon LA.

Centro de Investigación Príncipe Felipe, Valencia

16:45 OP15. Protein tyrosine phosphatase a novel target for the improvement of revascularization and survival of transplanted islet grafts

H. Figueiredo, A.L. Castillo Figueroa, A. García, R. Fernández-Ruiz, R. Malpique, R. Gomis

Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, CIBERDEM

17:00 OP16. Role of GATA6 in pancreatic β cell function

Anabel Rojas¹, Laura Villamayor Coronado¹, Raquel Araujo¹, Bernat Soria¹, Francisco Martín¹, David Cano²

¹*CABIMER, Sevilla, CIBERDEM*

17:15 OP17. Umbilical Cord Mesenchymal Stromal Cells Delay the Onset of Hyperglycaemia in the RIP-B7.1 model of type 1 diabetes

Esther Fuentes-Martin, Christian Lachaud C, Nadia Cobo-Vuilleumier, Franz Martin, Karim Hmadcha, Benoit Gauthier, Bernat Soria

¹*CABIMER, Sevilla, CIBERDEM*

17:30 – 18:00 Coffee break

18:00-19:15 Oral Presentation session 4

18:00 OP18. β -cell dedifferentiation and reduced neogenesis contribute to impaired β -cell mass regeneration in middle-aged rats

Noèlia Téllez, Marina Vilaseca, Yasmina Martí, Arturo Pla, Eduard Montanya
IDIBELL, Barcelona, CIBERDEM

18:15 OP19. Insulin secretion is inhibited by cortistatin-14 in pancreatic beta cells

M. Castellano-Muñoz, A. Rafacho, P. Alonso-Magdalena, E. Irlles, M. Bello, B. Merino, S. Soriano, P. Iborra, A. Ruiz, R. M. Luque, A. Nadal, I. Quesada
Universidad Miguel Hernandez, Alicante

18:30 OP20 Targeting inflammation in pancreatic islets

Júlia Rodríguez, Carlos Castaño, Gema Alcarraz-Vizán, Sara de Pablo, Mercè Obach, Joel Montané, Marcelina Párrizas, Anna Novials, Joan-Marc Servitja
Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, CIBERDEM

18:45 OP21. Exosomal miRNAs participate in the regulation of glucose homeostasis in mice

Marcelina Párrizas, Carlos Castaño, Maria Pallarés, Laura Brugnara and Anna Novials
Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona

19:00 OP22. Altered central and peripheral circadian clocks affecting energy metabolism in congenitally blind mice show differential entrainment by time-restricted feeding

Río-Martín A. R.¹, Fernández-Pérez, A.^{1,2}, de la Villa, P.³ and Vallejo, M.^{1,2}
¹*Instituto de Investigaciones Biomédicas Alberto Sols, CSIC/UAM, Madrid*, ²*CIBERDEM*

19:30-21:00 POSTERS AND REFRESHMENTS

21:00 DINNER

Friday, May 13th

9:00 PROGRAM 3 SESSION

*Chair: Antonio Zorzano (IRB, Barcelona)
Maria Inseser (Servicio Madrileño de Salud)*

9:00-9:30 State of the Art lecture

Treatment of Polycystic Ovary Syndrome in Adolescence: Towards a therapy targeting adipose tissue and insulin resistance

Lourdes Ibáñez (Fundación para la investigación y la docencia Sant Joan de Deu, Barcelona)

9:30-11:00 Oral Presentation session 5

9:30 OP23. Stimulated Occurrence and Biological Activity of Palmitoleic Acid and Its Isomers in Metabolic Diseases

*Clara Meana, Carlos Guijas, María A. Balboa and Jesús Balsinde
Instituto de Biología y Genética Molecular, CSIC, Valladolid*

9:45 OP24. Hyperactivation of mTORC1 in pancreatic β cells overexpressing human amylin causes a blockade in autophagic flux and an induction of apoptosis

*Guillén C, García Aguilar A, García Hernández M, Benito M
Facultad de Farmacia, UCM, Madrid*

10:00 OP25. Insulin receptor isoform α ameliorates glucose intolerance in diabetic mice

*O Escribano, S Diaz-Castroverde, A Gómez-Hernández, S Fernández, G García-Gómez, E Fernández-Millan, N Beneit, M Benito
Facultad de Farmacia, UCM, Madrid, CIBERDEM*

10:15 OP26. Metabolomics reveals impaired maturation of HDL particles in adolescents with hyperinsulinaemic androgen excess

*Samino S, Vinaixa M, Díaz M, Beltran A, Rodríguez MA, Mallol R, Heras M, Cabre A, Garcia L, Canela N, de Zegher F, Correig X, Ibáñez L, Yanes O
Universitat Rovira i Virgili, IISPV and Hospital Sant Joan de Déu, Reus*

10:30 OP27. Liver glycogen, a novel target to treat diabetes and obesity

*Iliana López-Soldado, Rebeca Iris Fuentes, Jordi Duran, Joan J. Guinovart
Institut de Recerca Biomèdica (IRB), Barcelona, CIBERDEM*

10:45 OP28. Genetic models rule out a major role of beta cell glycogen in the control of glucose homeostasis

Joan Mir-Coll^{1,2}, Jordi Duran^{1,3}, Felipe Slebe^{1,3}, Mar García-Rocha^{1,3}, Ramon Gomis^{2,3}, Rosa Gasa^{2,3}, Joan J. Guinovart^{1,3}

¹Institut for Research in Biomedicine, ³CIBERDEM

11:00 -11:30 Coffee break

11:30-13:00 Oral Presentation session 6

11:30 OP29. Characterization of serum microRNAs profile of polycystic ovary syndrome (PCOS), and obesity

María Insenser*, Mora Murri*, Elena Fernández-Durán, Jose L. San-Millán, Héctor F. Escobar-Morreale
Diabetes, Obesity and Human Reproduction Research Group, Hospital Universitario Ramón y Cajal & Universidad de Alcalá & Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, CIBERDEM

* These authors contributed equally to this work

11:45 OP30. BACE1 contributes to saturated fatty acid-induced ER stress, inflammation and insulin resistance in skeletal muscle cells

G. Botteri¹, L. Salvadó¹, A.M. Gómez-Foix², G. Montagut³, M.L. Ashford³, E. Barroso¹, X. Palomer¹, M. Vázquez-Carrera¹

¹Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy, University of Barcelona

12:00 OP31. ASCs from obese environment are more resistant to apoptosis: A potential role of survivin
Victoria Ceperuelo-Mallafre^{1,2} and Miriam Ejarque^{1,2}, Carolina Serena^{1,2}, Noelia Keiran^{1,2}, Catalina Núñez-Roa^{1,2}, Kelly Roche^{1,2}, Joan Vendrell^{1,2}, Sonia Fernández-Veledo^{1,2}

¹ Hospital Universitari de Tarragona Joan XXIII-Institut d'Investigació Sanitària Pere Virgili-Universitat Rovira i Virgili, Tarragona, ²CIBERDEM

12:15 OP32. Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells

Serena C., Keiran N, Ceperuelo-Mallafre V, Maymó E, Nuñez C, Ejarque M, Roche K, Vendrell J, Fernández-Veledo S

¹Hospital Universitari de Tarragona Joan XXIII. Institut d'Investigació Sanitària Pere Virgili. Universitat Rovira i Virgili, Tarragona, CIBERDEM

12:30 OP33. Mitofusin 2 links mitochondrial fitness with age-related metabolic disease and sarcopenia
David Sebastián, Eleonora Sorianoello, Jessica Segalés, David Sala, Maria Isabel Hernández-Álvarez, Antonio Zorzano
Institute for Research in Biomedicine (IRB Barcelona)

12:45 OP34. Mitofusin 2 is a phosphatidylserine transfer protein involved in liver cancer
María Isabel Hernández-Alvarez^{1,2,3}, David Sebastián^{1,2,3}, Saška Ivanova^{1,2,3}, Paola Bartocioni^{1,2,9}, Natalia Plana¹, Nuno Vasconcelos¹, Peddinti Gopalacharyulu⁴, Anna Adrover¹, Rui Castro⁵, Antonio Berenguer-Llargo¹, David Sala⁷, Tuulia Hyotylainen⁸, Matej Orešič⁸, Carles Cantó⁶, Manuel Palacin^{1,2,9}, and Antonio Zorzano^{1,2,3}

¹ Institute for Research in Biomedicine (IRB Barcelona), Institute of Science and Technology, Barcelona,

²Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Barcelona, ³CIBERDEM

13:00-13:45 CIBERDEM Platforms

Chair: Anna Novials (IDIBAPS, Barcelona)

Biorepositorio de Diabetes y Enfermedades Metabólicas CIBERDEM-IDIBAPS

Veronica Fernández Pascual (Technical coordinator Blood and Fluid Biobank, Biobank HCB-IDIBAPS, Barcelona)

Metabolomics Platform: new organization, objectives and research activities

Oscar Yanes (Coordinator Metabolomics Platform, Universitat Rovira i Virgili, Reus, CIBERDEM)

13:45-14:00 CLOSE OF MEETING

ORAL PRESENTATIONS

OP1. PCSK9 circulating levels and cetsp plasma activity are associated independently of lipid lowering therapies

Josefa Girona¹, Daiana Ibarretxe¹, Núria Plana¹, Sandra Guaita¹, Núria Amigó², Mercedes Heras¹, and Luis Masana¹

¹*Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, "Sant Joan" University Hospital, Universitat Rovira i Virgili, IISPV, Reus.* ²*Biosfer Teslab, Reus and Department of Electronic Engineering, Universitat Rovira i Virgili, IISPV, Tarragona.* ³*Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain.*

Aim: PCSK9 inhibition is a new powerful cholesterol-lowering therapy. Recently, it has been communicate that CETP inhibitors could influence PCSK9 levels as off-target effect. We have explored the relationship of PCSK9 levels and CETP activity in patients with metabolic diseases not on lipid lowering therapy.

Methods: Plasma CETP activity and PCSK9 levels were measured in 450 participants (mean age, 58 years; 49% women) who attended the metabolism unit because, metabolic syndrome (MetS) (78%), atherogenic dyslipidemia (32%), obesity (50%), diabetes (72%), and other risk factors (13%) after six week lipid lowering drug wash-out period.

Results: Plasma PCSK9 levels and CETP activity were significantly elevated in MetS (12,4%, p=0,004 and 11,2%, p<0,0001, respectively). Remarkably, plasma PCSK9 levels were positively correlated with CETP activity in whole population (r=0,256, p<0,0001) independently of age, gender, body mass index (BMI), systolic blood pressure (SBP), triglycerides, LDL-C and glucose. Individuals with the loss-of-function PCSK9 genetic variant rs11591147 (R46L) that have lower levels of PCSK9 (36,5%, p<0,0001) and LDL-C (17,8%, P=0,010) also was accompanied with lower CETP activity (10,31%, p=0,009). In multiple regression analysis, this association remained significant even after adjusting for gender, age, BMI, triglycerides, LDL-C, glucose, LCAT, SBP and MetS (p=0,003).

Conclusion: Our data reveal a significant association between PCSK9 and CETP regardless of lipid lowering treatment. This association is maintained in patients with lower PCSK9 genetically determined. The clinical implications of this metabolic relationship could be of interest, explaining in part the effect of PCSK9 and CETP inhibition on overall lipid profile.

OP2. A novel GLP-1/Glucagon receptor dual agonist improves non-alcoholic steatohepatitis and liver regeneration in mice

MP Valdecantos, V Pardo, E Fernández-Millán, C García-Monzón, AI Arroba, L Ruiz, A González-Rodríguez, F Escrivá, C Álvarez, C Barbas, A Konkar, J Grimsby, CM Rondinone, AM Valverde

Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM). Departamento de Bioquímica y Biología Molecular II, F. Farmacia, UCM. Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas. CEMBIO. Facultad de Farmacia. Universidad CEU.San Pablo-CEU. Medimmune Inc. Gaithersburg, MD-20878, USA

Background and aims. Since non-alcoholic steatohepatitis (NASH) is associated with impaired liver regeneration, we aimed to investigate the effects of a dual acting glucagon-like peptide-1(GLP-1)/glucagon (GCG) receptor agonist, termed G49, on NASH and hepatic regeneration.

Methods. C57/Bl6 male mice fed chow or methionine and choline-deficient (MCD) diet for one week were divided into 4 groups: C (chow diet plus vehicle), MCD (MCD diet plus vehicle), C+G49 (chow diet plus G49) and M+G49 (MCD diet plus G49). Following 2 weeks of treatment, partial hepatectomy (PH) was performed and mice were maintained on the same treatment schedule for an additional 2 weeks. Treatment effects on liver function, hepatic regeneration and comprehensive genomic and metabolic profiling was conducted throughout the study.

Results. NASH significantly improved in the M+G49 group manifested by reduced inflammation, steatosis, oxidative stress, apoptosis and increased mitochondria biogenesis. G49 treatment was also associated with a replenishment of hepatic glucose stores due to enhanced gluconeogenesis and reduced glucose utilization via pentose phosphate cycle (PPC) and oxidative metabolism. Following PH, treatment with G49 increased survival rates, restored the cytokine-mediated priming phase and enhanced proliferative capacity and hepatic regeneration ratio in mice on MCD diet. All NASH markers remained decreased in M+G49 mice after PH and glucose utilization was shifted to PPC and oxidative metabolism. Initiating G49 treatment immediately after PH was also effective in alleviating the pathological changes induced by the MCD diet.

Conclusion. Dual acting GLP-1R/GCGR agonists such as G49 represent a novel therapeutic approach for patients with NASH and particularly in those requiring PH.

OP3. Mitochondrial biogenesis induced by calorie restriction in white adipose tissue is mediated by PGC-1s co-activators: influence on glucose homeostasis

L. Cervela¹, M. de Marco¹, R. Pardo¹, P. Gama², P. García-Rovés², R. Simó¹, J. A. Villena¹

¹*Vall d'Hebron Institut de Recerca*, ²*Universitat de Barcelona*

Calorie restriction (CR) exerts multiple beneficial effects on health, including the prevention and amelioration of metabolic pathologies, such as insulin resistance and type 2 diabetes. Owing to its endocrine and lipid-storing functions, white adipose tissue (WAT) plays a pivotal role in the regulation of glucose homeostasis. However, besides the remarkable effects that CR has on adipose mass, little is known about the main cellular processes regulated by CR in WAT. To uncover the gene networks and cellular functions regulated by CR, we compared the gene expression profiles of WAT from mice subjected to CR (40%) for 16 weeks and mice fed ad libitum. Gene set enrichment analysis revealed that mitochondrial biogenesis was among the most significantly up-regulated processes by CR in WAT. CR dramatically increased the expression of mitochondrial genes, concomitantly with an increase in the expression of the coactivators PGC-1alpha and PGC-1beta. To determine if CR-induced mitochondrial biogenesis is mediated by PGC-1s, we generated an adipocyte-specific double knockout mouse in which the expression of PGC-1alpha and PGC-1beta was simultaneously ablated in adipose tissues (PGC1A/B-FAT-DKO mice). WAT of PGC1A/B-FAT-DKO mice fed ad libitum exhibited reduced expression of mitochondrial genes, decreased mitochondrial protein content and impaired mitochondrial respiration. Moreover, PGC1A/B-FAT-DKO mice failed to increase mitochondrial biogenesis and oxidative function in response to CR. Enhanced mitochondrial function in response to CR in WAT has been associated with better glucose homeostasis. However, PGC1A/B-FAT-KO mice responded to CR by improving glucose tolerance and insulin sensitivity to the same extent as wild-type mice. Our results demonstrate that PGC-1 coactivators mediate CR-induced mitochondrial biogenesis in WAT. Also, we show that mitochondrial biogenesis and full respiratory capacity in WAT is not required for the beneficial effects of CR on whole body glucose homeostasis, suggesting that other pathways maybe more relevant.

OP4. Topical administration of somatostatin prevents retinal neurodegeneration in a spontaneous model of type 2 diabetes

Lidia Corraliza, Patricia Bogdanov, Cristina Solà-Adell, Cristina Hernández, Rafael Simó

Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, CIBERDEM

Retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Among the neuroprotective factors synthesized by the retina, somatostatin (SST) is one of the most relevant. During DR, a downregulation of the SST retinal expression occurs. This imbalance could participate in the DR development. Topical administration of SST prevents retinal neurodegeneration in streptozotocin-induced diabetic rats but its effect in a spontaneous model of diabetes has not been examined. It should be noted that this experimental proof of concept constitutes a significant task of the EUROCONDOR (European Consortium for the Early Treatment of Diabetic Retinopathy) project (FP7-278040. <https://eurocondor.eu>).

On this basis, the aim of the present study was to assess the topical effect of SST and its action mechanism in early stages of DR in the db/db mice model. Mice were treated with either SST eye drops or vehicle during 15 days. Non-diabetic mice served as a control group. In summary, we found SST eye drops significantly prevent ERG abnormalities, decrease hallmarks of retinal neurodegeneration (glial activation and apoptosis). Regarding neuroprotection, a reduction of retinal glutamate levels and an increasing of GLAST expression were observed, as well as an augment in the pAKT/AKT ratio.

Our results suggest that topical administration of SST has a potent effect in preventing neurodegeneration induced by diabetes. Our findings open up a new preventive pharmacological strategy targeted to early stages of DR.

OP5. The cannabinoid sensitive receptors gpr55, gpr18 and cb1 might modulate inflammatory response in type 2 diabetes

S.Y. Romero-Zerbo, V. Espinosa-Jimenez, N. Cobo, B. Gauthier, F.J.Tinahones, G.Rojo, F.J. Bermudez-Silva
IBIMA-Hospital Regional de Málaga

Background and aims: Recent findings point to inflammation as a promising target to fight against type 2 diabetes (T2D). GPR55, GPR18 and CB1 are GPCRs and they are widely expressed throughout the body, including the immune system. There is a lack of information on the putative role of these receptors in modulating inflammation during T2D. In order to address this question, their expression was assessed in leukocytes from human subjects with pre-diabetes or diabetes, as well as in healthy subjects. Furthermore, inflammation was investigated after pharmacological manipulation of these receptors in a diet-induced animal model of T2D.

Material and Methods: 130 human subjects were selected based on previous history of pre-diabetes and T2D. Glucose handling was assessed by analysing fasting glucose and glucose tolerance. Leukocytes were isolated from peripheral blood samples and mRNA levels of GPR55, GPR18 and CB1 were quantified. On the other side, 10 weeks-old C57Bl/6J mice were rendered diabetic by feeding a high-fat diet and then treated with drugs targeting these receptors. Inflammatory cytokines were assessed by ELISA and V-PLEX pro-inflammatory panel kit. Pancreas and liver histopathology, macrophages and apoptosis were also studied.

Results: Pre-diabetic and diabetic subjects had decreased GPR55 levels in leukocytes when compared to controls. Indeed, GPR18 was increased in leukocytes from pre-diabetic subjects and GPR18 expression inversely correlated with plasma levels of the inflammatory adipokines adiponectin and leptin. No changes in CB1 expression was found among groups. On the other side, treatment of diabetic mice showed anti-inflammatory effects to varying degrees. GPR55/GPR18 activation but not CB1 blockade decreased plasma levels of the pro-inflammatory markers IL-6, KC-GRO and IL-5. Both treatments improved histology, decreased number of macrophages and apoptosis in the liver and pancreas.

Conclusions: Taken together, these results suggest that GPR55, GPR18 and CB1 might modulate the inflammatory response in type 2 diabetes.

OP6. Low-frequency and rare variants in type 2 diabetes mellitus by exome-sequencing

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Introduction: Type 2 diabetes mellitus (T2DM) is the result from the interaction of environmental, genetic and acquired factors. Low-frequency and rare variants could explain an important fraction of the estimated genetic component of the disease which could be found in the exome. This study has the aim to identify genetic variants in the exome in relation to T2DM in Spanish population.

Materials and Methods: Exome sequencing in 200 patients with T2DM and 200 Spanish healthy controls from CIBERDEM and National Biobank; all subjects had a BMI between 25-34.9 kg/m² and were 40 to 65 years old. Exome regions were captured and sequenced by NGS using Illumina systems. A bioinformatic analysis pipeline was used to perform quality controls, to align the reads to a reference genome and identify genetic variants.

