3rd European Fatty Liver Conference (EFLC2022)

Interorgan connection in NAFLD – from basic science to a multidisciplinary clinical approach

June 8 – June 10, 2022

Crowne Plaza Hotel
Maastricht, The Netherlands

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Accreditation and endorsement

The **3rd European Fatty Liver Conference (EFLC2022), MAASTRICHT, Netherlands, 08/06/2022-10/06/2022** has been accredited by the European Accreditation Council for Continuing Medical Education (EACCME®) with **18 European CME credits** (ECMEC®s). Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

Through an agreement between the Union Européenne des Médecins Spécialistes and the American Medical Association, physicians may convert EACCME® credits to an equivalent number of *AMA PRA Category 1 CreditsTM*. Information on the process to convert EACCME® credit to AMA credit can be found at www.ama-assn.org/education/earn-credit-participation-international-activities.

Live educational activities, occurring outside of Canada, recognised by the UEMS-EACCME® for ECMEC®s are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of the Royal College of Physicians and Surgeons of Canada.

To receive the certificate please e-mail the front desk: f.defauwes@maastrichtuniversity.nl

This academia-initiated event is endorsed by the United European Gastroenterology and Global Liver Institute (GLI).

Word of Welcome

Dear colleagues and friends,

Welcome to the **3rd European Fatty Liver Conference (EFLC2022)**:
Interorgan connection in NAFLD – from basic science to a multidisciplinary approach
June 8 till June 10, 2022

We are looking forward to welcoming scientists, clinicians, patient societies, nurse practitioners, health care policy makers, and members of the food and pharmaceutical industries to join the 3rd European Fatty Liver Conference (EFLC 2022), which will take place from June 8-10 in Maastricht, The Netherlands.

Interorgan connection in NAFLD: From basic science to a multidisciplinary clinical approach: Non-alcoholic fatty liver disease (NAFLD) impacts not only the liver but also plays a vital role in the genesis and progression of cardiometabolic diseases, diabetes, and cancer. As a consequence of the Western lifestyle, over 50% of the population is overweight or obese. When progressing to Non-alcoholic steatohepatitis (NASH), this foie gras humain, is responsible for increasing health care costs and decrease in quality of life.

Conference topics: Basic science mechanisms of steatosis, inflammation, fibrosis, and carcinogenesis and the clinical aspects, including drug therapy and nutritional approach.

Multidisciplinary: as the spectrum of NAFLD comprises hepatic and extrahepatic manifestations, our mission is to gather researchers, clinicians, and stakeholders in these fields.

The International NASH Awareness Day will be the main topic on Friday, with patient societies and food retailers joining the field's experts.

We wish you all a pleasant, fruitful, and interactive meeting here in Maastricht.

Warm greetings on behalf of the **EFLC2022 Organizing Committee**,
Ger Koek, Sven Francque, Bart Staels, Ronit Shiri-Sverdlov and Leen Heyens

EFLC2022 Organizing Committee

Ger H. Koek, Associate Professor, Gastroenterology and Hepatology, UM/MUMC+

Sven Francque, Professor of Medicine, Head of Gastroenterology and Hepatology, Antwerp University Hospital

Ronit Shiri-Sverdlov, Professor Hepatic Inflammation and Metabolic Health, UM/MUMC+

Bart Staels, Université de Lille, Institut Pasteur de Lille, CHU de Lille, INSERM U1011, Lille, France

Leen Heyens, PhD student, Faculty of Medicine and Life Sciences, Maastricht University and Hasselt University

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EFLC2022 Scientific Committee

Jerome Boursier, CHU Angers, France

Rui Eduardo Castro, University of Lisbon, Portugal

Onno Holleboom, Amsterdam UMC, The Netherlands

Nicolas Lanthier, Cliniques Universitaires Saint-Luc, UCLouvain, Belgium.

Franck Tacke, Charité Universitätsmedizin Berlin, Germany

Program

WEDNESDAY JUNE 8, 2022

16:00 – 17:00	Registration
Session 1: How to diagnose NAFLD/NASH today? *Chairs: Ger Koek & Sven Francque*	
17.00	Opening and welcome
17.00-17.20	*What is the role of genetic testing in NAFLD?* **Stefano Romeo**; University of Gothenburg, Sweden
17.20-17.40	*MicroRNAs as biomarkers: scientific plausibility and pathophysiological role* **Rui Eduardo Castro**; University of Lisbon, Portugal
17.40-18.00	*Non-invasive diagnostic tests for NASH: how to assess their accuracy in diagnosis and follow-up?* **Jerome Boursier**; CHU Angers, France
18.00-18.20	*NAFLD-NASH and the heart* **Onno Holleboom**; Amsterdam UMC, The Netherlands
18.20-18.40	*Referral pathways for patients with NAFLD* **Manolis Tscohatzis**; UCL Institute for Liver & Digestive Health, UK
18.40-19.00	Panel discussion

THURSDAY JUNE 9, 2022

08.00-08.30	Registration
08.30-08.35	Opening by the chairs
Session 2: Metabolic regulation and insulin resistance in NAFLD *Chairs: Ronit Shiri-Sverdlov & Bart Staels*	
08.35-09.00	*Glucose sensing and lipid metabolism in NAFLD* **Catherine Postic;** Institut Cochin, France
09.00-09.15	Abstract 1: *Investigating microRNAs to explain the link between cholesterol metabolism and NAFLD in humans: a systematic review* **Maurice Konings**; Maastricht University, The Netherlands
09.15-09.30	Abstract 2: *A common variant in the ketohexokinase gene is associated with fructosuria and cardiometabolic outcomes* **Amee Buziau;** Maastricht University, The Netherlands
09.30-10.00	*Insulin resistance & NAFLD* **Amalia Gastaldelli;** Institute of Clinical Physiology (IFC) of CNR of Pisa, Italy
10.00-10.15	Abstract 3: *Regulation of GDF-15 in obesity, type 2 diabetes and NAFLD* **Laurent L'homme;** UMR1011 INSERM, Institut Pasteur de Lille, Univ. Lille, France
10.15-10.30	Abstract 4: *The role of the hepatokine fetuin B in glucose homeostasis and adipose tissue function* **Kenneth Pasmans;** Maastricht University, The Netherlands
10.30-11.00	Break & visiting exhibitions
Session 3: Inflammation and immune mechanisms in NAFLD *Chairs: Luisa Vonghia & Leen Heyens*	
11.00-11.30	*deLIVERing a spatial cell atlas of the healthy and obese liver across species* **Charlotte Scott;** VIB-UGent Center for Inflammation Research, Belgium
11.30-11.45	Abstract 5: *Human hepatic in vitro models identify ANGPTL4, PDK4 and PLIN2 as potential pro-steatogenic mediators induced by elafibranor* **Joost Boeckmans;** Vrije Universiteit Brussel, Belgium
11.45-12.00	Abstract 6: *FAT10 in the senescense of hepatocytes and NASH development* **Lucie Bernard;** UMR1011 INSERM, Institut Pasteur de Lille, Univ. Lille, France
12.00-12.30	*NAFLD, immune cells and HCC* **Mathias Heikenwälder;** Deutsches Krebforshungszentrum, Germany

