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Halt-RONIN

Discovering chronic inflammation biomarkers that define key stages in the Healthy-to-NASH (non-alcoholic steatohepatitis) transition to inform early prevention and treatments strategies.

Grant Agreement No. 101095679

Halt-RONIN Newsletter May -September 2024

Our project aims to **discover chronic inflammation biomarkers that define critical stages in the Healthy-to-MASLD/MASH transition to inform early prevention and treatment strategies**.

Our second newsletter provides our latest news and aims to become a forum for analyzing relevant topics in the field of liver diseases and commenting on some of the most relevant articles published during these four months of the project. Halt-RONIN has reached the 21 months of life. This period has been filled with numerous exciting activities, all of which can be explored in our website: https://halt-ronin.com/news/

The meeting in Lisbon "2nd Annual Meeting" was a complete success, with lots of results and advances HaltRONIN project. It was celebrated on June 27-28, 2024, in Faculdade de Farmácia Universidade Lisbon, Lisbon, Portugal. It was organized by **the FFUL group, Faculdade de Farmácia Universidade Lisbon**. Speakers presented their advances and results in the different research lines of each group participating in the project, divided by work packages. The minutes and the programme for the meeting can be viewed at 2nd Annual Meeting

During these months, we have also organized the **International Seminar Series on Liver Toxicity and Steatotic Liver Disease**. These are taking place every third Wednesday of the month. We have celebrated two seminars:

Date	Speaker	Title
22 May	Prof Gerad A. Kullak-Ublik	Qualification and validation plan for biomarkers in DILI
19 June	Leo van Grunsven	Liver organoids for steatotic liver disease modelling: a new venue of opportunities
18 Sept	María Monsalve	Role of MAFLD-associated loss of mitochondrial plasticity in HCC development risk

You can watch the Seminars again here

Javier Cubero
Coordinator of Halt-RONIN
Complutense University, Madrid

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Interesting publications for the consortium:

1. A high-cholesterol zebrafish diet promotes hypercholesterolemia and fasting associated liver steatosis.

Zebrafish are an ideal model organism to study lipid metabolism and to elucidate the molecular underpinnings of human lipid-associated disorders. Unlike murine models, to which various standardized high lipid diets such as a high-cholesterol diet (HCD) are available, there has yet to be a uniformly adopted zebrasfish HCD protocol. In this study, we have developed an improved HCD protocol and thoroughly tested its impact on zebrafish lipid deposition and lipoprotein regulation in a dose- and time- dependent manner. The diet stability, reproducibility, and fish palatability were also validated. Fish fed HCD developed hypercholesterolemia as indicated by significantly elevated ApoB-containing lipoproteins (ApoB-LP) and increased plasma levels of cholesterol and cholesterol esters. Feeding of the HCD to larvae for 8 days produced hepatic steatosis that become more stable and severer after 1 day of fasting and was associated with an opaque liver phenotype (dark under transmitted light). Unlike larvae, adult fish fed HCD for 14 days followed by a 3 day fast did not develop a stable fatty liver phenotype, though the fish had higher ApoB-LP levels in plasma and an up-regulated lipogenesis gene *fasn* in adipose tissue. In conclusion, our HCD zebrafish protocol represents an effective and reliable approach for studying the temporal characteristics of the physiological and biochemical responses to high levels of dietary cholesterol and provides insights into the mechanisms that may underlie fatty liver disease.

Access to the original article:

Jin Y, Kozan D, Young ED, Hensley M, Shen MC, Wen J, Moll T, Anderson JL, Kozan H, Rawls JF, Farber SA, **A high-cholesterol zebrafish diet promotes hypercholesterolemia and fasting-associated liver steatosis, Journal of Lipid Research** (2024), doi: https://doi.org/10.1016/j.jlr.2024.100637.

Scientific Impact:

According web of science the Impact Factor (IF) of this journal was 5 (2023) and it shows a Journal Citation Indicator of 1.05. The Rank (by Journal Citation Indicator) situates this journal in the position 64/313 and the percentile 79.7, poisoning this in the Q1.

Degree of Originality and Innovation: This study introduces a refined high-cholesterol diet (HCD) protocol for zebrafish, using ethanol instead of diethyl ether for safety and accessibility. It comprehensively evaluates zebrafish lipid metabolism and steatosis, establishing zebrafish as a model for studying hypercholesterolemia and non-alcoholic fatty liver disease (NAFLD), broadening its application to cardiovascular and metabolic research.