Results: It was identified 21,822 SNPs in controls and 17,238 in cases with functional effect, present only in controls or cases that meet quality criteria. In particular, 160 and 132 SNPs were splicing variants, 1,817 and 1,614 SNPs were missense variants and 102 SNPs and 50 SNPs were stop variants in controls and cases, respectively.

Conclusions: We have identified a large number of genetic variants which may be involved in the development of T2DM or in the protection from it. Some of them are located in genes previously involved in T2DM.

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OP7. The autoimmune disease candidate gene dexi modulates virus-induced pancreatic beta cell inflammation and death

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Introduction and aims: The chromosome 16p13 region has been associated with several autoimmune diseases, including type 1 diabetes (T1D). CLEC16A is the most likely candidate gene in the region. It has been recently shown that the autoimmune-disease associated SNPs in intron 19 of CLEC16A regulate expression of a neighboring gene, DEXI. This suggests that DEXI may actually have an active role in the pathogenesis of T1D and other autoimmune diseases.

We presently analyze the role of DEXI in pancreatic beta cells exposed to double stranded RNA (dsRNA, a by product of viral infection) or infected with the diabetogenic virus Coxsackie Virus B5 (CVB5).

Material and methods: INS-1E cells, primary rat beta cells and human EndoC-bH1 cells were transfected with two different siRNAs targeting DEXI (inhibition of >70 %) and subsequently exposed to a synthetic analog of viral dsRNA (Polyinosinic-polycytidylic acid; PIC) or infected with CVB5. Viability was evaluated by Hoechst-Propidium iodide staining. Pro-inflammatory chemokine expression and ISRE reporter activity (measuring activation of the STAT1/STAT2 pathway) was evaluated in DEXI-silenced beta cells after PIC transfection. STAT1 signaling pathway activation was also assessed by Western blot for STAT1 phosphorylation.

Results: DEXI knockdown protected pancreatic beta cells against PIC- or CVB5-induced apoptosis (30% and 70% protection, respectively; $p < 0.001$). Silencing of DEXI decreased PIC-induced CXCL9, CCL5 and CXCL10 expression by 35-75% ($p < 0.01$) In addition, DEXI inhibition decreased activity of the STAT1 signaling pathway in response to PIC, as evidenced by decreased phosphorylation of STAT1 and reduced ISRE reporter activation (70% reduction; $p < 0.001$).

Conclusions: These results suggest that the autoimmune-associated gene DEXI regulates virus-induced pancreatic beta cell inflammation and death, and support DEXI as a potential candidate gene for T1D in the 16p13 region.

OP8. Fatty acids regulate TNFa levels released from adipocyte by changes in the methylation status of the TNFa promoter

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Circulating levels of TNF-alpha (TNFa) have being associated with several metabolic diseases. The factors regulating its synthesis and release could be considered therapeutic targets against the metabolic syndrome development. DNA methylation is a regulator of gene expression, which might play a role in both physiology and pathology through changes in protein levels. The aim of this study is investigate whether the effect of dietary fatty acids on TNFa released from adipocytes might be associated with modification on the TNFa promoter DNA methylation status.

A group of rats were assigned to three diets, each one with a different composition of saturated, monounsaturated and polyunsaturated fatty acids. Samples of visceral adipose tissues were taken for adipocytes isolation, in which released TNFa levels were measured, and for the methylation and expression studies. Additionally, 3T3-L1 cells were treated with C16, C18:1n9, and C18:2n6, with and without 5-Azacytidine (5-AZA). After treatments, cells and supernatants were harvested for the same analyses that were performed in the rat isolated adipocytes.

Released TNFa levels were different according to the diet or the fatty acid treatment, with the highest levels of the cytokine found in rats fed with the saturated diet ($p < 0.001$); and in cells treated with C16 ($p = 0.03$). TNFa promoter methylation levels were also different between diets ($p < 0.01$) or fatty acid treatments ($p = 0.03$); and in both cases, inversely correlated with released TNFa levels (Rat adipocytes: $r = -0.49$, $p = 0.04$; 3T3-L1 adipocytes: $r = -0.49$, $p = 0.05$) and relative gene expression (Rat adipocytes: $r = -0.47$, $p = 0.05$; 3T3-L1 adipocytes: $r = -0.68$, $p < 0.01$). Cells treated with 5-AZA displayed an increase in TNFa levels released to the medium regarding to the absence of 5-AZA, with no differences between fatty acids.

According to our results, dietary fatty acids regulation of TNFa levels released from adipocytes may be mediated by epigenetic modifications on the TNFa promoter DNA methylation levels.

OP9. Glycemic control and antidiabetic treatment trends in patients with type 2 diabetes cared for in primary care during 2007-2013 in catalonia

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Objectives: To assess the evolution of glycemic control and antidiabetic treatment prescription in patients with type 2 diabetes (T2DM) cared for by primary care teams in Catalonia during the 2007-2013 period.

Methods: A cross-sectional analysis repeated yearly using the same methodology. Data were extracted from the SIDIAP population database that includes electronic clinical records and pharmacy consumption of the entire population cared for by the Institut Català de la Salut. HbA1c \leq 7% and the individualized goal proposed by the RedGDPS in 2014 were used as glycaemic control criteria.

Results: total T2DM registered patients were 257,072 in 2007, 271,690 in 2008, 286,019 in 2009, 301,144 in 2010, 317,215 in 2011, 331,317 in 2012 and 343,969 in 2013. Mean age remained stable (68-69 years), while increases in the percentage of males (from 52.2 to 54.3%) and in diabetes duration (5.5 to 7.4 years) were observed. Regarding glycaemic control, no significant changes were observed: mean HbA1c remained around 7.2% and the percentage of patients reaching the HbA1c \leq 7 or the RedGDPS individualized goal, stayed around 55% and 75%, respectively. The percentage of patients not receiving antidiabetic drugs decreased from 28.1 to 19.4%, while monotherapy (31.8% to 36.2%), combination therapy (22.6% to 25.4) and insulin treatment (from 17.5 to 20%) increased progressively. The use of metformin and DPP4 inhibitors increased notably (48.5 to 68% and 0 to 13.2% of patients, respectively), while sulfonylureas and glitazones decreased (33.8 to 25.6% and 3.9 to 2.2%, respectively). The prescription of GLP-1 receptor agonists was very low (0 to 0.9%).

Conclusions: There have not been changes in glycaemic control during the 2007-2013 period in Catalonia, although the percentage of patients under pharmacological treatment increased notably.

OP10. ApoA-I mimetic administration, but not increase in ApoA-I-containing HDL levels, reduces tumour growth in mice with inherited breast cancer

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Diabetes, a situation that is associated with decreased HDL in plasma, has been related to increased cancer risk and mortality. Malignant proliferation of breast cancer tissue in women has been associated with changes in plasma lipid and lipoprotein levels. In post-menopausal women, low levels of high-density lipoprotein cholesterol (HDLc) have been associated with breast cancer risk. Decreased levels of apolipoprotein A-I (ApoA-I), the main HDL protein, has been found in patients with breast cancer. We aimed to test the effects of human ApoA-I hyperexpression and ApoA-I mimetic administration on tumor progression. The well-characterized mammary tumor virus-polyoma middle T-antigen transgenic mouse model of inherited breast cancer (PyMT) was used. PyMT were crossed with human ApoA-I transgenic mice or were treated with ApoA-I mimetic D-4F (10 mg/kg). PyMT-hApoA-I mice showed an increase in HDLc but tumor development was not prevented. In contrast, D-4F prevented tumor development in PyMT mice despite reducing HDLc levels. D-4F treatment significantly reduced tumor latency and mammary gland weight, and increased the percentage of normal or hyperplastic lesions whereas decreased the percentage of adenoma and carcinoma lesions. D-4F effects on tumor development were independent of 27-hydroxycholesterol. D-4F treatment reduced the levels of oxidized LDL (oxLDL) in plasma, which was found to be tumorigenic in human breast cancer. MCF-7 cells showed an increase in cell viability under oxLDL treatment but D-4F significantly counteracted this effect. In conclusion, ApoA-I mimetic, but not HDL levels, inhibits tumor growth in mice with inherited breast cancer and this is associated with a higher protection against LDL oxidative modification.

OP11. Genetic and pharmacological HDL-raising strategies partly rescue macrophage-specific reverse cholesterol transport in diabetic mice

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Background: In vivo macrophage-specific reverse cholesterol transport (RCT), one of the main atheroprotective properties of HDL, is thought to be impaired in diabetic patients and in mouse models. HDL raising strategies are suggested to exert favorable effects on HDL-mediated RCT in vivo, however the exact mechanisms involved in this process are less known.

Objective: To dissect the molecular mechanisms leading to improved in vivo RCT dynamics in a experimental diabetic setting.

Materials and methods: Diabetic (db/db) mice were used. The db/db mice were crossbred with human (h)apoA-I transgenic mice to obtain db/db mice overexpressing hapoA-I. Oral administration of a LXR agonist (T0901317; 20 mpk) was given to both control and db/db mice and compared with vehicle. Plasma and lipoproteins were assayed for chemical composition using comercial kits. Macrophage-specific RCT into liver and feces was assessed in vivo using [3H]-cholesterol as radiotracer. Tissues were assayed for mRNA relative levels by real time RT-PCR and protein abundance by western blotting analysis using specific antibodies, respectively.

Results: Kinetic studies in vivo revealed that radiolabeled cholesterol output from macrophage to feces was dramatically reduced (>-3-fold) in the db/db mice. Gene expression and protein analysis further revealed that the relative abundance of two key transporters involved cholesterol output to feces (Abcg5 and Abcg8) was dramatically decreased (>-2-fold) in the db/db mice. Both hapoA-I hyperexpression and LXR activation in the db/db mice favourably induced the delivery of the total tracer output from the liver into feces, being this effect in part attributed to a concomitant upregulation of the relative expression of Abcg5 and Abcg8 in the liver.

Conclusion: Our data indicate that impaired cholesterol transfer from macrophages to feces in diabetic mice is defective in part due to an altered liver-related gene expression of Abcg5 and Abcg8.

OP12. Identification of new mechanisms involved in the postnatal regeneration of endocrine pancreas: role of the autophagy in beta-cells and its relationship with IGF-2

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Aim: The developing endocrine pancreas undergoes substantial remodeling during the postnatal period, which triggers a transformation from fetal to adult phenotype of beta-cells. This beta-cell turnover is achieved by a transient wave of apoptosis that is temporally associated with a lack of expression of IGF-2 within islets. Because IGF-1R pathway is upstream of mTORC1, a negative regulator of autophagy, we expected autophagy to be activated in beta-cells during neonatal period. Thus, our study aimed to characterize the basal autophagic activity during endocrine pancreas remodeling and its contribution to neonatal beta-cell apoptosis.

Methods: Autophagy markers were measured by Western blot in the pancreas of rats on postnatal (PN) day 4, 14 and 23. Autophagosome formation was analyzed by TEM. Neonates were treated with rapamycin (3 mg/kg, 5 days) and beta-cell apoptosis was quantified by TUNEL assay. In order to determine the effect of IGF-2 on autophagy, in vitro studies were performed in INS-1E cells.

Results: Under basal conditions, a significant decrease of LC3II levels was observed in the pancreas of neonates on PN14 and PN23 compared with PN4. This decrease was accompanied by an accumulation of p62 (>2-fold vs. PN4). Similarly, TEM revealed a reduction in the number of autophagic vacuoles per beta cell in PN14 (49.7%) and PN23 rats (64.24%) compared with PN4 rats. Sub-chronic treatment with rapamycin, induced a significant reduction ($p < 0.01$) in the beta-cell apoptosis observed on PN14. Finally, kinetic experiments showed that long- but not short-term supplementation of INS-1E cells with IGF-2 resulted in a significant increase in LC3-II formation.

Conclusion: The blockage of autophagic activity in the endocrine pancreas seems to be required during postnatal remodeling in order to ensure the correct beta-cell turnover by apoptosis. This blockage might be associated to disappearance of islet IGF-2 expression.

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OP13. AAV-mediated specific overexpression of IGF1 in the pancreas prevents and counteracts type 1 diabetes

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Type 1 diabetes is characterized by progressive autoimmune destruction of insulin-producing beta-cells that leads to severe insulin deficiency. To prevent or counteract overt hyperglycemia therapeutic approaches aim at preserving the loss of pancreatic beta-cell mass, expanding the number of beta-cells, or interrupting the autoimmune process that destroys islets. Insulin-like growth factor 1 (IGF1) is a potent mitogenic and pro-survival factor for beta-cells, as well as having immunomodulatory properties. Here we report the first demonstration that overexpression of IGF1 specifically in the pancreas prevents and counteracts spontaneous autoimmune diabetes in NOD mice. Beta-cell-specific gene transfer was achieved through the use of AAV vectors combined with microRNA target sequences. This approach enabled robust, long-term IGF1 expression in the pancreas and prevented IGF1 production in off-target tissues. Treatment of pre-symptomatic NOD mice with AAV-IGF1 showed decreased lymphocytic infiltration of the islets and lower levels of expression of antigen-presenting molecules, inflammatory cytokines and chemokines important for tissue-specific homing of effector T cells, all of which prevented beta-cell loss. Furthermore, AAV-mediated IGF1 delivery to older mice with established pathology stopped autoimmune progression and reduced diabetes incidence to 20%, with sustained normoglycemia and normoinsulinemia in rescued animals. Collectively, these data suggest that AAV-based IGF1 gene delivery to beta-cells may represent a promising therapeutic approach for prevention and treatment of autoimmune diabetes.

OP14. Cellular patterning of insulin/IGF1 signalling by insulin receptor substrate 2 gene expression

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Changes in the sensitivity to insulin and insulin-like growth factor 1 (IGF1) are closely associated with ageing, metabolic disease, and cancer. However, our ability to track the sensitivity of individual cells to insulin/IGF1 in real time is extremely limited.

Emerging evidence points to a role for insulin in the maintenance and proliferation of adult stem cell populations, an observation that may underlie the chronic degeneration of organs such as the liver in patients with metabolic disease. Recent studies in *Drosophila* demonstrate that systemic insulin signals are locally interpreted by the stem cell niche in the brain and gut; enabling tissue-specific responses to changing environmental conditions. This novel concept of “local insulin signalling” has yet to be clearly demonstrated in mammalian tissues.

In our laboratory we have developed a promoter reporter system to track the cellular expression of insulin receptor substrate 2 (IRS2), a key mediator of insulin/IGF1 sensitivity. Using this novel approach we have observed that IRS2 gene expression is surprisingly non-uniform in cultures of human liver progenitor cells derived from human embryonic stem cells and human cancers. We show that cellular expression of IRS2 can be used as a proxy for insulin sensitivity, predicting the survival and proliferative response of individual cells within the cultures. “Local insulin signalling” mediated by IRS2 was also found to promote the differentiation of these cells into immature hepatocytes and limit maturation. These data provide novel insights into the cellular patterning of insulin/IGF1 sensitivity in mammalian cells by IRS gene expression and may help to identify links between alterations in insulin sensitivity, chronic liver disease and cancer.

OP15. Protein tyrosine phosphatase a novel target for the improvement of revascularization and survival of transplanted islet grafts

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Rational:To achieve long-term clinical success in islet transplantation, it is recognized that revascularization of islet graft must be promoted. Protein tyrosine phosphatase 1B(PTP1B) has been recently described as a negative regulator of angiogenesis through vascular endothelial growth factor-A (VEGFA) signaling by maintaining phospho-tyrosine levels. In this sense, our main aim is to investigate if PTP1B ablation contributes to the improvement of graft revascularization and survival.

Methodology:Diabetic BALB/c mice were transplanted into the anterior chamber of the eye, with 200 islets, isolated from PTP1B^{-/-} or PTP1B^{+/+} mice. Four groups of 12 animals were constitute: Non-transplanted; Control: transplanted with PTP1B^{+/+} islets; PTP1B^{-/-}: transplanted with PTP1B^{-/-} islets; and non-diabetic. Groups were followed for 28-days, measuring periodically weight and blood-glucose levels. In vivo, graft revascularization was evaluated, at day-7,15 and 28. Morphometric analysis was conducted on graft-containing eyes. Gene expression and protein detection were performed in islet samples.

Results-After 28 days the PTP1B^{-/-} group showed normalization of blood-glucose levels compared with the non-diabetic group and a 66% decrease relative control($p<0.05$) and a 70% decrease to the non-transplanted groups($p<0.01$). Graft functional-vascularization was accessed, in vivo, after 7,15 and 28 days post-transplant. PTP1B^{-/-} group, presented a significant increased vascular density (day-7:40%; day-15:59%; day-28:60%) and increased vascular area (day-7:1.9-fold; day-15:2-fold; day-28:2.3-fold), compared to control. Morphometric analysis, revealed a 5-fold increase on the percentage of β -cell expressing VEGFA on PTP1B^{-/-} grafts relative to control ($p<0.001$). Moreover, PTP1B^{-/-} islets showed, in vitro, a 3-fold increase in VEGFA expression ($p<0.05$) and, a 1.8-fold increase in relative VEGFA secretion ($p<0.05$).

Conclusion-Our results reveal PTP1B as a player on the revascularization of islet grafts, suggesting this, as a potential novel target on the improvement of islet transplantation.

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OP16. Role of GATA6 in pancreatic β cell function

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Wide Genome Association Studies (GWAS) in patients with neonatal diabetes associated to pancreas agenesis have revealed a high prevalence of this phenotype to de novo mutations in GATA6 and, although with less frequency, to mutations in GATA4. Recently our group has shown that GATA4 and GATA6 transcription factors play redundant roles in mice. While single inactivation of either Gata4 or Gata6 has no impact in pancreas formation, the simultaneous inactivation of both genes causes pancreatic agenesis. The block in pancreas development observed in Gata4/Gata6 double knockout mice is caused by defects in proliferation and differentiation of the pancreatic progenitors. Despite mutations in GATA6 are mostly associated to defects in pancreas formation, recent studies have described new mutations in this gene in patients with adult onset of diabetes, without obvious defects in pancreas organogenesis. These results reveal a new role of GATA6 in beta cell function. We aim to analyze the role of GATA6 in β cell function and glucose metabolism. We used GATA6 conditionally knockout mice in pancreatic progenitors. Our results show that while GATA6 KO mice are normoglycemic and glucose tolerance at 2 month of age, they develop glucose intolerance around 4- 5 months of age. This phenotype is likely due to a deficient insulin secretion in response to glucose. Indeed, gene expression profile analysis of GATA6 KO islets compared to control islets revealed downregulation of key genes involved in insulin secretion. Altogether, this data indicates that GATA6 regulates adult beta cell function by activating the expression of key genes involved in insulin secretion signaling pathway. This mechanism could explain the phenotype of diabetes observed in patients with mutations in GATA6.

OP17. Umbilical Cord Mesenchymal Stromal Cells Delay the Onset of Hyperglycaemia in the RIP-B7.1 model of type 1 diabetes

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In type 1 Diabetes Mellitus pancreatic beta cells are destroyed by autoreactive CD8+ and CD4+ T-cells. Immunotherapeutic interventions using monoclonal antibodies have failed to stop the process. Umbilical cord derived mesenchymal stromal cells (UC-MSCs) exhibit anti-inflammatory and immunomodulatory properties showing autoimmune protection capabilities. The RIP-B7.1 mouse is a model of experimental autoimmune diabetes that expresses the costimulator molecule B7.1 (CD80) on pancreatic beta cells. Induction of the autoimmune attack is tightly regulated through intramuscular immunization (IMM) using the preproinsulin cDNA in 8 weeks-old mice. We explored the effect of administering (allogenic) mesenchymal stromal cells from the mouse umbilical cord (UC-MSCs) to block or delay the immune attack and restore the functional beta cell mass in the RIP-B7.1 mouse. UC-MSCs were isolated from E17 FVB mice fetuses and characterized by flow cytometry. RIP-B7.1 mice were treated with vehicle or intraperitoneally transplanted with 2.5×10^5 or 5×10^5 UC-MSCs one week post IMM. Glycaemia was measured weekly. Eight weeks after IMM mice were sacrificed, pancreata extracted to perform histological or flow cytometry studies and plasma collected to analyze the inflammatory cytokine profile. UC-MSCs expressed the pericytes markers PDGFR- β , α -SMA, desmin, NG2, CD146, CD44 and CD29. Eight weeks after immunization, 100 % of immunized and non-transplanted RIP-B7.1 mice developed hyperglycaemia with insulinitis. The incidence of hyperglycemia at 8 weeks was decreased by 50 and 100 % in immunized mice transplanted with 2.5×10^5 and 5×10^5 UC-MSCs, respectively, correlating with reduced insulinitis. Pancreas cells showed a marked reduction in CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes and both M1 and M2 macrophages in UC-MSCs transplanted mice. UC-MSCs transplantation restored plasmatic levels of the proinflammatory cytokines IL-6, TNF α , and IFN γ . Our results suggest that UC-MSCs treatment could block or delay the development of hyperglycemia through suppression of inflammation and immune attack.