THURSDAY JUNE 9, 2022

12.30-12.45	Abstract 7: *Characterization of hepatic parenchymal hypoxia in a mouse model of nonalcoholic steatohepatitis* **Cédric Peleman;** University of Antwerp, Belgium
12.45-13.00	Abstract 8: *Investigating cDC involvement in the pathogenesis of non-alcoholic fatty liver disease* **Kathryn Waller**; Vlaams instituut Biotechnologie UGent, Belgium
13.00-14.00	Lunch & visiting exhibitions
Short abstract presentations	
14.00-14.05	Abstract 15: *Involvement of the alteration of the biological clock in the development of non-alcoholic fatty liver disease (NAFLD)* **Aurore Hebras;** UMR1011 INSERM, Univ.Lille, France
14.05-14.10	Abstract 24: *The progression of NASH to hepatocellular carcinoma is influenced by extracellular Cathepsins* **Hester van Mourik**; Maastricht University, The Netherlands
14.10-14.15	Abstract 22: *Hypoxia marker pimonidazole displays panlobular positivity in nonalcoholic Steatohepatitis* **Cédric Peleman**; University of Antwerp, Belgium
14.15-14.20	Abstract 17: *Liraglutide reduces hepatosteatosis in NAFLD and drug-induced fatty liver cell culture models through PPARγ signaling pathway* **Tea Omanovic Kolaric;** Josip Juraj Strossmayer University of Oijek, Croatia
14.20-14.25	Abstract 23: *Metabolic-associated fatty liver disease in Turkish individuals living in and outside of Turkey: a shared burden at different locations?* **Irem-Seray Gürtekin;** Maastricht University, The Netherlands
14.25-14.30	Abstract 25: *Pneumococcal immunization against oxLDL decreases tumor burden in NASH-derived hepatocellular carcinoma* **Lara Stoffels**; Maastricht University, The Netherlands
Session 4: Inter-organ crosstalk in NAFLD/NASH *Chairs: Rui Eduardo Castro & Patrick Rensen*	
14.30-15.00	*Adipose-liver crosstalk in NAFLD* **Patrick Rensen**; Leiden University, The Netherlands
15.00-15.15	Abstract 9: *Changes of non-invasive tests for liver steatosis and fibrosis by dual PPARa/g agonism in T2DM and coronary artery disease - Post-hoc analysis of the AleCardio RCT* **Vivian de Jong**; Julius Center, UMC Utrecht, The Netherlands
15.15-15.45	*Gut-Liver Axis and Microbiota* **Nathalie Delzenne;** Louvain Drug Research Institute (LDRI) – UCLouvain, Belgium

THURSDAY JUNE 9, 2022

15.45-16.00	Abstract 10: *Effect of bile acids on CD-1d-restricted antigen presentation to NKT cells* **Valentine Guinot;** UMR1011 INSERM, Institut Pasteur de Lille, Univ. Lille, France
16.00-16.30	*Clock perturbations as a common contributor to NAFLD, dyslipidemia and atherosclerosis* **Helene Duez;** UMR1011 INSERM, Institut Pasteur de Lille, France
16.30-17.00	Break & visiting exhibitions
Session 5: Complications of NAFLD/NASH *Chairs: Nicolas Lanthier & Ger Koek*	
17.00-17.30	*Modeling fibrosis and NAFLD in 3D liver cultures* **Leo van Grunsven;** Vrije Universiteit Brussel, Belgium
17.30-18.00	*Transcriptional control of hepatic stellate activation in liver fibrosis by BNC2* **Jerome Eeckhoute;** CR CNRS, INSERM UMR1011, France
18.00-18.30	Panel discussion

19.00- 23.00	Networking event with dinner at the hotel restaurant 'de Mangerie' (only for registered participants)

FRIDAY JUNE 10, 2022 – NASH AWARENESS DAY

08:25-08:30	Opening
Session 6: When and how should NAFLD/NASH be treated? *Chairs: Onno Holleboom & Leen Heyens*	
08.30-09.00	*Update on medical treatment for NAFLD and future perspective* **Sven Francque;** University of Antwerp, Belgium
09.00-09.15	Abstract 11: *Resmetirom reduces lipid load, restores THRB expression and prevents cell damage in a human stem cell based in vitro MAFLD model* **Alexandra Gatzios;** Vrije Universiteit Brussel, Belgium
09.15-09.45	*Lifestyle interventions in NAFLD: What should we aim to achieve in the clinical setting* **Kate Hallsworth;** NIHR Newcastle Biomedical Research Centre, UK
09.45-10.00	Abstract 12: *Run for your live(r): Exercise training at different times of day differentially modulates hepatic inflammation in early NAFLD* **Milena Schönke;** LUMC; The Netherlands
10.00-10.30	Break & visiting exhibitions
10.30-11.00	*How should the extrahepatic co-morbidities be treated?* **Raluka Pais;** Pitié-Salpêtrière Hospital, France
11.00-11.15	Abstract 13: *NAFLD and cardiometabolic health: Importance of visceral obesity and cardiorespiratory fitness* **Dominic Chartrand;** Université Laval, Canada
11.15-11.45	*Role of bariatric surgery in NAFLD* **François Pattou;** University Hospital Lille, France
Short abstract presentations	
11.45-11.50	Abstract 19: *Relationship between non-alcoholic fatty liver disease and coronary artery disease: A Mendelian randomization study* **Zhewen Ren;** Maastricht University, The Netherlands
11.50-11.55	Abstract 18: *Evaluation of liver fibrosis using the Fibroscan™ in the bariatric workup: recommendations for clinical practice* **Willy Theel;** Franciscus Gasthuis, The Netherlands
11.55-12.00	Abstract 20: *Getting GRIP on NASH: Implementation of an International Transmural Screening Program* **Vivian de Jong;** Julius Center, UMC Utrecht, The Netherlands
12.00-12.05	Abstract 16: *Deep phenotyping of high intensity interval training in patients with advanced stages of NAFLD* **Veera Houttu;** Amsterdam UMC, The Netherlands