Thematic Priority or Challenge Addressed and Contribution to the Scientific Debate: The study addresses how dietary cholesterol contributes to hypercholesterolemia and NAFLD, using zebrafish to model early human liver disease. Findings on fasting-induced steatosis and gene regulation provide new insights into the molecular links between cholesterol, adipose tissue, and liver dysfunction, particularly in liver fibrosis progression.

Methodological Contribution:

The study develops a reproducible, safe HCD protocol validated for zebrafish, incorporating lipid profiling, gene expression, and histology. Fasting-induced liver phenotypes resemble human disease states, with transcriptomic analysis offering a molecular basis for understanding lipid metabolism shifts due to HCD.

2. *Genetic risk accentuates dietary effects on hepatic steatosis, inflammation and fibrosis in a population-based cohort.*

Background & Aims
Steatotic liver disease (SLD), characterized by elevated liver fat content (LFC), is influenced by genetics and diet. However, whether diet has a differential effect based on genetic risk is not well-characterized. We aimed to determine how genetic factors interact with diet to affect SLD in a large national biobank.
Methods
We included UK Biobank participants with dietary intake measured by 24-hour recall and genotyping. The primary predictors were dietary pattern, *PNPLA3*-rs738409-G, *TM6SF2*-rs58542926-T, a 16-variant hepatic steatosis polygenic risk score (PRS), and gene-environment interactions. The primary outcome was LFC, and secondary outcomes were iron-controlled T1 time (cT1, a measure of liver inflammation and fibrosis) and liver-related events/mortality.
Results
A total of 21,619 participants met inclusion criteria. In non-interaction models, Mediterranean diet and intake of fruit/vegetables/legumes and fish associated with lower LFC, while higher red/processed meat intake and all genetic predictors associated with higher LFC. In interaction models, all genetic predictors interacted with Mediterranean diet and fruit/vegetable/legume intake, while the steatosis PRS interacted with fish intake and the *TM6SF2* genotype interacted with red/processed meat intake, to affect LFC. Dietary effects on LFC were up to 3.8-fold higher in *PNPLA3*-rs738409-GG *vs.* –CC individuals, and 1.4-3.0-fold higher in the top *vs.* bottom quartile of the steatosis PRS. Gene-diet interactions were stronger in participants with *vs.* without overweight. The steatosis PRS interacted with Mediterranean diet and fruit/vegetable/legume intake to affect cT1 and most dietary and genetic predictors associated with risk of liver-related events or mortality by age 70.
Conclusions
Effects of diet on LFC and cT1 were markedly accentuated in patients at increased genetic risk for SLD, implying dietary interventions may be more impactful in these populations.

Access to the original article:

Vicent L. Chen, Xiaomeng Du, Antonio Oliveri, Yanhua Chen, Annapurna Kuppa, Brian D. Halligan, Michael A. Province and Elizabeth K. Speliotes, **Genetic risk accentuates dietary effects on hepatic steatosis, inflammation and fibrosis in a population-based cohort,** *Journal of Hepatology* (2024), doi: https://doi.org/10.1016/j.jhep.2024.03.045

Scientific Impact:

According web of science the Impact Factor (IF) of this journal was 26.8 (2023) and it shows a Journal Citation Indicator of 5.97. The Rank (by Journal Citation Indicator) situates this journal in the position 3/143 and the percentile 98.3, poisoning this in the Q1 and D1.

Degree of Originality and Innovation: This study presents a novel investigation into gene-diet interactions affecting hepatic steatosis, inflammation, and fibrosis, exploring how genetic risk and diet quality influence liver health. Unlike previous research, it comprehensively examines these interactions in a large, community-based cohort (UK Biobank), showing that the impact of diet is significantly amplified in individuals with higher genetic risk, offering fresh insights into modifiable factors for liver disease prevention.
Thematic Priority or Challenge Addressed and Contribution to the Scientific Debate: The study addresses the critical challenge of understanding how genetic and dietary factors interact in the progression of steatotic liver disease (SLD). It contributes to the scientific debate by demonstrating that genetic risk is not fixed and can be modified through diet, particularly in individuals at elevated risk. These findings are significant for precision medicine, suggesting targeted dietary interventions for those with higher genetic susceptibility to liver diseases.
Methodological Contribution: Methodologically, the study utilizes data from the UK Biobank, including dietary patterns, genetic variants, and liver MRI measures, to explore gene-diet interactions on liver fat content and inflammation/fibrosis. This approach, combining genetics, diet, and advanced imaging, offers a comprehensive framework for assessing how lifestyle factors interact with genetic

predispositions in large, diverse populations, thus advancing research methods in liver disease.