OP18. β -cell dedifferentiation and reduced neogenesis contribute to impaired β -cell mass regeneration in middle-aged rats

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IDIBELL

Limitations in β -cell regeneration potential in middle-aged animals could contribute to the increased risk to develop diabetes associated with aging. We investigated the beta-cell regeneration of middle-aged Wistar rats in response to two different regenerative stimuli: partial pancreatectomy (Px+V) and gastrin administration (Px+G). Pancreatic remnants were analyzed 3 and 14 days after surgery. Beta-cell mass increased in young animals after Px and was further increased after gastrin treatment. In contrast, beta cell mass remained unchanged after Px and after gastrin treatment in middle-aged rats. β -cell replication and individual β -cell size were similarly increased in young and middle-aged animals, and β -cell apoptosis remained unchanged after Px in all groups. Surrogate markers of β -cell neogenesis and duct cell plasticity (extra-islet β -cells and nkx6.1 nuclear immunolocalization in regenerative duct cells) were all increased in young but not in middle-aged Px rats. The pancreatic progenitor-related transcription factors (neurog3 and sox9) were upregulated in islet beta-cells of middle-aged rats and were further increased after Px. Additionally, chromogranin A+ hormone- islet cells were exclusively identified in pancreases of middle-aged Px rats. In summary, the potential for compensatory β -cell hyperplasia and hypertrophy was retained in middle-aged rats, but β -cell dedifferentiation and impaired β -cell neogenesis limited β -cell regeneration.

OP19. Insulin secretion is inhibited by cortistatin-14 in pancreatic beta cells

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Cortistatin (CORT-14) is a neuropeptide with roles in endocrine secretion and central nervous system, with strong structural, pharmacological and functional homology to somatostatin (SST). However, the cellular mechanisms supporting these roles are poorly characterized. We studied the potential role of CORT-14 and SST in the insulin-secreting pancreatic β cells of the Islets of Langerhans. Using insulin secretion assays, we observed that both CORT-14 and SST reduced insulin release. The reduction in insulin secretion was paralleled by a decrease in glucose-induced calcium levels observed by fura-2 calcium imaging. Additionally, the effect of CORT-14 in β cell calcium load was blocked by specific SST-R5 receptor antagonists, suggesting a higher affinity of CORT-14 for this receptor. Using perforated patch clamp experiments we demonstrated that application of CORT-14 hyperpolarized pancreatic β cell thus decreasing action potential firing. Additionally, CORT-14 reduced calcium currents in whole cell patch clamp experiments. Our results suggest that the binding of CORT-14 to SST-R5 receptors leads to pancreatic β -cells hyperpolarization and triggers the inhibition of calcium channels, thus reducing calcium load and consequently insulin secretion.

OP20. Targeting inflammation in pancreatic islets

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Background. Islet inflammation has emerged as a key factor for the loss of functional β -cell mass in both type 1 (T1D) and type 2 diabetes (T2D). Immune modulatory therapy has been pursued for T1D and more lately for T2D although durable clinical efficacy and safety still needs to be achieved. Alpha1-antitrypsin (AAT) therapy offers great promise in protecting islets from inflammation. AAT is a circulating serine protease inhibitor that does not only inhibits serine proteases, but also exerts anti-inflammatory effects.

Aim. We sought to determine the protective effects of human AAT on β cells from cytokine-induced death in vitro and in a transgenic mouse model carrying the gene for human islet amyloid polypeptide (Tg-hIAPP) that accumulates amyloid deposits in pancreatic islets and present islet inflammation.

Results. We show that low doses of pro-inflammatory cytokines induced the activation of a transcriptional inflammatory program and β -cell death in mouse islets. Treatment with hAAT strongly prevented cytokine-induced islet cell death and apoptosis induced by exogenous cytokines. Clodronate liposome-mediated depletion of islet macrophages blocked cytokine-induced β -cell apoptosis, suggesting that hAAT partially mediates its protective effects by via resident macrophages. A global transcriptomics analysis revealed that hAAT treatment did not affected most of the transcriptional changes induced by cytokines, but interestingly resulted in a partial decrease of a selective set of pro-inflammatory genes and in an increase of stress-induced genes known to protect β cells. Finally, to test the efficacy of AAT in a model of T2D in vivo, we treated Tg-hIAPP mice with this anti-inflammatory agent. Remarkably, AAT was able to completely prevent the glucose intolerance present in these mice at 16 weeks of age.

Conclusions. Our results support the potential of hAAT as a strong candidate for the treatment of T1D and T2D and shed light on the mechanisms underlying this protective effect.

OP21. Exosomal miRNAs participate in the regulation of glucose homeostasis in mice

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In addition to hormones and other soluble factors, most cell types communicate by releasing exosomes. Exosomes are small vesicles particularly enriched in microRNAs (miRNAs) that are found in the blood and other fluids. Exosomes can be captured by acceptor cells, where they induce transcriptomic changes. Exosomal miRNAs have been shown to regulate tumor growth and metastasis, but their role in metabolic homeostasis is still unknown. Hence, we set up to explore the potential role of exosomal miRNAs in mediating tissue crosstalk during development of glucose intolerance.

MicroRNA profiling of plasma exosomes isolated from mice rendered glucose-intolerant by administration of a high-fat diet (HFD) show significantly higher levels of liver-enriched microRNAs such as miR-122 and miR-192, as compared with control plasma exosomes. Primary pancreatic islets or Min6 cells incubated in vitro with HFD exosomes display increased proliferation rate, but this effect is lost by prior dicer1 knockdown of the acceptor cells, thus suggesting miRNA involvement. Surprisingly, repeated injection of HFD exosomes through the tail vein results in induction of glucose intolerance in control mice being fed standard chow. These data suggest that exosomes are involved in the establishment of glucose intolerance, possibly by mediating cell-to-cell communication. Directed manipulation of circulating exosomes and their miRNA contents may then provide us with a novel therapeutic approach to treat type 2 diabetes.

OP22. Altered central and peripheral circadian clocks affecting energy metabolism in congenitally blind mice show differential entrainment by time-restricted feeding

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The hypothalamic suprachiasmatic nucleus (SCN) is a master regulator of circadian rhythmicity by synchronizing metabolic activity with daily light-dark cycles. The synchronizing function of the central molecular clock, consisting of self-regulated genes encoding transcriptional activators and repressors, is critically dependent on light-dependent stimuli reaching the SCN directly from the retina. In turn, the SCN translates this information to entrain molecular clocks in peripheral organs, which are essential to adapt metabolic activity to circadian variation. Here, we investigated the relative importance of the input pathway relying light information to the SCN for the function of central and peripheral clocks and for the circadian regulation of energy metabolism. We used Pitx3-deficient Aphakia (Pitx3Ak) mice, characterized by a congenital defect that severely disrupts eye development. Expression of clock genes was determined by RT-PCR, and energy metabolism was assessed by indirect calorimetry.

Electroretinogram recordings showed that Pitx3Ak mice were completely unresponsive to light. Indirect calorimetry revealed that, contrary to control mice, Pitx3Ak lacked cyclic day-night oscillations in oxygen consumption, energy expenditure, CO₂ production and respiratory exchange ratio. Expression levels of clock genes in the SCN and the liver of control mice showed night-day oscillations, but in Pitx3Ak mice they were reduced and showed no significant day-night variation. Time-restricted-feeding (TRF) allowing Pitx3Ak mice access to food only during the night resulted in the partial restoration of circadian rhythmicity in energy metabolism and expression of liver clock genes, but no change was observed in the expression of SCN clock genes. Our data show that congenital eye defects preventing the relay of light-dependent information to the SCN result in permanent defects in the expression of clock genes in the SCN. In addition, our results indicate that clock genes in peripheral tissues retain an autonomous capacity to be entrained by external stimuli in the absence of SCN-dependent activity.

OP23. Stimulated Occurrence and Biological Activity of Palmitoleic Acid and Its Isomers in Metabolic Diseases

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Atherosclerosis, a major cause for cardiovascular disease, can be initiated by the increased activation of endothelial cells lining the inside of the blood vessels. This activation may obey to multiple causes, such as diabetes or hyperlipidemia, and results in the endothelial cells releasing a variety of products with inflammatory potential that may attract monocytes and favor their infiltration into the subendothelial space. We have recently shown that human monocytes respond to free arachidonic acid, a well-defined secretory product of activated endothelial cells, by activating the de novo pathway of fatty acid biosynthesis, resulting in the formation of neutral lipids and the acquisition of a foamy phenotype due to the accumulation of cytoplasmic lipid droplets. In this work we describe that the neutral lipids of foamy monocytes are selectively enriched in a rather uncommon fatty acid, cis-7-hexadecenoic acid (16:n-9), a positional isomer of the more frequently described palmitoleic acid, which is strikingly absent from lipid droplets. Experiments addressing the biological role for 16:1n-9 indicate that this fatty acid prevents the inflammatory response of human monocytes and murine macrophages to bacterial lipopolysaccharide both in vitro and in vivo. Collectively, our results provide evidence that a previously unrecognized fatty acid, 16:1n-9, possesses anti-inflammatory activity that is comparable to that of omega-3 fatty acids and is clearly distinguishable from the effects of palmitoleic acid. Moreover, the selective accumulation in the neutral lipids of phagocytic cells of a rather uncommon fatty acid, reveals an early phenotypic change that may provide a biomarker of proatherogenicity, and a potential target for intervention in the early stages of cardiovascular disease.

OP24. Hyperactivation of mTORC1 in pancreatic β cells overexpressing human amylin causes a blockade in autophagic flux and an induction of apoptosis

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Facultad de Farmacia

Type 2 diabetes mellitus (T2DM) is a complex disease and it is considered epidemic in the world. Insulin resistance and pancreatic β cell dysfunction are the main contributors for the development to T2DM. As a consequence, β -cells compensate increasing its number and size in order to secrete more insulin and amylin. Increased mTORC1 signaling in β cells is commonly found along T2DM progression, leading to an increase of ER-stress and an accumulation of these proteins inside the cell. Recent data indicate that autophagy pathway protects from human amylin-induced proteotoxicity. In collaboration with Ana Novial's lab, we have observed that pancreatic β cells overexpressing human amylin (INS1E-hIAPP) present a blockade in autophagic flux, probably by its basal hyperactivation of mTORC1 signaling. This hyperactivation could be reverted by resveratrol or rapamycin treatment. In response to thapsigargin, INS1E-hIAPP presented a higher susceptibility to cell death compared with INS1E WT and INS1E-rIAPP. Furthermore, we detected an increased in the basal levels of ROS as well as the percentage of cells with depolarized mitochondria in INS1E-hIAPP. These data suggest an impaired capacity of mitochondrial elimination by mitophagy. For analyzing the consequences of overexpressing human amylin in extrapancreatic tissues we submitted with the conditioned media obtained from either INS1E-rIAPP or INS1E-hIAPP. We have collected the 48h-supernatants obtained from both the INS1E-rIAPP and INS1E-hIAPP and transferred to SHSY5Y human neuroblastoma cells for 24 h. We observed a decreased in cell survival as well as an increased in ER-stress and in autophagy levels in SHSY-5Y neuroblastoma cells. In summary, our data indicates that hIAPP overexpression in β cells increased mTORC1 signaling and inhibits autophagy. Whether amylin can be exported by the β cells as exosomes working on the neurons, remains to be established.

OP25. INSULIN RECEPTOR ISOFORM A AMELIORATES GLUCOSE INTOLERANCE IN DIABETIC MICE.

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Type 2 diabetes mellitus is a complex metabolic disease and its pathogenesis involves abnormalities in both peripheral insulin action and insulin secretion. Previous in vitro data showed that insulin receptor isoform A, but not B, favours basal glucose uptake through its specific association with endogenous GLUT1/2 in murine hepatocytes and beta cells. With this background, we hypothesized that hepatic expression of insulin receptor isoform A in a mouse model of type 2 diabetes could potentially increase the glucose uptake of these cells decreasing the hyperglycaemia and therefore ameliorating the diabetic phenotype. To test this hypothesis, we have developed recombinant adeno-associated viral vectors expressing insulin receptor isoform A or B under the control of a hepatocyte specific promoter. Our results demonstrate that hepatic expression of insulin receptor isoform A in diabetic mice, but not B, ameliorates the glucose intolerance. Consequently, it impairs the induction of compensatory mechanisms through beta-cell hyperplasia/hypertrophy that finally lead to beta-cell failure, reverting the diabetic phenotype in about 8 weeks. Our data suggest that long-term hepatic expression of insulin receptor isoform A could be a promising therapeutic approach for the treatment of type 2 diabetes mellitus.

OP26. Metabolomics reveals impaired maturation of HDL particles in adolescents with hyperinsulinaemic androgen excess

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Hyperinsulinaemic androgen excess (HIAE) in prepubertal and pubertal girls usually precedes a broader pathological phenotype in adulthood that is associated with anovulatory infertility, metabolic syndrome and type 2 diabetes. The metabolic derangements that determine these long-term health risks remain to be clarified. Here we use NMR and MS-based metabolomics to show that serum levels of methionine sulfoxide in HIAE girls are an indicator of the degree of oxidation of methionine-148 residue in apolipoprotein-A1. Oxidation of apo-A1 in methionine-148, in turn, leads to an impaired maturation of high-density lipoproteins (HDL) that is reflected in a decline of large HDL particles. Notably, such metabolic alterations occur in the absence of impaired glucose tolerance, hyperglycemia and hypertriglyceridemia, and were partially restored after 18 months of treatment with a low-dose combination of pioglitazone, metformin and flutamide.

OP 27. Liver glycogen, a novel target to treat diabetes and obesity

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Background: Diabetes and obesity have reached epidemic proportions worldwide. Obesity results from a prolonged imbalance between energy intake and energy expenditure. The regulation of energy intake appears as a plausible approach to reduce the impact of this pathological condition. In diabetes and obesity, liver glycogen has been proposed as another player in the regulation of food intake, and its levels can be manipulated to ameliorate the diabetic state.

Hypothesis: We propose that increased liver glycogen stores in diabetes and obesity contribute to decreased appetite. We aim to unravel the mechanism by which liver glycogen content regulates food intake.

Results: We have crossed transgenic mouse lines with increasing levels of hepatic glycogen with various mouse models of diabetes and obesity. We found that liver glycogen stores ameliorate the diabetic state, decrease food intake and maintain energy status in the liver. To address the contribution of the hepatic branch of the vagus nerve in the regulation of food intake, we have performed hepatic vagotomy and sham operation on mice that overexpress protein targeting to glycogen (PTG KIn) in the liver. One week after the operation, mice received a high-fat diet (HFD), which was maintained for 14 weeks. The hypophagia observed in response to a HFD was antagonized by hepatic branch vagotomy. This observation suggests that the hepatic vagus nerve participates in the regulation of food intake in response to the accumulation of glycogen in the liver.

Conclusions: Liver glycogen accumulation participates in the regulation of food intake in models of diabetes and obesity by triggering signals from the liver that are carried to the brain by vagal sensory neurons. Modulation of this signal and/or hepatic glycogen levels may provide a potential strategy by which to target diabetes and obesity.

OP28. Genetic models rule out a major role of beta cell glycogen in the control of glucose homeostasis.

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AIMS/HYPOTHESIS:

Glycogen accumulation occurs in beta cells of diabetic patients and has been proposed to partly mediate glucotoxicity-induced beta cell dysfunction. However, the role of glycogen metabolism in beta cell function and its contribution to diabetes pathophysiology remain poorly understood. We investigated the function of beta cell glycogen by studying glucose homeostasis in mice with (1) defective glycogen synthesis in the pancreas; and (2) excessive glycogen accumulation in beta cells.

METHODS:

Conditional deletion of the *Gys1* gene and overexpression of protein targeting to glycogen (PTG) was accomplished by Cre-lox recombination using pancreas-specific Cre lines. Glucose homeostasis was assessed by determining fasting glycaemia, insulinaemia and glucose tolerance. Beta cell mass was determined by morphometry. Glycogen was detected histologically by periodic acid-Schiff's reagent staining. Isolated islets were used for the determination of glycogen and insulin content, insulin secretion, immunoblots and gene expression assays.

RESULTS:

Gys1 knockout (*Gys1* KO) mice did not exhibit differences in glucose tolerance or basal glycaemia and insulinaemia relative to controls. Insulin secretion and gene expression in isolated islets was also indistinguishable between *Gys1* KO and controls. Conversely, despite effective glycogen overaccumulation in islets, mice with PTG overexpression (PTGOE) presented similar glucose tolerance to controls. However, under fasting conditions they exhibited lower glycaemia and higher insulinaemia. Importantly, neither young nor aged PTGOE mice showed differences in beta cell mass relative to age-matched controls. Finally, a high-fat diet did not reveal a beta cell-autonomous phenotype in either model.

CONCLUSIONS/INTERPRETATION:

Glycogen metabolism is not required for the maintenance of beta cell function. Glycogen accumulation in beta cells alone is not sufficient to trigger the dysfunction or loss of these cells, or progression to diabetes.

OP29. Characterization of serum microRNAs profile of polycystic ovary syndrome (PCOS), and obesity María Insenser*, Mora Murri*, Elena Fernández-Durán, Jose L. San-Millán, Héctor F. Escobar-Morreale *Diabetes, Obesity and Human Reproduction Research Group, Hospital Universitario Ramón y Cajal & Universidad de Alcalá & Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) & Centro de Investigación Biomédica en Red Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain*

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Introduction. MicroRNAs (miRNAs) are small noncoding RNA sequences that regulate negatively gene expression at the post-transcriptional level. miRNAs play important regulatory roles in a variety of biological processes, including metabolism. Specific microRNAs have been demonstrated recently to exist abundantly and stably in serum bearing putative disease-specificity. The potential for detecting circulating miRNAs as biomarkers of several human diseases, such as diabetes, cardiovascular disorders, cancer and certain viral infections has been reported widely both in animal models and in human subjects. The aim of this study was to characterize the serum miRNAs expression profile in polycystic ovary syndrome (PCOS) while considering the influence of obesity.

Description of design/methods. We included 12 control women, 12 patients with PCOS and 12 men selected as to have similar body mass index (BMI) and age. Six subjects per group had normal weight (BMI < 25 kg/m²) whereas 6 subjects per group were obese (BMI > 30 kg/m²). Profiling of miRNA expression used miRCURY LNA™ Universal RT microRNA PCR, 4x Human panel I+II in 384well PCR plates.

Results. We analyzed the expression of 752 miRNA. Of them, we observed differences in the expression of 90 (12%) as follows: 27 showed differences between patients with PCOS, control women and men; 47 showed differences between obese and lean subjects; and 39 showed an interaction between obesity and group of subjects. Most interactions consisted in decreased expression or no change of several miRNAs in obese women compared with increased expression of the same in obese men. Women with PCOS showed expression patterns that were similar to those of men for some miRNA, or similar to control women for others. We studied the biological function of the putative targets of these miRNA.

Conclusions. The present results suggest that several serum miRNAs are influenced by sex, PCOS and obesity, and might serve as potential candidates to explain the impact of obesity on the pathogenesis of PCOS and related metabolic conditions.