FRIDAY JUNE 10, 2022 – NASH AWARENESS DAY

12.05-12.10	Abstract 14: *The Fibrosis-4 cut-off value for significant fibrosis is dependent on the type of non-alcoholic fatty liver disease patients* **Leen Heyens;** Maastricht University, The Netherlands & Hasselt University, Belgium
12.10-12.15	Abstract 21: *Reduced handgrip strength is correlated with a higher FLI in NAFLD patients with type 2 diabetes and obesity* **Leen Heyens;** Maastricht University, The Netherlands & Hasselt University, Belgium
12.15-12.45	Lunch & visiting exhibitions
Session 7: NAFLD awareness *Chairs: Ger Koek & Achim Kautz*	
12.45-12.55	*Burden of NAFLD in Europe, and health care costs* **Ger Koek;** Maastricht University Medical Center, The Netherlands
12.55-13.10	*Experience of a NAFLD patient*
13.10-13.30	*What is the role of patient advocacy groups in improving NAFLD awareness and how can the patient guideline help?* **Achim Kautz;** Deutsche Leberhilfe e.V., Germany
13.30-13.50	*Why and how to identify NAFLD in primary care: opportunities and challenges* **Christos Lionis;** University of Crete, Greece **Jean Muris**; Maastricht University, The Netherlands
13.50-14.10	*How can the food industry help to prevent NAFLD and associated diseases?* **Robert-Jan Koens;** Jumbo Supermarkten, The Netherlands
14.10-14.30	*Lessons learned from another field: the experience of the European Coalition of People living with Obesity* **Vicki Mooney**; Eurobesity, Ireland
14.30-14.50	*Lifestyle interventions in Healthcare and the role of Health Insurers* **Madelon Johannesma;** CZ, The Netherlands
14.50-15.30	Panel discussion
15.30-15.50	Award Ceremony: best oral presentations
Closing	

Biosketches

Prof. dr. Jerome Boursier

CHU Angers

Angers, France

Jerome Boursier (MD, PhD) is graduated from the Faculty of Medicine of Angers University, in 2006. In 2011, he presented his PhD entitled “Methodology to improve the accuracy of the non-invasive diagnosis of liver fibrosis in chronic hepatitis C”. In 2014, he did a research fellowship in Anna Mae Diehl Lab in Duke University, investigating the area of the gut microbiota and non-alcoholic fatty liver disease. He is Professor of Medicine Angers University, since 2016. His main field of research is non-alcoholic fatty liver disease, with an expertise in the non-invasive diagnosis of liver lesions (development of non-invasive tests, pathological significance, diagnostic and prognostic accuracy, relevance in clinical practice). Member of the French Association for the Study of the Liver (AFEF) and the European Association for the Study of the Liver (EASL), he has an international network and collaboration in this field of research, with the coordination of several multicentric studies including attached biobank. Jerome Boursier is now the Head of the HIFIH Lab (UPRES 3859, SFR 4208) at Angers University, and the Head of the Hepato-Gastroenterology Department at Angers University Hospital.

Non invasive diagnostic tests for NASH: how to assess their accuracy in diagnosis and follow-up?

Prof.dr. Rui Eduardo Castro

University of Lisbon

Lisbon, Portugal

Rui Castro completed his PhD degree in Pharmacy (Biochemistry) by the Faculty of Pharmacy, University of Lisbon (FF/UL) in 2006, having spent a total of 12 months at the Department of Medicine, University of Minnesota Medical School, MN, USA. In 2007, he was awarded with a European Association for the Study of the Liver (EASL) Grant to study the role of microRNAs during liver regeneration at the University of Minnesota. Castro is Assistant Professor at FF/UL since 2015. Early 2021, Castro started his own group at iMed.ULisboa – Liver Disease Diagnostics and Therapeutics Lab. The research being developed by Castro combines his solid background in the modulation of liver cell function by bile acids with his most recent discoveries in the microRNA field, to answer fundamental questions on the pathogenesis, diagnostic, and therapeutic targeting of liver diseases. In this regard, Castro's lab is taking advantage of well-established in vitro and in vivo models of disease, as well as human patient biopsies and clinical data, combined in multi-layered translational approaches, to ultimately bring microRNA-associated health technologies to the clinical setting. In particular, the role of microRNAs and extracellular vesicles (EVs) in inter-organ communication in the setting of obesity and metabolic disease; as well as its exploration for the diagnosis, treatment, monitoring and prevention of liver disease, constitutes a key research objective. He is member of the Executive Committee of iMed.ULisboa and E-Learning Education Committee member at both United European Gastroenterology (UEG) and EASL.

MicroRNAs as biomarkers: scientific plausibility and pathophysiological role

microRNAs (miRNAs) are well established players in NASH pathogenesis, contributing for liver lipotoxicity, oxidative stress, metabolic inflammation and fibrogenesis. Preclinical pharmacological-based modulation of such miRNAs display a broad range of actions on whole-body metabolism, inspiring new efforts in achieving its safe, successful translation into the clinic. Noteworthy, the role of miRNAs as biomarkers and diagnostic tools in NASH is increasingly evident, constituting an attractive alternative to liver biopsy. Circulating miRNA signatures have been described to identify steatohepatitis and fibrosis, further allowing for stratification of disease severity. Large multicentre collaborations are likely to lead to the discovery of clinically relevant diagnostic miRNA panels for NASH.

Prof.dr. Nathalie Delzenne

Faculty of Pharmaceutical and Biomedical sciences

UCLouvain

Louvain, Belgium

Nathalie M. Delzenne is Full Professor at the Faculty of Pharmaceutical and Biomedical sciences at UCLouvain (Belgium). She is President of the Louvain Drug Research Institute (170 researchers), co-leader of the Research Group in Metabolism and Nutrition, full member of the Royal Academy of Medicine in Belgium, founding member and past-President of the Belgian Nutrition Society, present chair of the scientific committee of ESPEN (European Society for Clinical nutrition and metabolism). She has been pioneer in the discovery of nutrients targeting the gut microbiota (prebiotics) and elaborated the molecular mechanisms behind their effects on the control of metabolic and behavioural diseases . Highly cited researcher 2018- 2021 – with nearly 280 peer-reviewed papers in the field https://orcid.org/0000-0003-2115-6082

The gut-liver axis revisited regarding microbiome-nutrients interactions

Recent studies support the involvement of dozens of metabolites that are produced by gut microbes in the control of gut-liver axis. Certain bile acids, amino-acid derived metabolites, gazes or short chain fatty acids, as well as microbial components like lipopolysaccharides or peptidoglycans for example, participate to the regulation of key gut functions (immunity, cell renewal, neuro-endocrine function, gut barrier) that influence host behavior and energy metabolism. Some of those microbial derived metabolites reach the liver through the portal vein, where they regulate inflammatory and metabolic patterns. The dysbiosis refers to alteration of the gut microbiota composition and functions that characterizes several pathologies such as metabolic altered fatty liver disorders (MAFLD), diabetes, obesity, cardio-metabolic diseases, but also depression or food/drink addiction. Our presentation will illustrate the key role of the gut microbiome in the management of liver disease. By using the model of gut microbial transfer from obese or alcohol-dependent patients to mice, we have evaluated the causal role of the microbial dysbiosis in behavioral and metabolic alterations. We, and others have shown that metabolomic and metagenomic analysis can help discovering new biomarkers of hepatic disorders severity, and that breath metabolome can reveal nutrients-microbiome interaction. Promoting the intake of dietary fibers that interact with the gut microbiome (prebiotics) in obese and alcohol-use disorder cohorts, appears as an interesting way to modulate the gut microbiome, but leads to variable outcomes. Interestingly, the initial gut microbiota composition, drug treatment and physical exercise are interdependent components that explain individual variability in terms of health improvement by nutritional approaches. In conclusion, unravelling gut microbiome-liver interactions may be useful for diagnostic and therapeutic purposes, but requires to overcome the difficulty to implement meta-data analysis in day-to-day medicine for all (related funding projects.