Interesting publications of the consortium:

1. *Control compounds for preclinical drug-induced liver injury assessment: Consensus-driven systematic review by the ProEuroDILI network*

Background & Aims: Idiosyncratic drug-induced liver injury (DILI) is a complex and unpredictable event caused by drugs, and herbal or dietary supplements. Early identification of human hepatotoxicity at preclinical stages remains a major challenge, in which the selection of validated in vitro systems and test drugs has a significant impact. In this systematic review, we analyzed the compounds used in hepatotoxicity assays and established a list of DILI-positive and -negative control drugs for validation of in vitro models of DILI, supported by literature and clinical evidence and endorsed by an expert committee from the COST Action ProEuroDILI Network (CA17112).
Methods: Following 2020 PRISMA guidelines, original research articles focusing on DILI which used in vitro human models and performed at least one hepatotoxicity assay with positive and negative control compounds, were included. Bias of the studies was assessed by a modified 'Toxicological Data Reliability Assessment Tool'.
Results: A total of 51 studies (out of 2,936) met the inclusion criteria, with 30 categorized as reliable without restrictions. Although there was a broad consensus on positive compounds, the selection of negative compounds lacked clarity. 2D monoculture, short exposure times and cytotoxicity endpoints were the most tested, although there was no consensus on drug concentrations.
Conclusions: Extensive analysis highlighted the lack of agreement on control compounds for in vitro DILI assessment. Following comprehensive in vitro and clinical data analysis together with input from the expert committee, an evidence-based consensus-driven list of 10 positive and negative control drugs for validation of in vitro models of DILI is proposed.

Access to the original article:

Antonio Segovia-Zafra, Marina Villanueva-Paz, Ana Sofia Serras, Gonzalo Matilla-Cabello, Ana Bodoque-García, Daniel E. Di Zeo-Sánchez, Hao Niu, Ismael Álvarez-Álvarez1, Laura Sanz-Villanueva, Sergej Godec, Irina Milisav, Pierre Bagnaninchi, Raúl J. Andrade1, M Isabel Lucena, José C. Fernández-Checa, Francisco Javier Cubero, Joana Paiva Miranda, Leonard J. Nelson.**Control compounds for preclinical drug-induced liver injury assessment: Consensus-driven systematic review by the ProEuroDILI network.** *Journal of Hepatology* 2024. doi: https://doi.org/10.1016/j.jhep.2024.04.026

Scientific Impact:

According web of science the Impact Factor (IF) of this journal was 26.8 (2023) and it shows a Journal Citation Indicator of 5.97. The Rank (by Journal Citation Indicator) situates this journal in the position 3/143 and the percentile 98.3, poisoning this in the Q1 and D1.

Degree of Originality and Innovation:
This study innovatively addresses a major challenge in drug development: predicting drug-induced liver injury (DILI) early in the preclinical phase. By establishing a consensus-driven list of 10 positive and 10 negative control drugs for in vitro DILI model validation, the study provides a standardized framework. This original contribution enhances the consistency and reliability of preclinical liver toxicity testing, helping researchers and regulators better assess the hepatotoxicity of new drug candidates.

Thematic Priority or Challenge Addressed and Contribution to the Scientific Debate: The study tackles the critical challenge of early identification of human hepatotoxicity in drug

development. It contributes to the scientific debate by highlighting the lack of consensus on reference drugs and testing conditions for in vitro DILI models. By proposing a unified list of control drugs, it fosters better validation of preclinical models, advancing the field of hepatotoxicity research and setting a new standard for safety assessments in drug development.