OP30. BACE1 contributes to saturated fatty acid-induced ER stress, inflammation and insulin resistance in skeletal muscle cells

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Beta-secretase/beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE1) is a key enzyme involved in Alzheimer's disease. A recent study reported that BACE1-deficient mice are protected against high-fat diet-induced glucose intolerance, although the mechanisms involved are not well-known. In the present study we examined whether BACE1 contributes to palmitate-induced inflammation and insulin resistance. BACE1 expression and protein levels were increased by palmitate exposure and the BACE1 inhibitor Merck3 reduced palmitate-induced ER stress, inflammation and insulin resistance in myotubes. The product of BACE1 activity, soluble APPbeta (sAPPb), mimicked most of the effects caused by palmitate. Interestingly, both palmitate and sAPPb reduced the expression and protein levels of PPARgamma co-activator 1 alpha (PGC-1a). Knockdown of BACE1 in myotubes and skeletal muscle of BACE1-deficient mice confirmed that this beta-secretase contributes to reduce PGC-1a levels. cAMP regulatory element-binding protein (CREB) binding to the PGC-1a promoter plays a key role in activating its expression in skeletal muscle. Our findings suggest that BACE1 reduces PGC-1a levels by attenuating CREB phosphorylation as the result of a reduction in cAMP levels and protein kinase A activity. Both, sAPPb and the interaction of BACE1 with adenylate cyclase seem to be responsible for these changes.

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OP 31. ASCs from obese environment are more resistant to apoptosis: A potential role of survivin

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Background&aim: Adult adipose tissue (AT) contains a pool of abundant multipotent stem cells, designated as adipose-derived stem cells (ASCs) that are able to replicate as undifferentiated cells, and to develop as mature adipocytes. Current available information indicates that ASCs are important players in the dynamics of AT remodeling, an ongoing process that is dysregulated in obesity. Thus, obesity appears to be linked to defective cellular turnover and remodeling of AT. It is known that ASCs from obese patients have enhanced proliferation, migration and differentiation capacity. The aim of this work is to study the sensitivity of ASCs to apoptotic stimulus and to gain insight into the molecular players involved.

Methods: ASCs were isolated from subcutaneous adipose tissue of lean and obese subjects. Serum and plasma were also collected. Apoptosis was measured using annexin-propidium iodide staining by flow cytometry. Gene and protein expression were assessed by qPCR and WB. Recombinant adenovirus was used to perform overexpression studies.

Results: Apoptotic stimulus, such as leptin and hypoxia, induced apoptosis in ASCs from lean but not from obese donors. The profiling of several anti- and pro-apoptotic proteins showed increased levels of survivin (anti-apoptotic) in obese AT and ASCs. These increased levels could be explained by higher protein stability, lower levels of protein ubiquitination and additionally, lower levels of mir-203 in obese ASCs. On the other hand, survivin promoter was more methylated, turning into decreased mRNA levels as a possible counteracting mechanism to such large amount of protein. We adenovirally overexpressed survivin in lean ASCs, and these cells developed more resistant to apoptotic stimulus. Furthermore, inflammatory stimuli, such as conditioned media from macrophages M1, enhanced survivin expression in ASCs from lean patients.

Conclusions: ASCs from obese environment are more resistant to apoptosis and survivin appears to play a key role. These results uncover that an obese environment could promote the ASC niche.

OP32. Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells.

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Adipose tissue-derived stem cells (ASCs) are proposed as an alternative stem cell source to bone marrow-derived cells for immune cell therapy. However, microenvironmental factors may contribute to the functionality of the stem cell population in human adipose tissue (AT). We hypothesized that the fat depot in addition to the donor phenotype controls the immunomodulatory capacity of ASCs. Focusing on obesity and type 2 diabetes (T2D) as metabolic disorders which might affect the immune response of ASCs, we compared the inflammatory response of ASCs isolated from subcutaneous and visceral AT from age-matched donors (lean n=4, body mass index [BMI] 21.98+-1.9; obese n=4 BMI 33.1+-2.1 and T2D n=4 BMI 35.3+-1.5). Obese and T2D-derived ASCs showed increased expression of inflammatory markers, activation of the NLRP3 inflammasome and higher migration, invasion and phagocytosis capacities than those derived from lean donors. Remarkably, ASCs derived from obese and T2D subjects exhibited a reduction in typical immunosuppressive activities attributed to stem cells. Accordingly, obese and T2D-ASCs were less effective in suppressing T cell and B cell proliferation, activating the M2 macrophage phenotype as well as in increasing TGF-B1 secretion than lean-derived ASCs. Overall, these data indicate that the metabolic phenotype of the donor compromises the immunomodulatory properties of ASCs, particularly in cells derived from visceral AT. These results are relevant not only for understanding the physiology of ASCs in terms of cell-based therapies but also for their role as key regulators of the immune response.

OP33. Mitofusin 2 links mitochondrial fitness with age-related metabolic disease and sarcopenia

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Mitochondrial fusion and fission proteins regulate mitochondrial quality control and mitochondrial metabolism. In turn, mitochondrial dysfunction is associated with aging, although its causes are still under debate. Here, we show that aging is characterized by a progressive reduction of Mitofusin 2 (Mfn2) in mouse skeletal muscle and that skeletal muscle Mfn2 ablation in mice generates a gene signature linked to aging. Furthermore, muscle Mfn2-deficient mice show unhealthy aging characterized by altered metabolic homeostasis and sarcopenia. Mfn2 deficiency impairs mitochondrial quality control, which contributes to an exacerbated age-related mitochondrial dysfunction. Surprisingly, aging-induced Mfn2 deficiency triggers a ROS-dependent retrograde signaling pathway through induction of HIF1a transcription factor and BNIP3. This pathway ameliorates mitochondrial autophagy and minimizes mitochondrial damage. Our findings reveal that repression of Mfn2 in skeletal muscle during aging is determinant for the loss of mitochondrial quality, contributing to age-associated metabolic alterations and loss of muscle fitness.

OP34. Mitofusin 2 es a phosphatidylserine transfer protein involved in liver cancer"

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The mitochondrial fusion protein Mitofusin 2 (Mfn2) plays a key role in the maintenance of normal mitochondrial metabolism and ER function. Here we show that Mfn2 protects against liver disease. Liver-specific Mfn2 ablation caused mild steatosis, inflammation, fibrosis and liver cancer. Moreover, reduced Mfn2 expression was detected in patients with non-alcoholic steatohepatitis (NASH), thereby suggesting that this protein is involved in the development of this disease. Mfn2 deficiency reduced phospholipid synthesis and the expression of phosphatidylserine synthases Ptdss1 and Ptdss2. Interestingly, Ptdss1/2 deficiency recapitulates the hepatic alterations driven by Mfn2 ablation. Consistent with this, Mfn2 and Ptdss1 interact in a mitochondria-associated ER membrane (MAM) complex. Moreover, Mfn2 binds phosphatidylserine (PS) and shows PS transfer activity in vitro and in vivo, which may favor mitochondrial phosphatidylethanolamine (PE) synthesis. Our findings support the notion that Mfn2 deficiency confers susceptibility to liver cancer caused by dysregulation of PS synthesis in MAMs and transfer to mitochondria.

POSTERS

P1. Ghrelin gene polymorphisms interact with olive oil intake and Type 2 Diabetes in a Spanish population. Egabro-Pizarra study

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Ghrelin plays an important role in glucose metabolism and homeostasis. Several studies have reported common variants in *ghrelin* gene (*GHRL*) related to Obesity, Metabolic Syndrome (MetS), and Type 2 Diabetes (T2D), becoming in a potential candidate gene. The aim of this study was to investigate Single Nucleotide Polymorphisms (SNPs) and the susceptibility to develop T2D, and look for possible interactions with diet.

Material and Methods: A cross-sectional population-based study with 892 individuals was performed in two andalusian towns; Cabra and Pizarra, aged between 40-65 years, 43,2% men-56,8% women, in risk to develop metabolic diseases (inclusion criteria: BMI > 25 and/or some MetS component by ATP III). All participants filled nutritional surveys and anthropometric measures were taken, as long as, blood pressure, biochemical parameters and OGTT in order to detect carbohydrate metabolism alterations. Mediterranean Diet adherence was calculated by "Score Predimed", specially olive oil intake was registered. A nested follow up study (2 years) was carried out in 277 individuals who presented alterations in glucose metabolism after a Oral Glucose Tolerance Test (OGTT) (75 mg/dL). DNA was isolated and representative tag SNPs of *GHRL* were selected to capture all the variability (rs10490815-rs10490816-rs1629816-rs2619507-rs26802-rs27647-rs35679-rs35683-rs4684677-rs696217). Genotyping was performed using TaqMan Open Array technology. We calculated Hardy-Weinberg equilibriums and SNPs not in equilibrium were excluded from the analysis. Logistic regression and lineal models adjusting by age, sex, BMI and T2D, were calculated to detect associations.

Results: In the cross-sectional study, variants rs10490815, rs10490816, rs2619507 were associated to T2D presence (p-value=0.001, p-value=0.001 and p-value=0.01, respectively according over dominant model). rs10490815 and rs35683 variants were associated to impaired glucose metabolism (IFG-IGT) according to the same model (p-value=0.05, p-value=0.002). Also, rs10490815 was associated with insulin resistance by HOMA-IR (p=0.05). In the follow-up study, we found associations with risk to develop T2D for the same variants described in the cross-sectional study. Related to interactions with olive oil intake, individuals presenting the risk genotypes for rs10490815 and rs35683 variants, if they exclusively consume olive oil, presented lower T2D prevalence compared to individuals who consume other oil types (p=0.01 and p=0.0004, respectively).

Conclusion: *GHRL* gene variants are associated with T2D presence and a protective interaction with olive oil intake exists in this individuals.

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P2. SIROLIMUS NEGATIVELY IMPACTS INSULIN SIGNALING, GLUCOSE UPTAKE AND UNCOUPLING PROTEIN-1 IN BROWN ADIPOCYTES

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New onset diabetes after transplantation (NODAT) is a metabolic syndrome that affects a large number of patients with chronic treatment with immunosuppressive agents (IAs) such as sirolimus (also known as rapamycin). IAs negatively modulate insulin sensitive-tissues such as skeletal muscle, liver and white fat. However, the effects of IAs on insulin action and thermogenesis in brown adipose tissue (BAT) have not yet been investigated. In this study, we have analyzed the impact of sirolimus in the insulin signaling and thermogenic gene-expression in brown adipocytes (BA). We found that sirolimus induced the degradation of insulin receptor substrate 1 (IRS1) leading to a decrease in insulin-mediated protein kinase B (Akt) phosphorylation. As a consequence, in sirolimus-treated BA, insulin-induced glucose transporter 4 (GLUT4) translocation to the plasma membrane and glucose uptake were decreased. Sirolimus triggered an early activation of N-terminal Janus activated kinase (JNK) thereby increasing serine 307 phosphorylation of IRS1 that preceded its proteasomal degradation. The negative effects of sirolimus on insulin signaling were prevented by a JNK inhibitor. In vivo treatment of rats with sirolimus for three weeks abolished insulin-mediated Akt phosphorylation in BAT. Sirolimus also inhibited norepinephrine (NE)-induced lipolysis, the expression of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) and uncoupling protein 1 (UCP-1), as well as basal mitochondrial respiration in BA. In conclusion, our results have demonstrated for the first time the unique role of brown adipocytes as target cells of sirolimus suggesting that insulin resistance in BAT might play a major role in NODAT development.

P3. INHIBITION OF PROTEIN TYROSINE PHOSPHATASE 1B IMPROVES IGF-I RECEPTOR SIGNALING AND PROTECTS AGAINST INFLAMMATION-INDUCED GLIOSIS IN THE RETINA

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Purpose: Insulin like growth factor-I receptor (IGF-IR)-mediated signaling plays an important role in retinal growth and survival and its failure may contribute to aggravate diabetic retinopathy (DR). Protein tyrosine phosphatase 1B (PTP1B) has emerged as a negative modulator of IGF-IR-mediated signaling but its role in the context of the proinflammatory milieu during DR remains unknown. We investigated the involvement of PTP1B in the cross-talk between stress pathways activated by proinflammatory cytokines and IGF-IR-mediated signaling in the retina.

Methods: We treated 661W photoreceptors and retinal explants with a mixture of cytokines containing tumor necrosis factor α (TNF α), interleukin 6 (IL6) and interleukin 1 β (IL1 β), and the IGF-IR signaling cascade was evaluated in the absence or presence of PTP1B.

Results: 661W retinal cells and retinal explants were sensitive to IGF-I in inducing IGF-IR and Akt phosphorylation. Stimulation with cytokines triggered an early activation of stress kinases (JNK and p38 MAPK), resulting in insulin receptor substrate 1 (IRS1) phosphorylation at serine 307 that precedes its degradation. Pre-treatment of 661W cells or retinal explants with cytokines for 24 hours induced IRS1 degradation and decreased IGF-I-mediated IGF-IR and Akt phosphorylation. PTP1B siRNA or PTP1B deficiency ameliorated the negative effects of cytokines on IGF-IR signaling. Treatment with cytokines increased GFAP expression in retinal explants and this response was ameliorated by a PTP1B inhibitor. GFAP was also reduced by the PTP1B inhibitor in retinas from db/db mice.

Conclusions: Targeting PTP1B might be useful for modulating the beneficial effects of IGF-I in retinal cells during DR.

P4. MODULATION OF MICROGLIA POLARIZATION DYNAMICS DURING DIABETIC RETINOPATHY IN DB/DB MICE

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Aims/Hypothesis: Retinal diseases linked to inflammation are often accompanied by macrophage/microglial cells activation. However, the dynamics between M1 (pro-inflammatory) and M2 (anti-inflammatory) polarization of microglia during diabetic retinopathy (DR) has not been investigated and it might be therapeutically useful. We assessed microglia polarization in retinas from db/db mice and human diabetic donors and also the microglia-mediated anti-inflammatory effects of the bicyclic nojirimycin derivative (1R)-1-dodecylsulfinyl-5N,6O-oxomethylidenenojirimycin (R-DS-ONJ).

Methods: Visual function in mice was evaluated by electroretinogram (ERG). Expression of pro- and anti-inflammatory markers in the retina was analyzed by immunofluorescence, Western-blot and quantitative real-time PCR. Lipopolysaccharide (LPS)-mediated polarization profile was studied in Bv.2 microglial cells in the absence or presence of anti-inflammatory cytokines (IL4/IL13) or R-DS-ONJ.

Results: At 5 weeks of age, reduced ERG amplitude values of rod and mixed waves were detected in db/db compared to db/+ mice that correlated with elevated circulating endotoxemia and pro-inflammatory cytokines. At this early stage of DR, the marker of activated microglia Iba-1 co-localized with the M2 marker arginase-1 in the retina. Conversely, in retinas from 8 weeks old db/db mice Iba-1-colocalized with active caspase-1, a key component of the inflammasome, reflecting an opposite pattern of microglia polarization. Markers of activated microglia were detected in retinas of diabetic donors. Treatment of Bv.2 cells with LPS and IL4/IL13 or R-DS-ONJ switched the M1 response towards M2. In retinal explants from db/db mice, R-DS-ONJ induced a M2 response.

Conclusions/Interpretation: Modulation of microglia polarization dynamics towards a M2 status at early stages of DR offers novel therapeutic interventions.

P5. UCP-1 EXPRESSION INCREASES IN BAT OF TYROSINE HYDROXYLASE GENE HETEROZYGOUS MICE IN RESPONSE TO COLD

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Background

Brown adipose tissue (BAT), a highly innervated and irrigated tissue, is responsible for the adaptive response to cold, activating thermogenesis. Heat production in BAT is achieved by inducing the mitochondrial uncoupling protein (UCP-1) expression and the production of fatty acids generated by lipolysis. This response to cold is regulated by the release of noradrenaline from the innervating nervous system.

Objectives

We have studied the effect of cold exposure on BAT of heterozygous animals for tyrosine hydroxylase (Th), the limiting enzyme of the catecholamines synthesis pathway. Analysis of BAT during late stages of embryonic development and perinatal stages should give us information of the importance of this mutation in development of this target tissue.

Results

Staining with H&E showed some differences in the histology of tissue with a more “compact” appearance in TH+/- mice.

Analysis by immunohistochemistry and western blot confirmed the lower expression of TH in the BAT of the TH +/- mice. The catecholamine levels measured by ELISA showed a decreased in the dopamine and noradrenaline BAT content of TH +/- animals in response to cold. However, the reduced of catecholaminergic response to cold of the TH+/- BAT did not result in a diminished cold adaptation. The TH+/- mice showed similar Ucp1 induction after cold exposure to those of the wild type mice.

In addition, we found that some key genes in the BAT browning, such as Prdm16 and Fgf21, were increased in TH+/- mice in thermoneutrality conditions. Similarly to the BAT, the inguinal white adipose tissue (iWAT) of TH+/- mice responds normally to cold.

Conclusion

We are studying the compensatory mechanisms that allow normal UCP-1 expression in TH +/- mice BAT despite the lower expression of TH and catecholamines compared to wild type animals.

The analysis of other important tissue during thermogenesis, the iWAT, could help us to understand these mechanisms and the browning process.

P6. OBESITY AND CARDIOVASCULAR RISK: VARIATIONS IN VISFATIN GENE CAN MODIFY THE OBESITY ASSOCIATED CARDIOVASCULAR RISK

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Objectives

Our aim was to investigate if genetic variations in the visfatin gene (SNPs rs7789066/rs11977021/rs4730153) could modify the cardiovascular-risk (CV-risk) despite the metabolic phenotype (obesity and glucose tolerance). In addition we investigated the relationship between insulin sensitivity and variations in visfatin gene.

Material and Methods

A population-based study in rural and urban areas of the Province of Segovia, Spain, was carried out in the period of 2001-2003 years. A total of 587 individuals were included, 25.4% subjects were defined as obese [Body Mass Index (BMI) ≥ 30 Kg/m²].

Results

Plasma visfatin levels were significantly higher in obese subjects with DM2 than in other categories of glucose tolerance. The genotype AA of the rs4730153 SNP was significantly associated with fasting glucose, fasting insulin and HOMA-IR (Homeostasis model assessment-insulin resistance) after adjustment for gender, age, BMI and waist circumference.

The obese individuals carrying the CC genotype of the rs11977021 SNP showed higher circulating levels of fasting proinsulin after adjustment for the same variables.

The genotype AA of the rs4730153 SNP seems to protect from CV-risk either estimated by Framingham or SCORE charts in general population and in obese and non-obese. No associations with CV-risk were observed for the other studied SNPs (rs11977021/rs7789066).

Conclusions

In summary, this is the first study which concludes that the genotype AA of the rs4730153 SNP appear to protect against CV-risk in obese and non-obese individuals, estimated by Framingham and SCORE charts. Our results confirm that the different polymorphisms in the visfatin gene might be influencing the glucose homeostasis in obese individuals.

P7. Identification and functional characterization of 8 inactivating glucokinase mutations causing GCK-MODY

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Background: Glucokinase (GCK) is essential for glucose-stimulated insulin release from the pancreatic beta-cell, serving as a glucose sensor in humans. Heterozygous inactivating mutations in GCK cause monogenic diabetes subtype-GCK (GCK-MODY); characterized by stable mild fasting hyperglycemia and HbA1c. Over 700 different inactivating GCK mutations have been reported (HGMD), but only less than 20% of these mutations have been functionally characterized.

Objective: To explore the pathogenicity of 8 identified mutations in GCK through functional studies.

Methods: The promoter, 10 coding exons and exon/intron boundaries of GCK were amplified and directly sequenced (RefSeq:NM_000162.3). In silico software used: Mutation Taster, PolyPhen-2, SIFT. The biochemical effects of the different mutations on the catalytic activity and thermal stability of the glucokinase enzyme have been measured by enzymatic assays on bacterially expressed GST-fusion proteins.