Dr. Helene Duez

UMR1011 INSERM

Institut Pasteur de Lille

Lille, France

I received my PhD at the University of Lille in 2003 and became a post-doctoral fellow at the University of Toronto. I then joined Inserm in 2008 as an Associate research Professor and became a Research Director in 2018.

We focus our research on the link between the biological clock and cardiometabolic diseases. It has long been known that the biological clock plays a crucial role in various aspects of physiology ranging circadian rhythms in sleep patterns, blood pressure and immune response to metabolism. Disruptions of the clock caused by shift work, frequent jet lag, exposure to light at night and night-time eating increase the risk of developing metabolic (obesity, dyslipidemia, type 2 diabetes, NAFLD), inflammatory and cardiovascular (atherosclerosis, myocardial infarction) disorders. Focusing on the nuclear receptors and clock components Rev-erbs, we have demonstrated that the Rev-erbs controls the circadian expression/activation of genes and proteins involved in hepatic lipid and bile acid metabolism, muscle mass and mitochondrial function, in macrophage response to DAMPs through the control of NLRP3 and is important the the myocardial response to ischemia/reperfusion injury.

Clock perturbations as a common contributor to NAFLD, dyslipidemia and atherosclerosis

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming a global health problem. NAFLD is associated with a greater risk of cardiovascular disease (CVD). NAFLD and CVD share several common risk factors including obesity, insulin resistance and type 2, as well as atherogenic dyslipidemia (ie increased triglycerides and sdLDL, low HDL-cholestrol levels). In addition, (metabolic) inflammation is a common feature/driver of NAFLD and CVD. The biological generates circadian rhythms in various aspects of physiology, including (hepatic) lipid metabolism and inflammatory pathways. Disruptions of the clock caused by shift work, frequent jet lag, exposure to light at night and night-time eating increase the risk of developing metabolic (obesity, dyslipidemia, type 2 diabetes, NAFLD), inflammatory and cardiovascular (atherosclerosis, myocardial infarction) disorders. Thus, clock alterations may be seen as a common contributor to NAFLD and CVD.

Dr. J. Eeckhoute

CR CNRS, Inserm U1011

Lille, France

Jérôme Eeckhoute earned his PhD in molecular biology from the University of Lille in 2003 owing to studies defining the functional consequences of diabetes-associated mutations of the nuclear receptor HNF4A. He subsequently moved to the Dana-Farber cancer institute (Harvard Medical School, Boston, USA) where he was involved in defining the transcription factor hierarchy and interplay with chromatin involved in regulatory activities of the estrogen receptor. Ever since his recruitment by the CNRS in 2008, he has been focusing on applying functional genomics to decipher molecular mechanisms involved in (dys)regulation of cell/tissue-specific transcriptional regulatory programs with a special emphasis on nuclear receptors and chromatin/epigenomics. Recent interests lie in the characterization of molecular entities and mechanisms responsible for establishment and maintenance/loss of cell identity in liver pathophysiology.

Transcriptional control of hepatic stellate activation in liver fibrosis by BNC2

Dr. Amalia Gastaldelli

Institute of Clinical Physiology (IFC) of National Research Council (CNR) &

Institute of Life Sciences of Sant'Anna School of Advanced Studies

Pisa, Italy

Current positions: Research Director at the Institute of Clinical Physiology (IFC) of National Research Council (CNR) and Affiliate Professor at the Institute of Life Sciences of Sant'Anna School of Advanced Studies in Pisa, Italy. Adjunct Professor of Medicine at the Diabetes Division of University of Texas Health, in San Antonio, TX, USA. President of the NAFLD study group of EASD and of the EGIR study group (European Group for the study of Insulin Resistance).

Research focus: physiological mechanisms that regulate glucose and lipid metabolism, and that are altered in metabolic diseases as NAFLD and diabetes. I am leading a multidisciplinary group (biologists, chemists, mathematician) and we use a translational approach to the study of human physiology, e.g., mass spectrometry coupled with stable isotope tracers for the evaluation in vivo of glucose and lipids metabolism and imaging tools as PET to study organ metabolism in collaboration with many institutions in Europe and United States. PI in national and international scientific projects, participation di clinical trials, partner of several EU grants. Member of the EASL EASD EASO committee for writing the European clinical guidelines for management of NAFLD (2016)

Bibliometric indicators: more than 300 original articles and 30 book chapters with >21000 citations; **H index** of 74 in Scopus and 87 in Google Scholar and a **total impact factor** IF>2400 https://www.scopus.com/authid/detail.uri?authorId=7004210893

Insulin resistance and NAFLD

The prevalence of non-alcoholic fatty liver disease (NAFLD) is now 25% in the general population but increases to more than 55% in subjects with obesity and/or type 2 diabetes. Simple steatosis (NAFL) can develop into more severe, i.e., non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma and death. Several metabolic mechanisms have been indicated as primary promoters of NAFLD and of progression to NASH, in particular insulin resistance, mitochondrial dysfunction, synthesis and accumulation of lipotoxic lipids. Impaired insulin action in NAFLD is not limited to the liver but also occurs in other organs. Insulin resistance in the adipose tissue is the main driver of NAFLD due to excess release of fatty acid that are the primary source of hepatic lipids. In addition to triglycerides, other lipids are bioactive or toxic to liver cells and increase in relation to the degree of insulin resistance not only in the liver but also in muscles and adipose tissue. Not only lipids, but also amino acid metabolism is impaired in NAFL / NASH due to reduced insulin action in muscle and excess catabolism and release of amino acids into the circulation. Increased plasma levels of branched-chain and aromatic amino acids, glutamate, serine and glycine, are associated with an increase in liver fibrosis. In conclusion, insulin resistance induces metabolic changes not only in lipids but also in the amino acid profile that are linked to a more severe NAFLD phenotype. The causal relationship has yet to be fully clarified.