Methodological Contribution:

Methodologically, the study followed strict PRISMA guidelines, selecting 51 reliable studies from nearly 3,000 sources to build its evidence-based consensus. It emphasizes the need for a shift towards using human-relevant in vitro models, such as 3D multicellular cultures, and suggests cross-validation with animal models and clinical data. This systematic approach not only refines preclinical testing but also enhances the reproducibility and accuracy of DILI predictions.

2. Other publications:

Publications			
Title	**Authors**	**Title of the journal or equivalent**	**DOI**
Cathepsin D is essential for the degradomic shift of macrophages required to resolve liver fibrosis	Paloma Ruiz-Blázquez, María Fernández-Fernandez, Valeria Pinto et al.	Molecular Metabolism	https://doi.org/10.1016/j.molmet.2024.101989
EUS-PPG as an alternative to HVPG: Addressing discrepancies in MASLD-related portal pressure measurements	Jesús Rivera- Esteban, Daniel de la Iglesia García, Belén Agudo et al.	Endoscopy	
Pleiotropy of Progesterone Receptor Membrane Component 1 in Modulation of Cytochrome P450 Activity	Isabel S. Barata, José Rueff, Michel Kranendonk and Francisco Esteves.	Xenobiotics	https://doi.org/10.3390/jox14020034
Drug-induced Liver Injury in Latin America: 10-year Experience of the Latin American DILI (LATINDILI) Network	Fernado Bessone, Nelia Hernández, Inmaculada Medina-Caliz et al.	Clin. Gastroenterol Hepatol.	https://doi.org/10.1016/j.cgh.2024.06.030
Fecal microbiota transplantation from female donors restores gut permeability and reduces liver injury and inflammation in middle-aged male mice exposed to alcohol	Arantza Lamas-Paz, Marina Mesquita, Marcos Garcia-Lacarte et al.	Frontiers in Nutrition	doi.org/10.3389/fnut.2024.1393014
Identification of PRMT5 as a therapeutic target in cholangiocarcionoma	Jasmin Elurbide, Leticia Colyn, Maria U Latasa, et al.	Gut	10.1136/gutjnl-2024-332998

Communication and Dissemination activities:

Communication Activity Type	Description	Outcome
Congress	EASL liver Meeting 2024, Milan, Italy, 5-8 June. **OS-105-YI Collagen-integrin signaling re-establishes bile duct morphogenesis and promotes adult ductular regeneration.** Anabel Martínez Lyons.	Oral communication/ Journal of Hepatology 2024 vol. 80(S1) | S1–S77
Poster	The liver Meeting 2024, Milan, Italy, 5-8 June. **WED-528-YI Isolation and characterization of extracellular vesicles in faecal matter: implications for future biomarker discovery.** Héctor Leal Lassalle	Hepatology 2024 vol. 80(S1) | S1–S77
Poster	MDO Prague 2024, Prague, 7-10 July **Single Mutations in Cytochrome P450 Oxidoreductase Can Alter the Specificity of Human Cytochrome P450 1A2-Mediated Caffeine Metabolism**. Michel Kranendonk	Poster
Interview	Interview_Francisco Javier Cubero_ Solo el 5 % de las terapias estudiadas en animales llega a aprobarse para su uso en humanos_ Interview published in **Science Media Center**_https://sciencemediacentre.es/solo-el-5-de-las-terapias-estudia	Interview
Interview	Interview_Francisco Javier Cubero_HNN3.0 Helath-NCP-NET_ IMPACT SUCCESS STORIES_ Discovering chronic inflammation biomarkers that define key stages in the Healthy-to NASH (non-alcoholic steatohepatitis) transition to inform early prevention and treatment strategies (Halt-RONIN). https://hnn30.healthncp.net/pdf/success-stories-halt-ronin	Interview
Conference	AASLD SIG Hepatotoxicity group. 13 June. **The interplay of ER stress and autophagy in APAP toxicity.** Francisco Javier Cubero	Oral presentation
Meeting	23 Jornadas de avances en Hepatología. Málaga, Spain, 16-17 May. **Biomarcadores de diagnóstico y progresión en MASLD.** Javier Crespo	Oral presentation
Meeting	23 Jornadas de avances en Hepatología. Málaga, Spain, 16-17 May. **Hepatitis tóxica (DILI): una hoja de ruta para investigar en Europa.** Raúl J. Andrade.	Oral presentation