Results: Eight mutations identified in GCK were selected among the different mutations found in our lab. Four of them presented an autosomal dominant pattern, but three had a de novo mutation. Bioinformatic analysis predicted missense mutations to be damaging, but for 2 mutations at least one program did not predict a clear impact on protein function. Seven mutations were novel: p.Val55Ala, p.Ala176Glu, p.Ala201Thr, p.Gly328Val, p.Asp409Asn, p.Ser373_Val374delinsLysMet and p.[(Leu184IleGly193ArgAla201Pro)]. Only one was previously described: p.Gly246Arg. The biochemical characterization of these mutations has shown that all of them impair the kinetic characteristics of the enzyme and reduced the GCK activity. An exception is the mutation p.Ser373_Val374delinsLysMet that we could not assay the in vitro activity since the mutant protein was insoluble. The strongest kinetic effects are caused by mutation p.Ala176Glu and by the triple mutation that occurred in cis p.Leu184IleGly193ArgAla201Pro. These mutations have lost catalytic activity (activity index less than 0.01%). The mutation p.Val55Ala was the most thermally unstable

P8. Prevalence of Diabetes Mellitus in the Basque Country

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Aims: To assess the prevalence of Diabetes Mellitus (DM) and other impaired glucose metabolism in the Basque Country and their relationship with cardiovascular risk factors.

Methods: A population-based, cross-sectional, cluster sampling design study was carried out in adult Basque population (≥ 18 years). Eight hundred and forty seven participants completed a questionnaire on personal and family medical history and lifestyle. Anthropometric variables and blood pressure were measured and biochemical analysis and Oral Glucose Tolerance Test (OGTT; 75 g) were also performed.

Results: Total prevalence of DM was 10.6% (95% CI: 8.65-12.95). Among them 6.3% (4.79-8.22) had been previously diagnosed and 4.3% (3.04-5.92) were not aware that they had DM. Impaired glucose tolerance was present in 7.2% (5.53-9.15) and impaired fasting glucose in 3.8% (2.64-5.37). In total, 21.6% of the study population had some type of glucose metabolism disturbance with a higher rate among men (28.3 vs. 16.3%, $p < 0.001$) and increasing with age. Risk factors independently associated with the development of DM were: male sex, OR 4.58 (95% CI: 2.34-8.97); abdominal obesity, 2.80 (1.47-5.36); high triglyceride levels, 2.46 (1.26-4.81); high blood pressure, 2.40 (1.16-4.96); family history of DM, 2.30 (1.25-4.24); high LDL-c levels, 1.83 (1.01-3.31) and older age, 1.08 (1.05-1.10).

Conclusions: Prevalence of DM in the Basque Country is lower than in Spain and is independently associated with family history of DM and with cardiovascular risk factors as abdominal obesity, high blood pressure, high LDL-c levels and high triglyceride levels, which are also observed in the pre-diabetes status.

P9. A peptide mimic of suppressor of cytokine signaling 1 limits development and progression of experimental diabetic nephropathy

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Diabetes is the main cause of chronic kidney disease and end-stage renal disease worldwide. Chronic activation of Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway contributes to diabetic nephropathy by inducing genes involved in leukocyte infiltration, cell proliferation and extracellular matrix accumulation. Suppressors of cytokine signaling (SOCS) control the duration and amplitude of JAK/STAT signaling and are key physiological regulators of inflammation. This study examines whether a cell-permeable peptide mimicking the kinase inhibitory region of SOCS1 protects against nephropathy by suppressing JAK/STAT-mediated renal cell responses to diabetic conditions. In a mouse model combining hyperglycemia and hypercholesterolemia (streptozotocin diabetic apolipoprotein E-deficient mice), we observed that renal STAT1/STAT3 activation status correlates with the severity of nephropathy. Remarkably, administration of SOCS1 peptide at both early and advanced stages of diabetes ameliorated renal STAT1/STAT3 activity and resulted in renal protection, as evidenced by reduced albuminuria and renal histological changes (mesangial expansion, tubular injury, interstitial cell infiltrate and fibrosis) over time compared with mice receiving vehicle or mutant inactive peptide. SOCS1-treated mice exhibited a reduction of kidney leukocyte recruitment (T-lymphocytes and classical M1 macrophages) and decreased expression levels of pro-inflammatory and pro-fibrotic markers independently of glycemic and lipid changes. In vitro, SOCS1 peptide suppressed STAT activation and target gene expression, reduced migration and proliferation in mesangial and tubuloepithelial cells, and altered the expression of macrophage polarization markers. In conclusion, our study identifies SOCS1 mimicking as a feasible therapeutic strategy to halt the onset and progression of renal inflammation and fibrosis in diabetic kidney disease.

P10. CRITICAL ROLE OF PASK IN LIVER RESPONSE TO INSULIN, FASTING/FEEDING AND OXIDATIVE STRESS

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The PAS domain protein kinase (PASK) is a nutrient sensor, that in mammals is involved in the regulation of glucose and energy metabolism homeostasis. Furthermore, PASK-deficient mice exhibit protection against obesity and the development of hepatic steatosis and insulin resistance induced by high fat diets. We have identified PASK in hypothalamic areas involved in feeding behaviour. There, its expression was regulated under fasting/refeeding conditions and modulated by the antidiabetogenic agent: exendin-4. Additionally, the PASK deficiency alters the nutrient response of AMPK and mTOR pathway in areas involved in the control of food intake and liver.

Our recent findings, would expand the potential roles of PASK in the liver, in the context of glucose sensing, metabolic adaptation to fasting and feeding states, and besides its role in the maintenance of the redox status and oxidative stress.

In this sense, we have observed that exendin-4 (a stable analog of GLP-1) blocks hepatic PASK expression and PASK-deficient mice have disturbed the expression of mRNAs coding to glucokinase and glucokinase regulatory protein and glucokinase activity. Also PASK^{-/-} mice, presented altered expression of mRNAs coding to enzymes responsible for promoting the changes required to adaptative metabolic response to fasting and feeding states. Also an impaired liver insulin signalling pathway (expression and activation degree of AKT, GSK3 β) was observed.

Finally, PASK-deficiency also promoted expression of mRNAs coding to sirtuins and antioxidant enzymes such as Catalase, Cu/ZnSOD, MnSOD, suggesting an improved response to prevent toxicity of ROS (reactive oxygen species).

In conclusion, the enhanced phenotype of PASK^{-/-} mice, in relation of its capacity to prevent the obesity promoted by high fat diet, is accompanied by a better protection facing to oxidative stress status. Furthermore, the exendin-4 used as anti-diabetogenic agent can potentially mimic the phenotype found in PASK^{-/-} mice.

P11. Mechanisms of action of Calcium Dobesilate in the early stages of diabetic retinopathy

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Diabetic retinopathy (DR) is the most frequent complication in diabetes. Although DR has been considered a microcirculatory disease, there is growing evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR, which participates in the microcirculatory abnormalities that occur in DR. For this reason, in our group we find new pharmacological alternatives to prevent retinal neurodegeneration and progression of the microvascular impairment that occur in DR.

In the present study, we assess the effect of calcium dobesilate (CaD) in preventing neurodegeneration and vascular impairment in db/db mice model. The results have shown a significant decrease of reactive gliosis and apoptosis in the retina of CaD treated animals respect to non-treated. Moreover, we have observed a significant reduction of total retinal thickness and a diminished cell number in non-treated animals, showing that CaD preserved retinal morphology. CaD abrogates GLAST downregulation induced by RD, and therefore prevents the increase of glutamate caused by the RD.

CaD prevented the increase of endothelin-1 (ET-1), and also arrested the up-regulation of both endothelin receptors, Endothelin A (ETA-R) and Endothelin B Receptor (ETB-R). Finally, CaD increases the sealing function of the BBR, thus preventing the albumin leakage and reduces the expression of VEGF.

A significant improvement of ERG parameters was observed, revealing a clear impact of global retinal function.

In conclusion, CaD treatment could be effective in ameliorating the neurodegeneration in early diabetic retinopathy and in arresting microvascular impairment.

P12. IMPACT OF EPIDERMAL FATTY ACID BINDING PROTEIN ON 2D-NMR-ASSESSED DIABETIC DYSLIPIDEMIA AND RELATED DISORDERS

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Background

The role of circulating FABP5 on metabolic alterations is under active evaluation. On the other hand, FABP5 SNPs (rs454550 and rs79370435) seem to modulate its effect.

Objectives

Our aim was to examine the role of circulating FABP5 levels and its main SNPs in atherogenic dyslipidemia (AD) assessed by 2D-NMR and related metabolic and inflammation markers. We hypothesized that circulating FABP5 may be a biomarker for metabolic risk.

Methods

We studied 459 subjects admitted to the metabolism unit because of lipid metabolism disturbances and/or associated disorders. After a 6-week lipid-lowering drug wash-out period, anamnesis and physical examination were performed. Carotid intima-media thickness (cIMT) was measured by ultrasound. FABP5, FABP4, lipids, metabolic proteins, and enzymes were determined by biochemical methods. The lipid profile was assessed by NMR. The rs454550 and rs79370435 FABP5 gene variants were also determined.

Results

The FABP5 plasma levels were positively correlated with adiposity, glucose metabolism, and lipolysis parameters and were associated with AD, as assessed by NMR. There was a significant positive correlation between hsCRP and FABP5. The presence of type 2 diabetes, obesity, metabolic syndrome, or AD was associated with higher FABP5 plasma levels ($P < .005$). The FABP5 concentrations, but not those of FABP4, were higher in patients with carotid plaques. FABP5 was a main determinant of plaque presence according to logistic regression analysis. The rare rs454550 allele was hyper-represented in nonobese subjects ($P = .011$).

Conclusions

FABP5 is a biomarker of adiposity-associated metabolic derangements that include AD thus underscoring the concomitant presence of inflammation. FABP5 is associated with increased subclinical atherosclerosis.

P13. Oxidative stress markers and altered Semmes Weinstein monofilament test in type 2 diabetic subjects

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INCLIVA

Aim: to analyze the association of oxidative stress markers (glutathione system: GSH, GSSG and GSH/GSSG ratio and malonyldialdehyde (MDA) with altered Semmes-Weinstein (SWM) test in type 2 diabetic (T2DM) patients.

Subjects and methods: 70 T2DM, 34 with altered SWM, were studied. Patients with altered SWM test showed significantly higher values of GSSG (3.53 ± 0.31 vs 3.31 ± 0.36 mmol/ml, $p < 0.05$) and MDA (MDA: 1.78 ± 0.19 vs 1.84 ± 0.15 nmol/ml. In T2DM subjects, altered SWM test was independently related to age, HbA1c and GSSG levels.

Conclusion: We have found an alteration in the glutathione system and MDA values as markers of OS in subjects with altered detection of pressure sensibility. This finding could be important for understanding how OS affects proprioceptive sensibility in diabetic patients

P14. Gene expression profiling of subcutaneous adipose tissue in morbid obesity using a focused microarray reveals potentially favorable changes of factors related to lipid processing Josep Julve¹, Eva Pardina², Roser Ferrer², Joana Rossell², Juan Antonio Baena-Fustagueras³, José Manuel Fort⁴, Albert Lecube⁵, Julia Peinado-Onsurbe²

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The impact of bariatric surgery-mediated weight loss on the gene expression of factors related to lipid and glucose metabolism in subcutaneous adipose tissue (SAT) in morbid obese is still poorly defined.

Objective: The aim of this study was to analyze the gene expression of factors involved in lipid and glucose processing in subcutaneous adipose tissue in postoperatively morbid obese patients.

Materials and methods: Plasma and tissue samples were obtained from a subcohort of 15 out of 34 patients undergoing RYGB at baseline and 12 mo. after surgery. Plasma samples and tissue biopsies were assayed for chemical analysis. Subcutaneous fat depot biopsies were assayed for the expression of genes using tissue-specific focused microarrays.

Results: RYGB-induced weight loss produced a favorable decrease in the fat depot mass, insulin resistance and lipid levels in plasma of our postoperatively obese. At baseline, we identified that LEP, ADIPOQ, LIPE, LPL, CD36, PAI1, NOS2, SLC2A4, FABP4, UCP2, and PPARG were predominantly overexpressed in SAT compared with visceral adipose tissue (VAT). Compared with baseline, a significant upregulation of molecular targets of glucose and lipid metabolism (SLC2A4 and PPARGC1; +1.7- and +1.8-fold, respectively) and downregulation of molecular targets of lipid (PNPLA2, AQP7, FABP4, and CD36; -1.3-, -1.4-, -1.3-, and -1.3-fold, respectively) and energy (UCP2; -1.3-fold) metabolism, and vascular function (PAI1; -2.5-fold) were observed in SAT biopsies of postoperatively morbid obese. Notably only the change in the gene expression of CD36 was directly associated with that of BMI (Spearman $r=+0.78$; $P=0.048$) and SAT (Spearman $r=+0.76$; $P=0.041$).

Conclusion: Our data reveal that RYGB-induced weight loss is associated with significant changes in the gene expression of factors related to metabolic homeostasis of SAT which in turn might be reflecting favorable changes in its physiology.

P15. Consumption of polyunsaturated fat improves the saturated fatty acid-mediated impairment of HDL antioxidant potential

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Scope: A high-saturated fatty acid and high-cholesterol-containing (HFHC-SFA) diet is considered to be a major risk factor for cardiometabolic disease, whereas the polyunsaturated (PUFA) fatty acids are potentially atheroprotective. The present study aimed to compare the effects of a HFHC-SFA and HFHC-PUFA diets on two major antiatherogenic functions of HDL, the HDL antioxidant function and the macrophage-to-feces reverse cholesterol transport.

Methodology: Experiments were carried out in mice fed a low-fat, low-cholesterol (LFLC) diet, an HFHC-SFA diet or an HFHC-PUFA diet in which SFAs were partly replaced with an alternative high-linoleic and alpha-linolenic fat source.

Results: The HFHC-SFA caused a significant increase in serum HDL cholesterol and phospholipids as well as elevated levels of oxidized HDL and oxidized LDL. Replacing SFA with PUFA significantly reduced the levels of these oxidized lipoproteins and enhanced the ability of HDL to protect LDL from oxidation. The SFA-mediated impairment of HDL antioxidant potential was not associated with the cholesterol content of the diet, obesity or insulin resistance. In contrast, the effect of the HFHC diets on fecal macrophage-derived cholesterol excretion was independent of the fatty acid source.

Conclusion: SFA intake impairs the antioxidant potential of HDL and increases serum levels of oxidized lipoprotein species whereas the antioxidant potential of HDL is enhanced after PUFA consumption.

P16. CD5L decreases macrophage TNF secretion and enhances foam cell formation under high-glucose conditions

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Cardiovascular disease prevalence is very high in patients with type 2 diabetes mellitus, but the underlying mechanisms are incompletely understood. The macrophage is a key cellular player in the atherosclerotic process. Both macrophage scavenger receptor CD36 and its ligand CD5-Like protein (alternative named Apoptosis Inhibitor of Macrophages, AIM), actively participate in the molecular events that lead to plaque formation in atheromatous disease. The main goal of this study was to determine the role of CD5L in the homeostasis of macrophages under hyperglycemic and atherogenic conditions.

Methods

Two sources of macrophages were used, namely, THP1 cells and monocyte-derived primary macrophages, purified from peripheral blood from healthy, non-diabetic donors. Macrophages were cultured in normal (5mM) or high (15mM) glucose concentrations, and stimulated with bacterial lipopolysaccharide (LPS) and oxidized-LDL (ox-LDL). The ability of CD5L to modify macrophage secretion of the pro-inflammatory cytokine TNF was measured by ELISA. Moreover, CD5L effect on macrophage ox-LDL uptake and foam cell formation was assessed by flow cytometry. Expression of relevant receptors for oxLDL uptake (CD36, SRA) and cholesterol efflux (SRB1, ABCA1, ABCG1) was assessed by real time qPCR analysis.

Results

High glucose induced a pro-inflammatory TNF response in THP1 macrophages and in primary macrophages that was inhibited by CD5L. Moreover, high glucose enhanced ox-LDL- uptake and foam cell formation in THP1 macrophages, which was further increased upon CD5L expression. Real-time qPCR analysis showed that mRNA levels of scavenger receptor CD36 was significantly higher in macrophages expressing CD5L, and further enhanced under high glucose conditions; SRA mRNA was higher in macrophages expressing CD5L independently of glucose condition while the mRNA expression of reverse cholesterol transporters such as SRB1, ABCA and ABCG is under analysis.

Conclusions

Taken together, our data support a key role of CD5L in suppressing the pro-inflammatory and enhancing the pro-atherogenic effect of hyperglycemia in macrophages.

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P17. In-vitro differentiation of insulin-producing cells from human embryonic stem cells: the CABIMER protocol

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Sixteen years ago our group was the first to publish that insulin producing cells have been derived from mouse embryonic stem cells (mESCs). On October 29, 2014, ViaCyte (San Diego, California) announced the first-in-the-world implant of progenitors of insulin-producing cells derived from human embryonic stem cells (hESC) in a type 1 diabetes patient. ViaCyte strategy consists in implanting non-mature progenitors that may thereby follow the maturation process inside the patient body. In the past two years, four more papers described efficient methods to obtain more mature insulin-producing cells from human embryonic stem cells. Instrumental knowledge obtained from early mouse development, the sequential expression of the transcription factors and the signalling pathways involved in human beta-cell formation have been applied to the differentiation processes; in this context the application of such developmental principles to stem cells (human ESC and/or iPS) seems to be the key for a successful differentiation to obtain functional insulin-expressing cells. Reported signalling pathways and factors used in pluripotent stem cell's differentiation to obtain functional insulin secreting cells are the result of almost ten years of investigation, however the complexity of this process reveals that it has not been still achieved. The major problems of directing stem cells differentiation to beta-cell are the low reproducibility of published differentiation protocols and the low amount of insulin-secreting cells obtained at the end of the differentiation processes which lack maturation. Exogenous small molecules, such as nitric oxide, promote endoderm differentiation in mESC, whilst cyclopamine, miRNAs, and resveratrol ameliorate the maturation process of β -cell-like cells obtained from an optimized differentiation protocol of human ESCs. Our protocol efficiently generates insulin-producing cells that contain and release insulin and normalize blood glucose after transplantation in STZ-induced hyperglycaemic NOD-SCID mice.

P18. Safe and cost-effective use of mesenchymal stromal cells in cell therapy of critical ischaemia of the limbs for diabetic patients

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Mesenchymal stromal cells (MSCs) have been established as promising candidates for cell therapy due to their contribution to homeostasis, repair and support of tissues and to their anti-inflammatory, antiproliferative, immunomodulatory, trophic and pro-angiogenic properties. Intra-arterial administration of autologous adipose-derived MSCs (AdMSCs) has been used to test safety and feasibility for treatment of critical ischaemia of the limbs of 30 diabetic patients without the possibility of revascularization. Phase I-IIa open label, randomized, dose scalable clinical trial (NCT01257776) with transplantation of 0,5x10⁶ cells/kg and 1x10⁶ cells/kg patient body weight have been performed. Neovascuogenesis (digital subtraction angiography and MetaMorph v6.3 quantification and clinical improvement (Rutherford-Becker classification, University of Texas Diabetic Wound Scale, ABI and TcpO₂) was compared at baseline and at follow-up. AdMSCs from diabetic patients release more PAI-1 and less tPA, when exposed to serum of diabetic patients. Moreover, AdMSCs from diabetic patients proliferate more, release more tissue factor, migrate less, are more adipogenic and less osteogenic than control AdMSCs. After 12-months of follow-up all patients presented improvement in all clinical parameters. The clinical outcome was consistent with neovascuogenesis. Only minor effects have been described 30 min after infusion (local hyperemic reaction and headache). Two patients after the infusion of high cell doses presented a prothrombotic response. After corrective and preventive actions the trial was re-authorized by the Spanish Agency of Medicines and Medical Devices. Cost of CIL process in Spain is 16.000 euros on average, whilst effective treatments cost 10.000-15.000 euros. based on these observations a multicenter (Phase II) study is being designed.