Dr. Kate Hallsworth

Liver Unit

Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH)

Newcastle, UK

Dr. Kate Hallsworth is a Senior Clinical Academic Physiotherapist within the Liver Unit at Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH), UK. She has published world-leading research in the field of lifestyle management for patients with NAFLD. She led the first study assessing the effects of resistance exercise in NAFLD and subsequent trials investigating different physical activity/exercise modalities. She also led the first study assessing the feasibility and acceptability of using a very-low calorie diet to manage patients with advanced NAFLD, which showed very exciting results in terms of patient uptake, adherence and improvements in key clinical outcomes. Her translational research focusses on the use of lifestyle interventions in the management of NAFLD/NASH. This includes physical activity measurement in this patient population and assessing the impact of different exercise modalities on liver, metabolic and cardiac health. Her more recent research has looked at why lifestyle interventions are not being optimised in clinical practice and what tools are necessary to aid healthcare professionals to support patients to make lifestyle changes. She led the development of an evidence-based NAFLD-specific digital intervention (VITALISE-interVention to promote lIfesTyle change in non-Alcoholic fatty LIver diseaSE) which was co-designed with patients and will be trialled within the NHS in the coming months. Kate is passionate about improving the care of patients with NAFLD and is driving service redesign to ensure a multidisciplinary, holistic approach to disease management, offering patients tailored lifestyle interventions that meet their needs. She was part of an international group working on an EASL NAFLD Patient Guideline and national groups working on developing BASL Quality Care Standards for patients with NAFLD and BSG Liver Transplant Guidelines for NASH. Kate is an advocate for Public/Patient Involvement and Engagement and has a close relationship with the British Liver Trust and national patient representative group LIVErNORTH. She has also helped to develop a website to support patients diagnosed with liver cancer and their relatives/carers providing input on lifestyle interventions (https://www.livingwithlivercancer.co.uk/).Kate was awarded a prestigious Rising Star Award from EASL in 2021 for her work which focuses on putting people with, or at risk of, liver disease at the heart of her practice.

Lifestyle interventions in NAFLD: What should we aim to achieve in the clinical setting?
In the absence of approved drug therapy, lifestyle interventions remain the cornerstone of NAFLD management. This session will cover the EASL–EASD–EASO Clinical Practice Guidelines for the management of NAFLD in relation to lifestyle treatment, and the evidence that the guidelines were based on. The session will highlight different treatment options for both diet and physical activity/exercise and the importance of tailoring interventions to individual patients. The session aims to provide practical tips for use in the clinical setting and will highlight useful techniques to support patients to make and sustain lifestyle changes.

Prof. dr. Mathias Heikenwälder

Department Chronic Inflammation and Cancer

German Center for Cancer Research (DKFZ

Heidelberg, Germany.

Prof. Mathias Heikenwälder is a trained molecular biologist, with expertise in immunology and a strong link to translational research evoked by 10 years of work and expertise in a Pathology Institution (Clinical Pathology, University Hospital Zurich). Since October 2015 he is Department head at the German Cancer Research Center (DKFZ) in Heidelberg focusing on the link between chronic inflammation and cancer. The Heikenwälder laboratory aims to understand the different immune signatures of chronic inflammatory human diseases using relevant mouse models - with the final aim is to generate models of chronic inflammation potentially used for pre-clinical research. Thus, the Heikenwälder laboratory focuses on comparative studies of human and animal tissues, recapitulating human disease on a histo-pathological and pathophysiological level. The laboratory engages in classical molecular biology techniques complemented with sophisticated ways to receive as much information from tissue samples through histology (e.g. light microscopy/ immune fluorescence/ FISH/ in situ hybridization), other in vivo imaging techniques (e.g. MRI) as well as through FACS analyses for tissue homogenates. At the same time, the Heikenwälder laboratory is also interested in the systemic functional effects of pathologies and the interplay between several affected non-lymphoid tissues and the immune system. Finally, testing several therapeutic compounds in a single use and combinatorial fashion is one of the goals of employing established and stratified pre-clinical mouse models. Prof. Heikenwälder publishes his work in high-ranking journals and has established himself as an international leader in the field of liver cancer. Mathias Heikenwälder is the third most frequently cited German-speaking researcher in the field of cell biology in the last 5 years and was one of the Highly Cited Researchers (Cross Fields) (Web of Science Group) in 2019, 2020 and 2021. The research community has widely cited his publications, but they have also shifted fatty liver and liver cancer research in new directions - relevant to the day-to-day management of patients with fatty liver or liver cancer.

Immune mechanisms driving NASH and liver cancer

In association with the pandemic spreading of obesity and metabolic syndrome, the prevalence of NAFLD-related HCC is increasing almost exponentially. In recent years, many of the underlining multifactorial causes of NAFLD have been identified, and the cellular mechanisms sustaining disease development have been dissected up to the single-cell level. Still, there is still an urgent need to provide clinicians with more therapeutic targets, with particular attention on NAFLD-induced HCC, where immune checkpoint inhibitors do not work as efficiently. Whereas much effort has been invested in elucidating the role of innate immune response in the hepatic NAFLD microenvironment, only in the past decade have novel critical roles been unraveled for T cells in driving chronic inflammation toward HCC. The metabolic and immune microenvironment interact to recreate a tumor-promoting and immune-suppressive terrain, responsible for resistance to anticancer therapy. Here, I will illustrate the cellular crosstalk with other immune cells, regulatory networks or stimulatory effects of these interactions, and role of the metabolic microenvironment in influencing immune cell functionality in the context of fatty liver disease and subsequent liver cancer.

Dr. Onno Holleboom

Department of Vascular Medicine

Amsterdam UMC

Amsterdam, The Netherlands

Dr. A.G. (Onno) Holleboom MD PhD is an internist registered in Vascular Medicine and Endocrinology, faculty member of the Department of Vascular Medicine and Assistant Professor at Amsterdam UMC. He initiated a multidisciplinary outpatient clinic for NAFLD together with hepatology and including clinical trials. Within the NAFLD-NL consortium and supported by the Dutch MLDS gastroenterology and hepatology foundation, he aims to develop care paths for NAFLD together with LUMC and Radboud MC. Together with prof. Max Nieuwdorp, he runs a research group with 6 PhD candidates focussing on genetic and gut microbial drivers of NAFLD in three cohort studies, as well as more fundamental work on lipid droplet and inflammatory pathways. He has published 60 peer-reviewed articles, including in Circulation and Cell Metabolism and received various prestigious grants, a.o. from The Netherlands Organization for Scientific Research and the Amsterdam UMC Fellowship.

NAFLD-NASH and the heart

Dr. Madelon Johannesma

CZ, The Netherlands

Dr. M.C.G. Johannesma holds a MSc in Health Science and Epidemiology and a PhD in Health, Medicine and Life Sciences. Currently she is working as program manager Health Care Innovation at CZ, a Dutch health insurance company. On that account she is involved in several projects concerning life style in order to stabilize or prevent chronic diseases. She is a member of the expert group prevention at Zorgverzekeraars Nederland (ZN). Another focus in her work is Personalized Medicine, in this program the focus is on implementation of predictive tools such as advanced diagnostics and Decision Support Systems to get the right treatment for the right person in the right place.

Lifestyle interventions in Healthcare and the role of Health Insurers

At current, 50% of Dutch people aged 18 and older were overweight and almost 15% obese. Obesity is linked to many diseases, such as type 2 diabetes mellitus, cardiovascular disease and various cancers. From January 2019, the Combined Lifestyle Interventions (CLI) is part of basic health insurance in the Netherlands for people with overweight or obesity. The CLI promotes healthy lifestyle changes by focusing on behaviour change resulting in weight loss. Being part of basic health insurance policy makes the CLI easily accessible for the target population. However, there is an increasing demand to broaden the current target population for the CLI and integrate life style in the healthcare pathway of many different diseases. The presentation will outline the limitations and opportunities from a health insurance perspective taken into account the national law an regulations.

Achim Kautz

Kautz5 gUG

Cologne, Germany

Achim Kautz is CEO of Kautz5 gUG – a non-profit project and consulting company for patient-centred care research and health concepts. He is actively advocating for liver patients since more than 20 years. Over these years he was CEO of the German patient organization "Deutsche Leberhilfe e.V." and co-initiator of the European Liver Patients´ Association (ELPA) and the World Hepatitis Alliance (WHA). He has been part of a number of EU declarations and WHO resolutions mainly in the liver field and serves as a member of the consultancy group in the Ministry of Health in Germany looking after the national elimination strategy for HIV, viral hepatitis and STDs. He is the initiator of many metabolic disease related health projects and active in NASH population research on national and international level. He is also co-initiator of the International Liver Cancer Movement (ILCM) with over 60 PAGs and scientific organizations globally. He is also a consultant for scientific associations and senior special advisor for Burson Cohn & Wolfe (BCW).

What is the role of patient advocacy groups in improving NAFLD awareness and how can the patient guideline help?

Looking to the patient advocacy landscape in NAFLD we must note there are no specific advocacy groups existent, thus far. Therefore, patient groups from the fields of liver, diabetes and obesity need to take on NAFLD advocacy. Parallelly there is a high need to build up national and pan-European NAFLD communities.
One important tool to start the process is a co-created aligned easy to understand guideline for patients living with NAFLD or NASH. This was the effort of a wide group of supporters and contributors coming from hepatology, gastroenterology, family medicine, nutrition, public health and patient groups and has been published in J Hep Reports in September 2021. Based on this, a working group under the leadership of Sven Francque (Antwerp University Hospital), Diane Langenbacher and Achim Kautz (both Kautz5 gUG) developed together a lay summary "*Non-alcoholic fatty liver disease (NAFLD): How you can reduce the risk for your liver and for other health issues?"* including 14 key steps for patients to take responsibility for their liver and to start the NAFLD/NASH management, pro-actively.
For the first-time patients with NAFLD/NASH have an empowering tool comparing high level evidence recommended with what is provided currently in their country care setting, building the foundation of further advocacy and awareness.
More awareness is needed especially in those with advanced fibrosis (stage F3 +) and those who are at high risk of NAFLD/NASH. Due to high rates of co-morbidities patient groups from the field of diabetes and obesity play a vital role increasing such awareness. In this context, GPs are also highly important as they address the risk to their obvious at-risk patients and need to start screening in a simple way by ultrasound and use of FIB-4. The lay version represents a valuable resource to distribute proper information.

Prof. dr. Christos Lionis

Faculty of Medicine

University of Crete

Heraklion, Greece

Christos Lionis is a medical doctor and Professor with a strong interest in primary care and public health education, practice, and research. Christos has served the Clinic of Social and Family Medicine (CSFM) at the School of Medicine, University of Crete (http://www.fammed.uoc.gr/Joomla/) since 1995 (March) initially as an Assistant Professor and in 2009 as a Professor of General Practice and Primary Health Care and CFSM Director. He is also appointed as Guest Professor of General Practice in the Institute of Health and Medicine at the University of Linkoping, Sweden (2018), a position that he continues to hold today. As part of his work with the CFSM, Christos has been involved both as PI and collaborator in multiple large European and international research collaboratives and research proposals funded by the EU and international agencies. His work has focused on capacity building of primary health care and public health in Greece and internationally. Christos has a passion for education and has a played a key role in the development medical training, continuing medical education, and quality standards in primary care and public health nationally. He has developed a strong collaboration with WHO especially during the past two years. Christos is also involved in an editorial and advisory capacity with a number of international journals. He also is a member of the Executive Board of various professional organisations including that of WONCA Working Party on Mental Health where he is currently elected as Chair. He has been awarded as Honorary Fellow for the Royal College of General Practitioners (2009), the World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians (WONCA) (2016) and the European Society of Cardiology (2017). Christos has published 423 papers in international journals that they cited in PubMed. Since 2019, Christos is a member of the European Commission Expert Panel on Effective Ways of Investing in Health.

Why and how to identify NAFLD in primary care: opportunities and challenges

Vicki Mooney

Eurobesity

Dublin, Ireland

I am a Patient Advocate, Public Speaker, Media Personality & the Executive Director of the ECPO. I am a founding member of the EASO Patient Council, a Disease Experience Expert Panel (DEEP) representative and I sit on the Global Obesity Patient Alliance (GOPA) team, I also chair the board of directors for the ICPO (Irish Coalition for People living with Obesity).

Originally from Ireland, I now live in Lanzarote with my fiancé & 3 children. I use my voice to help address the stigma, bias and discrimination perpetrated against people living with Obesity and other chronic NCDs which obesity is a gateway to. Whilst not only working on various projects across Europe, and now globally, to ensure we not only see better access to quality treatment, management and education, but also to help drive forward prevention of Obesity across Europe'

Lessons learned from another field: the experience of the European Coalition of People living with Obesity

This presentation will open up the conversation on the link between obesity and NAFLD from the lived experience of a person living with Obesity.

Prof. dr. Jean Muris

Maastricht University

Department of Family Medicine

Maastricht, The Netherlands

Dr Jean W.M. Muris (1959) is a Full Professor at Maastricht University, a leading Dutch university known for its innovation.

He was the Strategic Chair in Asthma and COPD in Primary Care at the research school CAPHRI (www.caprhi.nl), and since 2017 has been the Chair of the Department of Family Medicine/General Practice at Maastricht University.

After completing his studies in medicine at the University of Maastricht in 1983, Jean immediately continued his education in General Practice Training and primary care research. In 1985, he participated in an experimental research doctoral programme that was recognized by the Dutch College of General Practitioners http://nhg.org, which formed his strong foundation in research. During this time and extending to 1993, he also worked as a General Practitioner, serving health care center Withuis in Venlo and from 1993 until now as an Academic GP for Health Care Centre Geulle.