P19. Altered Ocular Metabolites in Irs2-Deficient Mice: Potential Biomarkers of Retinal Degeneration

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The majority of patients with Type 1 diabetes and >60% of patients with type 2 diabetes develop diabetic retinopathy (DR), emphasizing the crucial role of both glycemic control and insulin sensitivity in the underlying pathology of this debilitating complication. Although DR is an ocular disease, systemic factors modulate its development and progression but the precise mechanisms have not yet been defined due to paucity of suitable animal models. Insulin signaling in the retina regulates neuronal development, growth, survival, and anabolic synthesis, as opposed to the largely metabolic role of these pathways in peripheral tissues. Indeed, mice deficient for Irs2 display significant photoreceptor loss and defective vision, suggesting that this model represents a tool for understanding the role of hyperglycemia, insulin resistance, and systemic inflammation in DR. As neurodegeneration is an early event in the pathogenesis of DR, we are using Irs2-deficient mice to unravel the mechanisms that contribute to neuronal apoptosis, with the ultimate goal of identifying novel targets for detection and treatment of the early stages of DR. Here we report the utility of analyzing whole mouse eyes by NMR as a tool for studying the molecular basis of DR. Eyes were harvested from pre-diabetic (glucose < 120), female Irs-2 mice and their WT controls and processed for metabolomics analysis. The results reveal that defective insulin signaling leads to significant metabolic changes in the eye including carbohydrate metabolism pathways (e.g., UDP-glucose, lactate, glycerol), lipids, and neurotransmitters (e.g., glutamate, aspartate, glycine). The metabolic signature of Irs2-deficient eyes is consistent with cellular energy deprivation and failed neurotransmission, even in the pre-diabetes state, identifying a potential cause of photoreceptor degeneration observed in these mice. Hence, analysis of ocular metabolites in the appropriate model may provide clues to the underlying pathology of DR and is an attractive tool for development and testing of effective treatments.

P20. A model for diabetes induction in pre-clinical human islet transplantation

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Streptozotocin (STZ) is a cytotoxic glucose analogue that causes β -cell death and is widely used to induce experimental diabetes in rodents. The sensibility of β -cells to STZ is species-specific, and human beta cells are resistant to the cytotoxic action of STZ. In rodents, islet isolation and transplantation are planned ahead of time and diabetes is induced several days before transplantation to avoid the toxic effects of STZ on grafted islets. In contrast, the availability of human islets depends on organ donation, and prospective diabetic recipients must be kept in the Animal Care Facility for unpredictable lapses of time, requiring daily treatment with insulin to survive. Taking advantage of the resistance of human islets to STZ, we have investigated a model of human islet transplantation in which experimental diabetes is induced after transplantation. Methods: Human and mouse islets were transplanted under the kidney capsule of athymic nude mice, and 10-14 days post transplantation mice were intraperitoneally injected with five consecutive daily doses of STZ (70 mg/Kg body weight) or vehicle. Grafts were harvested a) on the last day of STZ injection and β -cell death, mass and vascular density were determined; b) on day 30 after STZ injection to assess metabolic evolution. Results: After STZ injection, β -cell death increased and β -cell mass was reduced in mouse islet grafts compared to vehicle-injected animals, but did not change in human islet grafts. Vascular density was not modified by STZ in either group, but was lower in human islets grafts. Animals transplanted with rodent islets developed hyperglycemia after multiple-dose STZ-injection. Animals transplanted with human islets remained normoglycemic, and developed hyperglycemia after graft harvesting. Thus, STZ had no effects on β -cell death, mass and function of human transplanted islets. Conclusion: We provide a new, more convenient and affordable, model for human islet transplantation in which diabetes can be induced after islet transplantation.

P21. Early and chronic undernutrition followed by a hypercaloric diet increases the risk of metabolic syndrome in wistar rats

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Understanding the biology of adipose tissue is crucial in view of the considerable evidence of links between increased production of some adipocyte factors and several metabolic complications, such as insulin resistance and type-2 diabetes. Different studies have suggested an increased risk of obesity in human subjects who have a previous history of undernutrition. However, this is controversial. To investigate this apparent paradox, we have developed an experimental model of early undernutrition followed by rehabilitation with a moderately high-lipid cafeteria formula in Wistar rats.

In white adipose tissue of undernourished-rehabilitated rats, we found whole insulin resistance, inflammatory cues and signs of oxidative stress. On the other hand, a marked ectopic lipid deposition in liver and skeletal muscle were also observed. Surprisingly, these alterations were not associated with obesity in terms of body weight. These facts prompt us to study the underlying molecular mechanisms. We found that food restriction followed by refeeding induced a pronounced infiltration of both brown adipocytes and macrophages in white adipose tissue (visceral and subcutaneous pads). Concomitantly, a marked increase in NPY was also seen in this tissue.

On the basis of these results, we conclude that early-chronic undernutrition followed by rehabilitation with moderately high-lip diets enhances some alterations characteristic of the metabolic syndrome. Some of the targets analyzed in this work, like UCP1 and NPY, have been investigated for the treatment of obesity.

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P22. Targeting aberrant hepatocellular growth using a novel allosteric inhibitor of IRS proteins: NT-157

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Hyperinsulinaemia promotes aberrant liver growth during development whilst insulin resistance is associated with chronic liver disease and hepatocellular carcinoma (HCC). Increased expression of insulin receptor substrate 2 (IRS2), a key downstream mediator of insulin/IGF1 receptor signalling, has previously been linked to hepatocarcinogenesis, although controversy remains as to how broadly this finding applies to large patient cohorts. In this study we investigated the role of IRS2 as a mediator of hepatocellular proliferation using the bipotent human HepaRG cell line, HCC derived HepG2 cells and in vivo studies using adenovirus to express IRS2 in mouse liver. Exogenous expression of IRS2 promoted steatosis, glycogen accumulation as well as aberrant hepatocellular growth in all three models due to increased signalling flux through the insulin/IGF1 axis. However, only in HepG2 cells was IRS2 able to promote anchorage-independent and SCID-xenograft growth. Gene silencing experiments in HepaRG cells showed that endogenous IRS2 promoted hepatocellular differentiation of progenitor-like cells via the canonical insulin receptor, whereas in the HepG2 model IRS2 promoted anchorage-independent growth via the IGF1 receptor. We tested this hypothesis using NT-157, a novel allosteric inhibitor of IRS proteins - which targets the interaction between IRS proteins and the IGF1 receptor. NT-157 had no effect in blocking *de novo* human hepatogenesis in HepaRG cells (>20uM), whilst it was highly effective in blocking HepG2 tumorigenesis in vitro (<2uM). These data suggest we can potentially intervene to target the protumorigenic effects of IGF1/IRS2 signalling in HCC whilst maintaining the regenerative potential of insulin/IRS2 signalling in progenitor cells of the damaged liver parenchyma.

P23. Epigenetic Pdx1 Regulation by Nitric Oxide Enables Differentiation of Embryonic Stem Cells Towards Functional Pancreatic Beta-Cells

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Pdx1 (Pancreatic and duodenal homeobox 1) is a transcription factor that regulates the pancreatic development and the differentiation and functionality of beta cells. Our group has shown that the treatment of mouse embryonic stem cells (ESC) with high concentrations of NO increases Pdx1 expression. In the present study we report that the increase of Pdx1 expression after NO exposure is associated with the release of Polycomb Repressive Complex 2 (PRC2) and the histone acetyltransferase P300 from its promoter. Furthermore, it was observed some changes in bivalent marks of histone H3K27me3 and H3K4me3 on Pdx1 promoter, but no changes in acetylated histone H3 occupancy. Moreover, site specific changes of Pdx1 promoter methylation were found. The knowledge of the regulation of the gene Pdx1 has allowed us to develop a protocol to differentiate mouse ESC towards insulin producing cells. The protocol consists of adding to the culture medium 0.5 mM DETA-NO for 19 hours, 100 µM valproic acid for 6 days, 50 µM P300 inhibitor for 20 hours and a final step of suspension culture to form aggregates for 3 days. This simple and cost effective differentiation protocol allows the generation of cells stably expressing markers of beta-cells and enables the generation of glucose-responsive monohormonal insulin producing cells. Currently, the protocol is being replicated in human ESC with promising results.

P24. BACE2 SUPPRESSION AMELIORATES β -CELL DYSFUNCTION INDUCED BY HUMAN ISLET AMYLOID POLYPEPTIDE (IAPP) OVEREXPRESSION

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BACE2 (β -site APP-cleaving enzyme 2) is a β -protease that has been found in the brain, where it is thought to play a role in the development of Alzheimer's disease (AD). It has also been localized in the pancreas, where it seems to play a physiological. Amyloidogenic diseases, including AD and type 2 diabetes (T2D), have been reported to share the accumulation of abnormally folded and insoluble proteins that interfere with cell function. In the case of T2D, amylin (IAPP) deposits have been shown to be a key feature of the disease. The aim of the present study was to investigate the effect of BACE2 modulation on β -cell alterations induced by IAPP overexpression.

IAPP-heterozygous mice, BACE2-KO mice and their respective controls were used to analyze their phenotype after 16 weeks with high-fat diet (HFD) feeding. Afterward, these two models were crossed in order to analyze the impact of BACE2 suppression on β -cell alterations observed in hIAPP-Tg mice.

The glucose tolerance test (GTT) of hIAPP-Tg mice revealed glucose intolerance with respect to the wild type animals. These animals showed a low secretory response of insulin after the glucose challenge (25% decrease $p < 0.05$, vs. control littermates). On the other hand, BACE2-KO mice showed better glucose homeostasis than their wild type counterparts when fed with HFD (28% decrease in AUC of GTT). Moreover, BACE2-KO mice fed with a HFD showed an 18% reduction in body weight ($p < 0.05$), indicating that deletion of BACE2 protects against HFD. The crossed animals (hIAPP-TgxBACE2-KO) presented a significant improvement in glucose tolerance as compared to hIAPP-Tg mice (18% decrease in AUC) that was not accompanied by an increase in insulin sensitivity. This improvement indicated a potential beneficial effect of BACE2 deletion on β -cell function.

The inhibition of BACE2 compensates glucose tolerance defects induced by hIAPP overexpression in the β -cell. Thus, targeting BACE2 may represent a good therapeutic strategy to improve β -cell function in T2D.

P25. Decreased expression of CDKN2A/2B in human diabetes and coronary artery disease associate with T cell imbalance

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INCLIVA

Type 2 diabetes mellitus (T2DM) and coronary artery atherosclerotic disease (CAD) have been associated with single nucleotide polymorphisms located in the vicinity of the CDKN2A/2B/BAS genes. Activation of T-cells and decreased levels of regulatory T (Treg)-cells create a chronic inflammatory state that facilitates insulin resistance and CAD. In this study, the connection between CDKN2A/2B/BAS gene expression and leukocyte phenotype was investigated in human subjects exhibiting T2DM and CAD and in atherogenic diet-fed apoE^{-/-} mice. Gene expression analysis in leukocytes from T2DM and T2DM-CAD subjects demonstrated reduced mRNA levels of the CDKN2A splice variant 1 (p16Ink4a) (a decrease of 74% and 95%, p<0.02 and p<0.008), CDKN2B (a decrease of 66% and 85%, p<0.01 and p<0.005) and CDKN2BAS (a decrease of 89% and 96%, p<0.03 and p<0.04) compared with human controls. Protein expression analysis demonstrated diminished levels of p16Ink4a and p15Ink4b in T2DM (p16Ink4a: reduction of 30%, p<0.03; p15Ink4b: reduction of 33%, p<0.05) and T2DM-CAD (p16Ink4a: 34%, and p<0.02; p15Ink4b: reduction of 43%, p<0.01) patients compared with controls. T2DM and T2DM-CAD patients also displayed an increase in activated (CD3+CD69+)T-cells (24% and 29%; p<0.03 and p<0.02) and a 72% and 51% increase in the proinflammatory CD14++CD16+-monocyte subpopulation (p<0.0007 and p<0.04). T2DM and T2DM-CAD subjects had a 15% and 22% decrease in (CD4+CD25+CD127-)Treg-cells (p<0.04 and p<0.003) and a 17% and 25% reduction in plasmatic IL4 (p=0.05 and p<0.005). Interestingly, Treg levels inversely correlated with HOMA-IR index in all subjects. In agreement with the above studies, atherogenic diet-fed apoE^{-/-} mice displayed decreased expression of Cdkn2a/2b genes (58% and 86%, p<0.03 and p<0.00001), augmented activated T-cells (78%, p<0.02), a 48% increase of proinflammatory Ly6Chi-monocytes (p<0.02) and a 60% reduction of Treg/Th17 ratio (p<0.03), compared with controls. Thus, T2DM and CAD progression associate with decreased CDKN2A/2B/BAS gene expression and with T-cell and monocyte imbalance.

P26. EPITHELIAL TO MESENCHYMAL TRANSITION IN HUMAN BETA, ALPHA, DELTA AND PP CELLS EXPANDED IN VITRO

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Aim

The concept of epithelial-to-mesenchymal transition (EMT) of cultured pancreatic beta cells has been carefully demonstrated by lineage tracing. In vitro, adult human beta cells transition into a highly proliferative mesenchymal cell population. The presence of EMT in other pancreatic endocrine cell populations is unknown. The aim of this study was to determine the EMT process in endocrine pancreatic cells.

Material and Methods

Human islets were isolated from pancreas of 12 multiorgan donors (age 54±18 y.o; BMI 26±4; islet purity 70±15%), dissociated into single cells and purified by magnetic cell sorting using PSA-NCAM antibody. The resulting cell fraction was seeded for monolayer culture and split 1:2 weekly. Co-expression of the mesenchymal marker vimentin in endocrine cells (immunofluorescence) was used to evaluate EMT. Gene expression was determined by qRT-PCR. Beta cell apoptosis was quantified by double-staining using TUNEL assay and insulin antibody. To inhibit EMT, cells were exposed to A83-01, an inhibitor of TGF-β type I receptor ALK5 kinase. Inhibition of EMT was evaluated by qRT-PCR and immunofluorescence techniques.

Results

A progressive reduction in the percentage of endocrine markers and a gain of vimentin was found along the passages. PP-positive cells were not found beyond P2 and the presence of insulin, glucagon, and somatostatin positive cells was residual at P4. The percentage of apoptotic beta cells was low, suggesting that the reduction in insulin expressing cells was not due to increased beta cell death. Co-expression of endocrine and mesenchymal markers was early detected in culture and increased subsequently up to P4. Changes in gene expression were also suggestive of EMT process. A83-01 treatment further confirmed the occurrence of EMT in endocrine cells based on the reduction in the percentages of co-expressing cells and the gene expression levels.

Conclusion

All four types of adult human pancreatic endocrine cells undergo EMT in vitro.

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P27. Low Nitric Oxide induces a Hypoxia-like Response in Pluripotent Stem Cells

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Expansion of stem cells under conditions that maintain their pluripotency is a suitable goal in any cell therapy program. We have reported that low concentrations of the Nitric Oxide (NO) donor diethylenetriamine/nitric oxide adduct DETA-NO promotes the expansion of these type of cells and the mechanisms involved in this action are yet to be fully characterized. On the other hand, it has been reported that hypoxia prevents differentiation of hESCs and is required to maintain a pluripotent state. We show here that NO triggers a hypoxia-like response in hiPSCs when grown under normoxia, thus maintaining pluripotency. The expression of pluripotency genes such as Nanog, Sox-2 and Oct-4 increases in cells cultured with low NO with respect to normoxia. A similar pattern is observed in cells cultured under hypoxia. We also have found that low NO stabilizes Hypoxia Inducible Factors HIF1 α and HIF 2 α . Because of the close relationship between hypoxia, energy metabolism, mitochondrial function and pluripotency, we have analyzed by q RT-PCR the expression of genes involved in glycolytic pathway such as HK2, LDHA and PDK1. We further analyzed the expression of genes involved in mitochondrial biogenesis such as PGC1 α , TFAM and NRF1 and we have observed that low NO maintains the same pattern expression as observed in hypoxia. In addition, mitochondrial membrane potential showed a slight decrease in cells exposed to low with NO in the normoxic condition. We have analyzed other metabolic parameters of mitochondrial function such as oxygen consumption and we have found that NO regulates mitochondrial function and mimics Hypoxia Response in human ESCs. An understanding of the action of low NO in the cellular response to hypoxia might be instrumental for the optimization of culture media for in vitro expansion of pluripotent stem cells.

P28. Expression of Alx3 in the hypothalamic arcuate nucleus regulates energy metabolism and body composition

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Neurons located within different hypothalamic nuclei integrate glucose and hormone signaling to contribute to the regulation of metabolic homeostasis by balancing caloric input and energy expenditure. One of these nuclei, the arcuate nucleus (ARC) plays a central role in the regulation of food intake by processing signals related to hunger or satiety, but the mechanisms by which these signals are processed for the systemic control of energy metabolism in the hypothalamus are largely unknown. Previous studies demonstrated that the homeodomain transcription factor Alx3 is important for the maintenance of pancreatic islet-dependent glucose homeostasis. In the present study, we describe the expression of Alx3 in the ARC and provide evidence for a possible role in the central control of energy homeostasis.

We found that food intake in Alx3-deficient mice was reduced relative to wild type animals, but we observed no differences in body weight, suggesting the existence of a metabolic imbalance. Indirect calorimetry demonstrated the presence of reduced oxygen consumption and energy expenditure without affecting locomotor activity. There was a small reduction in respiratory exchange ratio during the night-to-light transition. ¹⁸F-Fludeoxyglucose uptake measured by positron emission tomography as well as functional diffusion-weighted magnetic resonance Imaging (DWI) in response to fasting confirmed the existence of altered neural activity in the ARC of Alx3-deficient mice. Immunofluorescence, RT-qPCR and western blot experiments revealed the expression of Alx3 in POMC and NPY neurons in the ARC. Alx3-deficient mice also showed increased body fat mass relative to lean mass, increased expression of proadipogenic genes in adipose tissue, and increased adipocytes size relative to control mice. Our data support the notion that Alx3 expressed in the hypothalamic ARC contributes to the regulation of energy homeostasis and relative body composition.

P29. Liposomes mimicking apoptotic β -cells: a new immunotherapy for type 1 diabetes

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Currently, the aetiology of type 1 diabetes mellitus (T1DM) remains unknown and strategies to cure this disease are not available. To prevent and cure T1DM, a sine qua non condition is the arrest of the autoimmune β -cell destruction. An ideal immunotherapy should restore immunological self-tolerance, avoiding systemic side effects and allowing islet regeneration.

A physiological strategy to avoid autoimmunity is apoptosis. Apoptotic cells are a source of autoantigens, and when they are phagocytosed, induce tolerance and promote cell replacement. Based on these features, liposomes mimicking apoptotic β -cells have been created as immunotherapy for T1DM. Liposomes are vesicles clinically used for drug delivery and are safe and biocompatible.

Liposomes rich in phosphatidylserine (PS), a membrane component exposed during apoptosis and the main signal for phagocytosis, were loaded with β -cell autoantigens. PS-liposomes showed multivesicular morphology and optimal size for phagocytosis. PS-liposome induced tolerogenic dendritic cells (tolDCs), capable of inducing self-tolerance. TolDCs display low levels of costimulatory molecules, secrete prostaglandin E₂, a lipid mediator with suppressive function, and impair autoreactive T cell proliferation. The tolerogenic potential of PS-liposomes has been confirmed in non-obese diabetic (NOD) mice, the spontaneous model of T1DM. When administered to prediabetic NOD mice, PS-liposome signal was detected in lymph nodes, spleen and pancreas. Importantly, a single dose of PS-liposomes administered at pre-clinical stage, arrests β -cell destruction, reduces insulinitis, and permanently prevents T1D. PS-liposome treatment results in a fast expansion of antigen-specific T cells that could prove to possess regulatory function.

Therefore, PS-liposomes mimicking apoptotic β -cells can be an effective antigen-specific immunotherapy, constituting a promising strategy to arrest autoimmunity in T1DM and other autoimmune diseases.

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P30. PROGNOSTIC FACTORS AND PATHOPHYSIOLOGY OF DIABETES REMISSION AFTER METABOLIC GASTRIC BYPASS, SLEEVE GASTRECTOMY AND GREATER CURVATURE PPLICATION: A RANDOMIZED CONTROLLED TRIAL

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Introduction: 39% of morbidly obese patients have type 2 diabetes mellitus (T2DM) and 80% of them can achieve diabetes remission after bariatric surgery. However, there are few randomized studies comparing the metabolic results of different surgical techniques and the hormonal mechanisms involved.

Objective: To study and compare the improvement of T2DM and the hormonal pathways following three surgical techniques: metabolic gastric bypass (mGBP), sleeve gastrectomy (SG) and greater curvature plication (GCP).