His PhD, completed in 1993, was on the topic *'Non acute abdominal complaints in primary and secondary care'*.

In addition to his post-graduate research and teaching position at the University, Jean runs a primary care practice. He finds that maintaining an academic/research footing as well as a practical footing in medicine have been the key to his success; one practice informing and benefiting the other.

Prolific in his research, he has published over 200 works, a list of which can be viewed at Orcid and at Google Scholar. He is the secretary-general of the European Society of Primary Care Gastroenterology (www.espcg.eu).

Currently, his research focuses on educational research in primary care specialist training and methods to help stop smoking and manage respiratory diseases, with a particular emphasis on asthma and COPD. His gastro expertise is on irritable bowel syndrome, non-alcoholic fatty liver disease, reflux complaints, functional dyspepsia and celiac disease. He is a member of several multidisciplinary guideline committees on gastro and respiratory diseases in and outside the Netherlands.

Why and how to identify NAFLD in primary care: opportunities and challenges

Dr. Raluca Pais

Pitié-Salpêtrière Hospital

Paris, France

Dr Raluca Pais is MD PhD in Hepatogastroenterology Department and Institute of Cardiometabolisme and Nutrition, Pitié Salpetriere Hospital, Paris, France. She first completed her medical training at University of Medicine and Pharmacy “Iuliu Hatieganu” Cluj Napoca, Romania. She earned her doctoral degree at the University Pierre et Marie Curie, Paris VI for her work on the natural history and progression of non-alcoholic fatty liver disease, liver and cardiovascular related comorbidities related to NAFLD.

With the support of AP-HP (Assistance Publique Hopitaux de Paris) and ICAN (Institut of Cardiometaboolism and Nutrition) she founded at Pitié Salpetriere Hospital, the NASH Clinic, the first hospital structure for the multidisciplinary care of patients with metabolic steatosis in France. This structure aims toanticipate and intercept the complications of NASH (early atherosclerosis, arterial hypertension, diabetes, etc.) and offer personalized care to each patient, taking into account their clinical phenotype, personal history and environment to ensure the best possible compliance with medical recommendations.

Dr Pais is also part of several European Consortia: EU projet Horizon 2020 EPOS (Elucidating Pathways of Steatohepatitis), Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) funded by the European Innovative Medicines Initiative 2 and EU-PEARL (EU Patient-Centric Clinical Trial Platforms) , a strategic partnership between the public and private sectors to shape the future of clinical trials.

How should the extrahepatic co-morbidities be treated?

Prof.dr. Francois Pattou

Lille University

Lille, France

Professor of General Surgery, Lille University, Lille. France
Head, General and Endocrine Surgery, University Hospital, Lille. France
Director, Translational research on Diabetes,
Univ Lille, CHU Lille, Pasteur Institute Lille, Inserm UMR 1190, Lille, France
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François Pattou (56 years) became professor of Surgery at the University of Lille in 2002, and head of the Department of General and Endocrine Surgery at Lille University Hospital in 2005. FP also leads an INSERM research unit devoted the clinical development of biotherapies for treating diabetes (U1190), and a funding member of the European Genomic Institute for Diabetes (EGID).

FP's research is devoted to the surgical treatment of endocrine and metabolic diseases and focused on cell therapy for type 1 diabetes and metabolic surgery for type 2 diabetes. He has authored more than 4O0 papers in high profile journals, and is the principal investigator of several ongoing clinical trials in islet cell transplantation and metabolic surgery. He also coordinates Precinash a private-public consortium devoted too the study of NASH. The recipient of numerous research grants from national and international institutions (Programme d'Investissements d'Avenir, European Commission, Innovative Medicine Initiative, Agence Nationale de la Recherche, Juvenile Diabete Research Fundation, Fondation Francophone pour le recherche sur le diabète), François Pattou has been awarded by several prizes (National Academy of Medicine, Rachmine Levine scientific achievement award, Matmut award, Fondation de la recherché Médicale).

Ten selected publications

1. **Pattou F**, Kerr-Conte J, Wild D, In vivo imaging of transplanted human beta cells with GLP1 Rc scan. New Engl J Med 2010;363:1989-90

2. Caiazzo R, Lassailly G, Leteurtre E, Baud G, Verkindt H, Raverdy V, Buob D, Pigeyre M, Mathurin P, **Pattou F**. Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study. Ann Surg. 2014 Nov;260(5):893-8.

3. Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese PatientsLassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, Raverdy V, Leteurtre E, Dharancy S, Louvet A, Romon M, Duhamel A, Pattou F, Mathurin P. Gastroenterology. 2015 Aug;149(2):379-88; quiz e15-6. doi: 10.1053/j.gastro.2015.04.014. Epub 2015 Apr 25.

4. Bonner C, Kerr-Conte J, Gmyr V, Queniat G, Moerman E, Thévenet J, Beaucamps C, Delalleau N, Popescu I, Malaisse W, Sener A, Deprez B, Abderrahmani A, Staels B, Pattou F. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. Nature Med 2015;21(5):512-7.

5. Baud G, Daoudi M, Hubert T, Raverdy V, Pigeyre M, Hervieux E, Devienne M, Ghunaim M, Queon A, Pigny P, Klein A, Caiazzo R, **Pattou F**. Bile diversion decreases sodium dépendent glucose intestinal uptake after Roux-en-Y Gastric Bypass (RYGB). *Cell Metab* 2016 23, 547–553

6. Raverdy V, Baud G, Pigeyre M, Verkindt H, Torres F, Preda C, Thuillier D, Gélé P, Vantyghem MC, Caiazzo R, **Pattou F**. Incidence and Predictive Factors of Postprandial Hyperinsulinemic Hypoglycemia After Roux-en-Y Gastric Bypass: A Five year Longitudinal Study. *Ann Surg* 2016;264:878-885.

7. Vantyghem MC, Chetboun M, Gmyr V, Jannin A, Espiard S, Le Mapihan K, Raverdy V, Delalleau N, Machuron F, Hubert T, Frimat M, Van Belle E, Hazzan M, Pigny P, Noel C, Caiazzo R, Kerr-Conte J, **Pattou F**. Ten-Year Outcome of Islet Alone or Islet After Kidney Transplantation in Type 1 Diabetes: A Prospective Parallel-Arm Cohort Study. *Diabetes Care.* 2019 Nov;42(11):2042-2049.

8. Vantyghem MC, de Koning EJP, **Pattou F**, Rickels MR. Advances in β-cell replacement therapy for the treatment of type 1 diabetes. *Lancet*. 2019 Oct 5;394(10205):1274-1285.