Methods: Prospective, randomized controlled single-center study in patients with T2DM and morbid obesity. 45 patients aged 49.4 ± 7 years, BMI 39.4 ± 1.9 kg / m², initial HbA_{1c} $7.7 \pm 1.9\%$; were randomly assigned (1:1:1) to the 3 surgical techniques. At baseline, one and 12 months a standard meal test was performed to determine GLP-1, GLP-2, PYY, Ghrelin and glucagon concentrations.

Results: Twelve months after surgery, total weight loss was higher in mGBP compared with SG and GCP (-35.2 ± 8.1 vs. -27.8 ± 5.4 vs. -20.5 ± 6.8 kg, $p = 0.007$, $p < 0.001$, respectively). HbA_{1c} at one year was significantly lower in mGBP compared with SG and GCP (5.09 ± 0.62 vs 6.21 ± 0.82 vs $6.61 \pm 1.30\%$, $p = 0.001$, $p = 0.001$). The percentage of patients with diabetes remission was higher in mGBP 80% vs. 53.3% vs. 20%. GLP-1 area under the curve (AUC) at month one and 12 was greater in mGBP than after SG and GCP. In the multiple regression analysis the absence of insulin treatment and the increase in GLP-1 AUC from baseline to month one were the factors associated with T2DM remission.

Conclusions: mGBP is the technique that has shown a higher rate of weight loss and T2DM remission. Factors associated with improved glycemic control are those that accompany a less evolved diabetes, and an enhanced secretion of GLP-1.

P31. Long-term exposure to low doses of Bisphenol A alters calcium handling in pancreatic β -cells by ER β -mediated reduction of R-type calcium currents

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Bisphenol A (BPA) is a monomeric compound widely used in the manufacture of multiple plastics and epoxy resins present in daily products (e.g. food containers and beverage cans). Besides, it is used for industrial purposes (e.g. metal coating of several materials and paints). It is well known that BPA acts like a xenoestrogen via extranuclearly located estrogen receptors, ER α and ER β . Evidence links BPA exposure and incidence of metabolic disorders, including diabetes mellitus and obesity. However, the molecular mechanisms involved are still under research.

We have investigated the effect of long-term exposure to environmentally relevant doses of BPA (1nM) on pancreatic beta-cell function. We recorded calcium signals using fura-2 microfluorimetry. In addition, whole-cell patch clamp recording was used to measure calcium currents. Gene expression of different ion channel subunits were analyzed by quantitative RT-PCR.

Incubation with low doses of BPA elicited a decrease in $[Ca^{2+}]_i$ in response to high extracellular K^+ (20 and 65 mM). This effect presented a dose-response shape with a maximum at 1 nM BPA. Results obtained using the patch-clamp technique confirmed that whole Ca^{2+} -current was reduced by 20%. The pharmacological profile suggested that calcium-current regulated by BPA was of the R-type. This effect was associated with a lower mRNA expression of $C_v2.3$ channels compared to Control. A combined strategy using specific agonists, antagonists and knockout mice show that these functional alterations are mediated by the estrogen receptor β (ER β).

P32. The autophagic protein DOR/TP53INP2 is a novel regulator of brown adipose tissue metabolism

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Brown adipose tissue-mediated thermogenesis is an important effector on energy dissipation and can have an impact on total energy balance. Due to the recent discovery that adult humans have active brown adipose tissue depots, this tissue has emerged as a novel target for the treatment of metabolic disorders. Diabetes and obesity-regulated protein, DOR, also called TP53INP2, is a nuclear cofactor that exits to the cytosol and stimulates autophagy in cellular models, in *Drosophila melanogaster* and in skeletal muscle under in vivo conditions. Moreover, we found that DOR mRNA levels are markedly induced in brown adipose tissue under situations characterized by enhanced thermogenic activity such as cold exposure. DOR is also a negative modulator of white adipogenesis and its ablation causes augmented adiposity in mice. In this regard, tamoxifen-inducible total DOR knockout (DOR KO) mice presented increased brown adipose tissue weight and lipid droplet size, downregulation of key genes involved in brown adipogenesis and thermogenesis, and cold intolerance. Using brown preadipocytes, we also found that DOR is required for brown fat differentiation. DOR loss-of-function in these cells resulted in reduced lipid accumulation, mitochondrial content and expression of brown adipocyte marker genes, as UCP1 and PGC1 α . Therefore, we generated a Myf5-DOR knockout mice to study the impact on tissue homeostasis of DOR ablation in brown adipose precursor cells. Preliminary data on brown adipose tissue from Myf5-DOR knockout mice showed a gene expression profile comparable to the global DOR KO mice model. All these results suggest that DOR has a crucial role controlling whole body energy homeostasis by modulating brown adipose tissue metabolism.

P33. Brown adipose tissue-specific insulin receptor inactivation is sufficient to aggravates atherosclerotic process

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Aims: Obesity is one of the major risk factors for the development of cardiovascular diseases and is characterized by abnormal accumulation of adipose tissue, including perivascular adipose tissue (PVAT). However, brown adipose tissue (BAT) activation reduces visceral adiposity. To demonstrate that severe brown fat lipoatrophy produced by brown adipose tissue-specific insulin receptor (IR) inactivation might accelerate atherosclerotic process, we generated a new mouse model without IR in BAT and without apolipoprotein E (BATIRKO; ApoE^{-/-} mice) and assessed vascular and metabolic alterations associated to obesity. In addition, we analyzed the contribution of the adipose organ to vascular inflammation.

Methods and Results: Brown fat lipoatrophy induces visceral adiposity, mainly in gonadal depot (gWAT), severe glucose intolerance, high postprandial glucose levels and a severe defect in acute insulin secretion. BATIRKO; ApoE^{-/-} mice showed greater hypertriglyceridemia than the obtained in ApoE^{-/-} and hypercholesterolemia similar to ApoE^{-/-} mice. BATIRKO; ApoE^{-/-} mice, in addition to primary insulin resistance in BAT, also showed a significant decrease in insulin signaling in liver, gWAT, heart, aorta artery and tPVAT. More importantly, our results suggest that severe brown fat lipoatrophy aggravates the atherosclerotic process, characterized by a significant increase of: lipid depots, atherosclerotic coverage, lesion size and complexity, increased macrophage infiltration and proinflammatory markers expression. Finally, an increase of TNF-alpha and leptin as well as a decrease of adiponectin by BAT, gWAT and tPVAT might also be responsible of vascular damage.

Conclusions: Our results suggest that severe brown lipoatrophy aggravates atherosclerotic process. Thus, BAT activation might protect against obesity and its associated metabolic alterations.

P34. Immunoregulatory properties of human adipose derived stem cells (hASCs) in inflammatory diseases. Differences between Crohn's disease and obesity

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Background: Inflammation and dysbiosis are common in several highly prevalent diseases, such as Cohn's disease (CD), obesity and type 2 diabetes (T2D). Visceral fat depot plays a decisive role in the inflammatory environment observed in these pathologies. Specifically, in CD subjects there is frequently an increase of mesenteric fat-tissue which is called "creeping fat (CF)". However, CD patients show an aggressive local inflammatory response whereas obese and T2D patients develop a subtle chronic inflammatory status. The main objective of this study was to identify key factors involved in triggering the immune innate response of adipose tissue (AT). Specifically, we focus on the potential role that human adipose-derived stem cells (hASCs) play in local and systemic inflammation in the setting of CD and obesity.

Methods: We isolated hASCs from subcutaneous (SAT) and visceral adipose tissue (VAT) of a well-characterized cohort including 4 groups: inactive CD (in remission), active CD, obese, and obese subjects with diabetes (T2D). hASCs were immunophenotyped by flow cytometry using positive and negative markers according to the International Society of Cell Therapy.

Results: hASCs obtained from CD subjects shown a significantly greater migratory and invasive capacity than those derived from obese subjects, regardless of the presence of T2D. This distinctive difference in phenotype between CD versus obese hASCs was observed in both active and inactive CD donors and in both depots SAT and VAT. The phagocytic capacity was also higher in CD subjects compare obese subjects but in this occasion in inactive CD donors showed lower phagocytic activity to compare active CD donors. Accordingly, AT inflammation markers (IL-6, TNF α , IL-1B, MCP-1) indicated that both active and inactive CD subjects present a higher environment compared to obese subjects. Remarkably, we demonstrate that CD produces a detrimental effect not only on mesenteric adipose tissue-resident stem cells, but also on the subcutaneous fat depots.

P35. Lipin-2 Regulates NLRP3 Inflammasome Activation in Macrophages

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Cardiovascular disease is one of the most frequent inflammatory diseases. Atherosclerosis is the primary cause for cardiovascular disease, and diabetes increases the risk several-fold by enhancing the formation and/or progression of atherosclerotic lesions, a process in which abnormally-activated monocytes and macrophages appear to play a major role. Macrophages and other cell types are recruited to the subendothelial space of the arterial wall, where they engulf modified lipoproteins and become foam cells that constitute the core of atherosclerotic plaques. In a wide range of metabolic conditions, including atherosclerosis and Type 2 diabetes, the NLRP3 inflammasome is activated and contributes to disease by producing the pro-inflammatory cytokines IL-1 β and IL-18. In the present work we have investigated the role of Lipin-2, a key enzyme in lipid metabolism, in the activation of the NLRP3 inflammasome. We found out that depletion of lipin-2 in macrophages promotes an increased expression of the proinflammatory cytokine IL-1 β , which depends on the overactivation of NF- κ B and MAPK by LPS. Cells lacking lipin-2 alter lipid cellular levels increasing ATP receptor activity, leading to overstimulation of the NLRP3 pathway, thereby triggering IL-1 β and IL-18 production in an ASC and caspase-1 dependent manner. The absence of lipin-2 may modify lipid membrane lipids, leading to an increase of ATP receptor activity, potassium efflux from the cell, and assembly of the inflammasome and its activity. Our studies confirm a protective role for lipin-2 in the pathology of inflammatory diseases mediated by classical activation of inflammasoma NLRP3, and may open new avenues for controlling metabolic diseases in which macrophages may contribute to the development of the disease.

P36. Type 1 Diabetes and Polycystic Ovary Syndrome: Systematic Review and Meta-Analysis

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Background: A few small studies have reported increased prevalences of polycystic ovary syndrome (PCOS) and symptoms of androgen excess in women with type 1 diabetes.

Purpose: To perform a systematic review and meta-analysis of studies evaluating androgen excess symptoms and PCOS in women with type 1 diabetes.

Data sources: Entrez-PubMed and Scopus electronic databases.

Study selection: We selected studies addressing androgen excess signs, symptoms and disorders in girls, adolescents and adult women with type 1 diabetes.

Data extraction: Main outcome measures were prevalences of PCOS, hyperandrogenemia, hirsutism, menstrual dysfunction, and polycystic ovarian morphology (PCOM).

Data Synthesis: Nine primary studies involving a total of 475 adolescent or adult women with type 1 diabetes were included. The prevalences of PCOS and associated traits in women with type 1 diabetes were 24% (95CI: 15% - 34%) for PCOS, 25% (95CI: 17% - 33%) for hyperandrogenemia, 25% (95CI: 16% - 36%) for hirsutism, 24% (95CI: 17% - 32%) for menstrual dysfunction and 33% (95CI: 24% - 44%) for PCOM. These figures are considerably higher than those reported earlier in the general non-diabetic population.

Limitations: The data collected in the original studies were heterogeneous in terms of age, race, ethnicity and criteria used for the diagnosis of PCOS, yet we used a quality-effects model in the meta-analyses to overcome this limitation.

Conclusions: PCOS and its related traits are frequent findings in women with type 1 diabetes. PCOS may contribute to the subfertility of these women by a mechanism that is not directly dependent on the glycemic/metabolic control among other negative consequences for their health. Hence, screening for PCOS and androgen excess should be included in current guidelines for the management of type 1 diabetes in women.

P37. Role of DOR/TP53inp2 on mitophagy

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Type 2 diabetes is a disease characterized by a state of insulin resistance leading then to a progressive decrease in beta cells and insulin secretion. These effects are related to defects in mitochondria, particularly in the skeletal muscle and liver, both fundamental tissues in maintaining glucose homeostasis.

On the other hand, the accumulation of damaged mitochondria has been proposed as a key issue in the pathogenesis of many common age-related diseases, including Parkinson's disease (PD). Several works suggest that two of the genes involved in familial forms of PD, PINK1 and Parkin, act in a common pathway regulating mitochondrial quality control.

Under mitochondrial depolarization, Parkin, an E3 ubiquitin ligase is recruited to dysfunctional mitochondria by PINK1 and phosphorylated ubiquitin. After that, Parkin ubiquitinates mitochondrial outer membrane proteins to induce a wide range of outcomes, from proteasomal degradation to vesicle formation, motility arrest, and mitochondrial autophagy.

DOR/TP53INP2 is a nuclear protein located in promyelocytic leukaemia nuclear bodies under basal conditions. Some years ago our group reported that DOR is abundantly expressed in insulin-sensitive tissues and highly repressed in the muscle of obese diabetic rats. However, under conditions characterized by the activation of autophagy or cellular stress DOR exits the nucleus in response to cellular stress or activation of autophagy and possibly participates as a cofactor to target material to the autophagosome. Then, it is reasonable to propose that DOR could target cellular organelles such as mitochondria to autophagosomes. Preliminary data from our laboratory indicated that DOR is recruited to the mitochondria upon mitochondrial depolarization (induced by CCCP, an inductor of mitophagy). Based on that, we have focused our project into the potential effects of DOR on mitochondria.

P38. Genetic models rule out a major role of beta cell glycogen in the control of glucose homeostasis

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Glycogen accumulation occurs in beta cells of diabetic patients and has been proposed to partly mediate glucotoxicity-induced beta cell dysfunction. However, the role of glycogen metabolism in beta cell function and its contribution to diabetes pathophysiology remain poorly understood. We investigated the function of beta cell glycogen by studying glucose homeostasis in mice with (1) defective glycogen synthesis in the pancreas; and (2) excessive glycogen accumulation in beta cells.

Conditional deletion of the *Gys1* gene and overexpression of protein targeting to glycogen (PTG) was accomplished by Cre-lox recombination using pancreas-specific Cre lines. Glucose homeostasis was assessed by determining fasting glycaemia, insulinaemia and glucose tolerance. Beta cell mass was determined by morphometry. Glycogen was detected histologically by periodic acid-Schiff's reagent staining. Isolated islets were used for the determination of glycogen and insulin content, insulin secretion, immunoblots and gene expression assays.

Gys1 knockout (*Gys1* KO) mice did not exhibit differences in glucose tolerance or basal glycaemia and insulinaemia relative to controls. Insulin secretion and gene expression in isolated islets was also indistinguishable between *Gys1* KO and controls. Conversely, despite effective glycogen overaccumulation in islets, mice with PTG overexpression (PTGOE) presented similar glucose tolerance to controls. However, under fasting conditions they exhibited lower glycaemia and higher insulinaemia. Importantly, neither young nor aged PTGOE mice showed differences in beta cell mass relative to age-matched controls. Finally, a high-fat diet did not reveal a beta cell-autonomous phenotype in either model.

Glycogen metabolism is not required for the maintenance of beta cell function. Glycogen accumulation in beta cells alone is not sufficient to trigger the dysfunction or loss of these cells, or progression to diabetes.

P39. Mfn2 ablation leads to metabolic rewiring

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Metabolic rewiring is triggered under metabolic stress and is a general feature of several metabolic pathologies such as cancer, obesity as well as upon immune system activation. Alterations in gene expression during metabolic rewiring lead to changes in different metabolic pathways in order to sustain energetic and biosynthetic requirements of the cell under stress conditions. Our laboratory described that the expression of Mfn2, a mitochondrial fusion protein, is lower in type 2 diabetic patients than in healthy individuals. Moreover, Mfn2 loss-of-function induces mitochondrial fragmentation, oxidative stress, mitochondria respiration leakage and chronic activation of ER stress in liver and in skeletal muscle. All these signals leads to insulin resistance and glucose intolerance. In this work we have investigated the consequences of Mfn2 deficiency on cellular metabolism. To this end, we used Mfn2 knockout (KO) MEF cells, and Mfn2 knockdown (KD) 3T3-L1 preadipocytes or C2C12 myoblasts to detect alterations in energetic and biosynthetic pathways. In order to investigate changes in biosynthetic pathways we analyzed the incorporation of ¹⁴C-glucose and ¹⁴C-glutamine to newly synthesized lipids and proteins. Our results indicate that Mfn2 loss-of-function causes a decreased synthesis of lipids and proteins dependent on glucose, and promotes glutamine-dependent anaplerosis. In addition, gene expression studies revealed that the unfolding protein response (UPR) is chronically activated in Mfn2 ablated cells and leads to the induction of several genes involved in the Krebs cycle and amino acid metabolism causing metabolic rewiring. On the other hand, using lentiviral-mediated knockdown of some of these genes, we stimulated the synthesis of lipids and proteins dependent on glucose. In conclusion, Mfn2 modulates cellular metabolism through UPR activation and determines metabolic fate.

P40. Anti-Inflammatory Roles of Secreted Group V Phospholipase A2

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Diabetes Mellitus type 2 and atherosclerosis are closely related diseases. Dyslipidemia, inflammation and hyperglucemia, key features of diabetes disease, are all well-known causes of atherosclerosis. Macrophages can be divided into two different subsets: classically-activated macrophages, induced by interferon-g plus lipopolysaccharide, (M1), and alternatively-activated macrophages, induced by interleukin-4, (M2). These macrophages have been described in vivo in atherosclerotic lesions. M1 have been shown to promote the progression of atherosclerosis, and are more abundant within lesions of progressing plaques. M2 are characteristic of regressing atherosclerotic plaques. Macrophages constitute a major source of extracellular phospholipase A2, a family of enzymes with a significant role in atherosclerosis. sPLA2-V is involved in the establishment and progression of atherosclerotic plaques, and the regulation of immunoinflammatory response mounted by the macrophages. sPLA2-V hydrolyzes choline phospholipids on the surface of VLDL and LDL particles and leads to structural changes that diminish clearance of the lipoproteins, thereby increasing their residence time in the circulation and susceptibility to chemical modification. We sought to determine the actions of sPLA2-V on macrophages and key contributors to the chronic inflammatory state that is characteristic of the plaque. We show that in human monocyte-derived macrophages sPLA2-V is strongly up-regulated by IL-4 but not by IFN γ plus LPS. Hence, sPLA2-V constitutes a marker for human alternatively activated macrophages. Further, we show that the increased expression of sPLA2-V in IL-4-treated macrophages serves to regulate the cellular levels of ethanolamine phospholipids (LPE) that are necessary to support the elevated phagocytic response that these cells exhibit. Our results suggest that, in human atherosclerosis lesions, sPLA2-V could function as a bi-faceted enzyme, augmenting the atherogenicity of LDL particles, but, also accelerating clearance of debris and thus reducing inflammation.

P41. Expression profile of cell surface TLR2 and TLR4 on leukocytes in response to oral macronutrient challenges: influence of obesity and sex hormones

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Objective: The activation of immune cells is necessary for initiating chronic inflammation in cardiometabolic disorders. In order to study if macronutrients of the diet (glucose, fats and proteins) have a different effect on early steps of immune cell activation, we analyzed the cell surface expression of toll-like receptors (TLRs) and markers of leukocyte activation after their oral ingestion.

Design: We studied 53 individuals [17 women with polycystic ovary syndrome (PCOS), 17 non-hyperandrogenic women and 19 men] matched for age and body mass index. On alternate days participants were submitted to an oral glucose, lipid, or protein load (300kcal each). We investigated the postprandial activation state of leukocytes by analyzing TLR2, TLR4, CD11b, CD80, CD86, and CD36 cell surface expression.

Results: There was a significant interaction between glucose or lipid loads, group and obesity on leukocyte TLR2 expression. Men presented higher changes from neutrophil expressed TLR2 baseline levels compared with PCOS women after a glucose ($P = 0.022$) and lipid load ($P = 0.050$). Fasting leukocyte TLR4 expression and changes from baseline after each macronutrient load were higher in non-obese compared with obese individuals ($P < 0.01$). Monocyte CD86 expression was different between groups after protein intake ($P < 0.001$) and obese individuals had higher fasting monocyte CD36 levels after macronutrient challenges ($P = 0.001$). Leukocyte CD11b expression did not change after macronutrient loads. Regarding inflammatory predictors, obesity and fasting glucose were associated with white blood cell and neutrophil counts ($P < 0.001$), whereas insulin sensitivity predicted C-reactive protein, CH50, and complement C3 levels ($P = 0.05$).

Conclusions: Macronutrient challenges induce acute leukocyte activation impairing TLR2 and TLR4 expression. This effect may be influenced by obesity and could be relevant for cardiometabolic complications.

P42. IN THE SLC13A5, NKX6-1,ATG2B AND GRP120 GENES ASSOCIATE TO FETAL GROWTH AND BODY COMPOSITION IN NEWBORNS

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Background: Fetal growth and adult metabolic diseases could be regulated by epigenetic factors. Gestational diabetes, preeclampsia and prematurity are associated to an altered placental methylation. However, the epigenetic mechanisms underlying fetal growth restraint in non-complicated pregnancies remain unknown.

Objective: To assess DNA methylation profile in placenta and cord blood from newborns born appropriate- (AGA) or small-for-gestational-age (SGA), to identify new candidate genes of fetal growth.

Design and patients: Placentas and cord blood samples were collected at term delivery of infants, born either AGA [birthweight, -1.1 and 1.1 SD; N=30] or SGA [birthweight, < 2 SD; N=21]. Body composition was assessed by absorptiometry at age 15 days. Methylation profile was assessed using a human DNA array. Results were validated using bisulfite pyrosequencing (BSP). Selected genes were analyzed in cord blood by BSP. Placental expression was assessed by RT-PCR.

Results: In SGA newborns, the SLC13A5, NKX6-1 and ATG2B genes were hypermethylated in placenta (P<0.0001, P=0.008 y P=0.02, vs AGA, respectively) and cord blood (P<0.0001), while GPR120 was hypomethylated in placenta (P=0.0006) and hypermethylated in cord blood (P<0.0001). Weight and length at birth were negatively associated with methylation levels in all studied genes (P<0.007). HMW adiponectin was positively associated to ATG2B (P=0.03), while IGF-I and HOMA-IR were negatively associated to SLC13A5, NKX6-1 and GPR120 (P<0.002). Total fat and abdominal fat correlated inversely with the methylation status of SLC13A5, NKX6-1 y ATG2B (P<0.02) and directly with GPR120 (P<0.007). Placental expression of SLC13A5, NKX6-1 and ATG2B was reduced, and that of GPR120 increased in SGA infants (P=0.006, p=0.0003, p=0.0002, and p=0.009, respectively).

Conclusion: Several genes involved in the regulation of energy metabolism were found to be differentially methylated in placentas and cord blood of SGA newborns, and they were associated to a lower birthweight and lower total and abdominal fat at age 15 days.

P43. Recognition of a Sequence: More Growth before Birth, Longer Telomeres at Birth, More Lean Mass after Birth

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Background

Telomere length at birth is a major determinant of telomere length in late adulthood. However, the prenatal setting of telomere length is poorly understood. Individuals born large from non-diabetic mothers are at lower risk for later-life disorders than those born small, a feature of their longer health span being a higher lean mass that provides more muscle strength and that is already present in infancy.

Methods

At birth, we studied leukocyte telomere length (by quantitative polymerase chain reaction) in 103 small-, appropriate- or large-for-gestational-age (SGA, AGA, LGA) infants born after uncomplicated, term, singleton pregnancies. All infants were breastfed for ≥ 4 months. At 2 weeks and 12 months, body composition was assessed by dual X-ray absorptiometry.

Results

Telomere lengths were shorter in SGA newborns and longer in LGA newborns than in AGA newborns ($P < 0.0001$), also after adjustment for maternal age, pregestational body mass index, gestational weight gain, and gestational age. Telomere length at birth associated (all $P \leq 0.001$) to birthweight ($r = 0.50$) and to both lean mass ($r = 0.43$) and fat mass ($r = 0.48$) at age 2 weeks, but only to lean mass at 12 months ($r = 0.51$).

Conclusion

Higher weight and longer telomeres at birth are followed by more lean mass in late infancy.

P44. GNIP1 is an ubiquitin E3 ligase involved in skeletal muscle glycogen metabolism

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Aims/hypothesis: GNIP1 is a TRIM protein with recently demonstrated ubiquitin E3 ligase activity that interacts with glycogenin. These data suggest that GNIP1 could be a protein with a main role in the control of glycogen metabolism, although evidence based on functional analyses remains to be obtained. The aim of this study was to define GNIP/TRIM7 isoform expression pattern and to test their ubiquitin E3 ligase activity, and analyse the functional effects of GNIP1 on muscle glucose/glycogen metabolism.

Methods: The expression levels of GNIP/TRIM7 and the potential TRIM7 ubiquitin E3 ligase activity were analysed in comparison with GNIP1 and GNIP2. GNIP1 and TRIM7 were overexpressed in LHCN-M2 human cultured muscle cells and GNIP1 in gastrocnemius mouse muscle and studied their subcellular location and their effects on GS and GP activity, glycogen accumulation, phosphorylation levels and protein content of Akt/PKB, GSK3 and GS, glycogenin protein content and cell glucose uptake rate.

Results: The GNIP/TRIM7 isoform most expressed in human skeletal muscle is GNIP1, whereas in cardiac muscle only TRIM7 is expressed. GNIP1 and TRIM7 have autoubiquitination activity in vitro and localize in Golgi and cytosol respectively in LHCN-M2 myoblasts. GNIP1 increases glucose uptake in cultured LHCN-M2 myotubes and glycogen content, GS activity and phospho-GSK3 α/β (Ser21/9) and phospho-Akt/PKB (Ser473) protein content and decreases GS phosphorylation in Ser 640 in overexpressed gastrocnemius mouse muscle in vivo, whereas decrease blood glucose and lactate levels and body weight, without changing whole-body insulin or glucose tolerance in mouse.

Conclusions/interpretation: GNIP1 is an ubiquitin ligase with a markedly glycogenic effect in skeletal muscle.

P45. Lipin-2 inhibits Inflammasome Activation Induced by Palmitic Acid in Macrophages

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It is well known that metabolic diseases are characterized by elevated concentrations of circulating free fatty acids (FFAs), especially saturated fatty acids (SFAs), which activate macrophages through Toll-like receptors (TLR2/4). Previous studies in our laboratory showed that depletion of lipin-2, a member of the phosphatidic acid phosphatase lipin family involved in de novo lipid biosynthesis, promotes increased an inflammatory response to SFAs such as palmitic acid. Further studies demonstrated that this inflammatory response also involves the increased expression and release of IL-1b, a pro-inflammatory cytokine produced by the activation of the NLRP3 (NLR pyrin domain containing 3) inflammasome. The aim of this study was to further investigate the mechanism by which the lack of lipin-2 increases the inflammasome-dependent response to palmitic acid. We produced a human macrophage cell line (THP1) knockout for lipin-2 using the CRISPR/Cas9 technology by lentiviral transfection. Palmitic acid has the capacity to act as the first and second signal needed to fully activate the NLRP3 inflammasome. During the priming signal (signal 1) palmitic acid induces pro-inflammatory gene expression (IL1b, IL6) by activating via ERK and NFkB, possibly through TLR2/4. As signal 2, the fatty acid induces the formation of the NLRP3 inflammasome complex and activation of caspase-1, with the consequent maturation of pro-IL-1b into IL-1b. Lipin-2 depletion has effects on the priming phase of the inflammasome activation, by increasing p65 NF-Kb translocation to the nucleus as well as activation of ERK. During the second phase, the absence of lipin-2 exacerbates palmitic acid-inflammasome activation, increasing expression of pro-IL-1b and components of the NLRP3 inflammasome complex. Collectively, these data suggest that lipin-2 participates as a mechanism to dampen inflammasome activation during palmitic acid treatment. We are currently working to characterize the molecular mechanisms that govern these effects.

P46. Stem Cells isolated from adipose tissue of obese subjects show different methylation patterns that might compromise their biological functions

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Background: Adult adipose tissue contains a pool of abundant multipotent stem cells, designated as adipose-derived stem cells (ASCs) that are able to replicate as undifferentiated cells, and to develop as mature adipocytes. Available information indicates that ASCs are important players in the metabolic dynamics of the adipose tissue (AT) participating in the development of obesity and related comorbidities. We have proved that AT from obese individuals contains a dysfunctional population of human ASCs (hASCs). In this sense, we hypothesize that the hostile environment associated with obesity (as inflammation and hypoxia) could be the underlying cause of the defective properties of AT-resident stem cells through epigenetic modifications.

Methods: We isolated hASCs from subcutaneous (SAT) adipose tissue of lean (BMI 20-24,9 Kg/m²; N=6) and obese (BMI 30-34,9 Kg/m²; N=6) subjects. gDNA was extracted from all hASCs and its mature differentiated descendants. An Infinium Human Methylation 450 Bead Chip was performed for epigenome-wide association studies (a total of 4 comparisons were completed (Fig.1)). Gene expression of some affected genes were also validated by qPCR.

Results: Differentially DNA methylation profiles exist due to the obese environment in the hASCs niche (650 significant differentially methylated regions (DMR)) and these differences diminish in mature adipocytes (206 DMR). We showed that DNA methylation is quite static during the transition from stem to the fully mature adipocyte, and most of the differences observed are due to the obesity phenotype in the hASCs niche. Interestingly, most of these changes are located in transcribed regions, which have also been actively correlated with gene expression. Gene Ontology analysis revealed adipogenesis, inflammation and migration as the biological functions most significantly represented among the DMR identified in the hASCs niche.

Conclusion: Our study reveals that methylation status is significantly modified in an obese environment supporting hASCs dysfunction as a key regulatory event in obesity.

P47. The autophagic protein DOR/TP53INP2 represses adipogenesis and adiposity by modulation of β -catenin

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Insulin resistance and excessive fat accumulation are fundamental defects that precede the development of the metabolic syndrome and type 2 diabetes. Diabetes and obesity-regulated protein, DOR/TP53INP2, is a regulator of basal autophagy and interacts directly with Microtubule-associated protein light chain 3 (LC3) to promote autophagosome formation and protein degradation. In addition, DOR is downregulated in subcutaneous fat from obese human subjects, suggesting an adaptive role in this condition. In this regard, tamoxifen-inducible total DOR knock-out (DOR KO) mice display an increase in body weight without changes in food intake. Moreover, white adipose tissue depots of these mice are also increased compare to control littermates. In keeping with these data, DOR gain-of-function caused a repressed adipogenesis in human and mouse pre-adipocytes and conversely, DOR loss-of-function enhanced adipogenesis. The Wnt signaling pathway plays a well-established role in regulation of adipocyte differentiation. Stimulation of the pathway leads translocation of β -catenin to the nucleus where it regulates the expression of Wnt target genes. In this regard, Wnt signaling activation requires the relocation of Glycogen Synthase Kinase 3 (GSK3 β) to a late-endosomal compartment in order to promote cytosolic accumulation of β -catenin and its subsequent nuclear translocation. In human and murine preadipocytes, a downregulation of β -catenin levels is a prerequisite in order to initiate adipogenesis. In this connection, DOR loss-of-function in mice or in 3T3-L1 cells leads a decrease of β -catenin along with the increase of adipogenic master genes CCAAT/enhancer binding protein alpha (C/EBP α) and Peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2). In all, our data suggest that DOR negatively regulates adipogenesis and adiposity through modulation of β -catenin function.

P48. Lipin-2 Role in the Differentiation and Polarized Activation of Macrophages

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Obesity is a state of chronic low degree inflammation governed by inflammatory cells, such as macrophages, that can lead to the development of insulin resistance and hence to the establishment of the type 2 diabetes. The elevated saturated free fatty acids present in the adipose tissue of obese individuals have been described to change the activation state of macrophages from the antiinflammatory M2 polarization state, to the proinflammatory M1 polarization state. Polarized M1 macrophages in the obese adipose tissue contribute to maintain the inflammation state and the development of insulin resistance. We have recently described that Lipin-2, an enzyme that dephosphorylates phosphatidic acid to form diacylglycerol to be used for triacylglycerol synthesis, reduces the proinflammatory signaling mediated by elevated concentration of saturated fatty acids in macrophages. Based on its protective function we decided to extend our investigations to uncover whether lipin-2 could also be involved in macrophage differentiation and polarization. To that end we established an in vitro differentiation and polarization protocol for the human THP-1 promonocytic cell line to study by qPCR the expression of well-known differentiation and polarization markers in cells with different expression levels of lipin-2. Also, analysis of the expression of Lipin-2 revealed increased mRNA levels for the enzyme during macrophage differentiation and M1 polarization processes. Overall our results suggest that Lipin-2 may play an important role during differentiation/ polarization processes.

P49. Prevalent role of the insulin receptor isoform A in the regulation of hepatic glycogen metabolism and glucose homeostasis

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Aims/hypothesis: In the postprandial state, liver regulates glucose homeostasis by glucose uptake and conversion to glycogen and lipids. Glucose and insulin signaling finely regulate glycogen synthesis by several mechanisms. In this sense, insulin receptor isoform A (IRA) favors glucose uptake in hepatocytes as compared to isoform B (IRB). Thus, we hypothesized that IRA could increase glycogen synthesis by favoring glucose uptake and glycogen storage.

Methods: We addressed the role of insulin receptor isoforms on glycogen metabolism in vitro in immortalized neonatal hepatocytes. Regarding in vivo experiments, we expressed IRA and IRB specifically in the liver by using adeno-associated viruses (AAVs) in type 2 diabetic iLIRKO (inducible Liver Insulin Receptor Knockout) mice and studied the role of IR isoforms on glycogen synthesis and storage and glucose homeostasis.

Results: In immortalized hepatocytes, IRA expression induces an increased insulin signaling that was associated with an increased glycogen synthesis and storage. In addition, IRA expression in the liver of iLIRKO mice, but not IRB, induces an increased glycogen content and favors liver glycogen synthase dephosphorylation.

Conclusions: We now provide a new insight about the role of IRA in the regulation of glycogen metabolism in cultured hepatocytes and in the liver in a diabetic scenario. Our data strongly suggest that IRA, but not IRB, not only increases glucose uptake, but also favors glycogen synthesis and storage and glucose homeostasis. Therefore, we suggest that IRA expression in the liver could be an interesting gene therapy strategy for the treatment of hepatic insulin resistance and glucose intolerance.

P50. TP53INP2 function in liver metabolism and diabetes

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DOR/TP53INP2 is a nuclear protein that transactivates a number of nuclear hormone receptors and regulates myogenic and adipogenic differentiation. Upon cellular stress DOR rapidly shuttles from the nucleus to the cytoplasm into punctate structures named autophagosomes (1). DOR interacts with the LC3 family proteins through a consensus LIR motif, and over-expression of DOR in HeLa cells increased the number of autophagosomes under basal and stress-induced conditions, indicating that DOR is a positive regulator of autophagy (1, 2). In addition, DOR mRNA and protein levels are down-regulated in muscle and adipose tissue in diabetic and obese mice and in human samples (3, and unpublished results).

Our data show that DOR protein levels are also drastically down-regulated in livers of diabetic and obese mice. In order to assess the role of DOR in liver metabolism and its possible role in diabetes, we generated a liver-specific DOR KO mice (L-DOR), which we metabolically phenotyped. Moreover, since deregulation of hepatic autophagy has been shown to contribute to insulin resistance (4) and DOR regulates autophagy, we are also characterizing autophagy in our mouse model.

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P51. Essential role of IGF1R and IR in the regulation of brown adipose tissue development and thermogenesis

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Background and objectives: It is well established that insulin-like growth factor 1 (IGF1) and insulin are essential factors in the development and differentiation of brown adipose tissue and its function. We previously generated the first two BAT-specific knockout, lacking insulin receptor (BATIRKO) or insulin-like growth factor 1 receptor (BATIGFIRKO). The first one presents a severe BAT lipoatrophy, obesity prone upon ageing and inflammatory disease. The second one shows an impaired cold acclimation, moderate hyperinsulinemia and hypertriglyceridemia upon time and hepatic insulin insensitivity associated to lipid accumulation, with the outcome of a global insulin resistance.

Methods: We have generated mice lacking insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1R) in BAT. Also, we performed glucose and insulin tolerance test, analyzed the body fat by NMR, studied the morphology of different adipose tissues by hematoxylin and eosin stainings and measured the BAT functionality by cold exposure experiments.

Results: DKO mice show severe BAT atrophy (by 85%) with hypertrophic brown adipocytes. DKO at 3 months versus controls show a significant increase in body fat as estimated by NMR and also increased tissue-weights of different WAT depots (epididymal, retroperitoneal and mesenteric) and hypertrophic white adipocytes in those fat depots. We also found that DKO mice as compared with their controls failed to maintain body temperature when confronted to 4°C environment for 4 or 5 hours exposure. In addition, DKO mice showed a manifest insulin resistance, moderate hyperinsulinemia, fasting hyperglycemia and insulin insensitivity in liver, muscle, heart, inguinal WAT and BAT as compared with their controls at 12 months.

Conclusions: Overall, our data identify IR/IGF1R as essential regulators of BAT development and function, and, when disrupted, leads to defective thermogenesis, obesity prone and peripheral insulin resistance.

P52. Sirt3 prevents inflammation in the heart

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Background and objective

Sirt3 is a member of the sirtuin family of protein deacetylases that targets enzymes involved in energy metabolism processes, which also exerts a profound protective action against oxidative stress. As such, modulation of SIRT3 activity has been proposed as a promising therapeutic target for ameliorating metabolic diseases (e.g. diabetes) and associated cardiac disturbances. Therefore, the main goal of this study was to investigate the effects of Sirt3 modulation on the inflammatory processes in the heart.

Methods

Male mice with constitutive and systemic downregulation of Sirt3 expression and human cardiac AC16 cells were used in this study. Overexpression and gene silencing studies were carried out by Lipofectamine 2000-mediated transfection of TNF-alpha-treated AC16 cells with the corresponding plasmids or siRNA constructs.

Results

Deletion of Sirt3 in knockout mice induced an inflammatory profile in the heart characterized by enhanced expression of pro-inflammatory genes and enhanced DNA-binding activity of the transcription factor AP-1, which plays an essential role in the pathogenesis of cardiac hypertrophy. In agreement with this, Sirt3 overexpression in human cardiac cells partially prevented the pro-inflammatory response induced by TNF-alpha. Western-blot analyses revealed that c-Fos levels, one of the proteins that, together with Jun family members composes AP-1, were downregulated in nuclei of human cardiac cells overexpressing Sirt3. These changes correlated with the inhibition of the transcriptional activity of AP-1. It still remains to be elucidated which is the specific target of Sirt3 activity responsible for this anti-inflammatory effect.

Conclusion

Sirt3 exerts cardioprotective effects in human cardiac cells exposed to TNF-alpha and in the heart of mice by attenuating the inflammatory response.

P53. Enhanced adult human adult B-cell proliferation induced by an activating glucokinase gene mutation.

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Glucokinase gene (GCK) encodes for the enzyme glucokinase (GK) which, functioning as the "glucose sensor" governs glucose metabolism in pancreatic beta-cells. Studies performed in rodents established that beta-cell mass is controlled through changes in GK-mediated glucose metabolism and that an increase in the rate of glycolysis, by means of GK activation, results in a higher beta-cell proliferation rate. However, in isolated adult human islets, the effect of an enhanced GK-mediated glucose metabolism in beta-cell proliferation is still unknown. Islets from adult human cadaveric pancreases present beta-cell proliferation at a low but quantitatively rate. therefore, the possibility of boosting this basal beta-cell proliferation could open an opportunity to improve the outcome of cell therapy as a treatment of diabetes. Using lentiviral technology, we endogenously activated GCK, in human islets, by introducing the activating GCK mutation V91L (GCK-V91L-islets). These islets presented increased metabolic activity, glucose-induced sensitivity and insulin release. Likewise, GCK-V91L-islets present a 4.5 fold increase in beta-cell proliferation when compared with non-transduced islets. An increased apoptotic rate was also observed in GCK-V91L-islets. Our data indicate that the residual beta-cell proliferation existing in isolated adult human islets can be boosted by endogenous activation of GK using lentiviral transduction, providing novel interventions to enhance beta-cell mass for cell therapy for diabetes.

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