9. Lassailly G, Caiazzo R, Ntandja-Wandji LC, Gnemmi V, Baud G, Verkindt H, Ningarhari M, Louvet A, Leteurtre E, Raverdy V, Dharancy S, **Pattou F***, Mathurin P*. Bariatric Surgery Provides Long-term Resolution of NASH and Regression of Fibrosis. Gastroenterology. 2020;159(4):1290-1301

10. Data-driven subgroups of type 2 diabetes, metabolic response, and renal risk profile after bariatric surgery: a retrospective cohort study. Raverdy V, Cohen RV, Caiazzo R, Verkindt H, Petry TBZ, Marciniak C, Legendre B, Bauvin P, Chatelain E, Duhamel A, Drumez E, Oukhouya-Daoud N, Chetboun M, Baud G, Ahlqvist E, Wierup N, Asplund O, Laferrère B, Groop L, Pattou F. Lancet Diabetes Endocrinol. 2022 Mar;10(3):167-176.

Role of bariatric surgery in NAFLD

Dr. Catherine Postic

Institut Cochin

Paris, France

Catherine Postic is a research group leader in the Department of Endocrinology Metabolism and Diabetes at the Cochin Institute, INSERM U1016, CNRS UMR 8104 Université de Paris Cité.
Catherine Postic earned her doctorate at the University Paris Diderot in Paris. She completed post-doctoral work at Vanderbilt University in Nashville, TN USA. During this post-doctoral fellowship she was trained in the field of molecular biology and achieved the making of a conditional locus for the glucokinase (gck) gene, a key gene of glucose metabolism.
With this approach she has explored the cell-specific function of glucokinase in liver and pancreatic ß-cells and demonstrated the importance of this enzyme in the sensing of glucose. Her long time interest in the control of hepatic metabolism prompted her to study the function and the regulation of the transcription factor ChREBP. Using both physiological and metabolic approaches her group made significant advance in understanding the regulation of ChREBP by glucose and assessing its function in metabolic diseases such as Non Alcoholic fatty Liver Disease (NAFLD).She recently got interested in another important glucose sensor, the O-GlcNAc transferase (OGT), and is currently studying its role in liver physiology.

Glucose sensing and lipid metabolism in NAFLD

Glucotoxicity is a phenomenon that initiates a vicious cycle in which chronic hyperglycemia leads to the development of type 2 diabetes. Among the mechanisms involved, it is described that a fraction of glucose, metabolized in the hexosamine biosynthetic pathway, induces O-GlcNAcylation of intracellular proteins. O-GlcNAcylation is a reversible post-translational modification controlled only by two enzymes, OGT, which adds a molecule of N-Acetyl Glucosamine (GlcNAc) to serine or threonine, and OGA, which removes it. Our laboratory reported that O-GlcNAcylation of key effectors of metabolism contributed significantly to gluco-lipotoxicity in liver (Kuo 2008, Guinez 2011) and pancreatic β cells (Fardini 2014). In order to determine the metabolic consequences of a targeted deletion of the OGT enzyme in in the context of NAFLD, our laboratory generated mice constitutively deficient for OGT in the liver (OGTLiverKO). Surprisingly, these mice do not exhibit major disruption of metabolic homeostasis but show a severe liver phenotype with the presence of numerous regeneration nodules visible after weaning. RT-PCR and western blot analyzes showed that the expression of cyclins A2, B1 and D1 was significantly increased in the liver of OGTLiverKO mice compared to controls, suggesting an exacerbated proliferative state. Immunohistological analyzes revealed the presence of pro-inflammatory cells and signs of fibrosis in the spans surrounding the regeneration nodules. This was associated with a significant increase in markers of inflammation (TNFα) and fibrosis (TGFβ), suggesting significant liver injury in OGTLiverKO mice. Full characterization of this novel mouse model is currently ongoing.

Prof. dr. Patrick C.N. Rensen

Leiden University

Leiden, The Netherlands

Patrick Rensen received his PhD (cum laude) in 1992 at Leiden University, and is professor Metabolic Aspects of Vascular Disease at Leiden University Medical Center, Leiden, The Netherlands, and guest professor at Xi'an Jiaotong University, Xi'an, China. He is Established Investigator of the Dutch Heart Foundation, chairman of the European Lipoprotein Club (ELC), board member of the Scandinavian Society for Atherosclerosis Research (SSAR), and board member of the Leiden University Fund (LUF) Committee for Academic Expenditure. His research group studies the role of lipid and glucose metabolism in cardiometabolic diseases, with a main focus on modulation of energy metabolism as a strategy to prevent and treat obesity and associated diseases including NAFLD, type 2 diabetes and atherosclerotic cardiovascular disease. He currently investigates novel strategies to target NAFLD, including lifestyle-related approaches (e.g., exercise, dietary fiber intake) and pharmacological approaches (e.g., FGF21, incretins, DHCR24 inhibitors), taking account the role of thermogenic adipose tissues, the gut microbiome and the biological clock. To this end, he combines mechanistic experiments in a humanized mouse model for cardiometabolic diseases (i.e., APOE*3-Leiden.CETP mice) with human intervention studies in metabolically compromised individuals. Current grant support includes the Dutch Research Council (NWO), Dutch Heart Foundation (DHF), Dutch Diabetes Research Foundation (DFN), European Federation for the Study of Diabetes (EFSD), NovoNordisk Foundation, Dutch Digestive Foundation (MLDS) and the Chinese Scholarship Council (CSC). He currently co-authors approx. 340 publications (H-index 58) in peer-reviewed scientific journals (e.g., Cell, Cell Metab, Nat Med, Nat Commun, Eur Heart J, Circulation, Gut), and is Editorial Board member of Atherosclerosis.

Adipose-liver crosstalk in NAFLD

The main research focus of our group is modulation of energy metabolism to target obesity and related cardiometabolic diseases. We recently set out to evaluate experimental strategies to combat NAFLD, via lifestyle-targeted and pharmacological approaches, mainly by employing APOE*3-Leiden.CETP transgenic mice. Using this well-established model for human cardiometabolic diseases including NAFLD, we recently studied biological mechanisms underlying protective effects of various recombinant hormones (e.g., FGF21, GLP1 and GIP) in NAFLD. By combining extensive metabolic phenotyping with immune cell profiling, we revealed that FGF21R agonism and GLP1R/GIPR agonism strongly inhibit diet-induced NAFLD progression, involving activation of thermogenic adipose tissues. In addition we evaluated a novel strategy to target NAFLD by inhibition of DHCR24, the enzyme that mediates the conversion of desmosterol into cholesterol, using our selective DHCR24 inhibitor SH42. Treatment with SH42 increased hepatic desmosterol to selectively activate LXR in macrophages without inducing lipogenesis in hepatocytes. As such, this novel strategy appeared very successful in reducing hepatic inflammation as well as lipid accumulation. Results of these ongoing studies will be presented, and relevance for treatment of NAFLD in humans will be discussed.

Prof. dr. Stefano Romeo

University of Gothenburg

Department of Molecular and Clinical Medicine

Gothenburg, Sweden

Stefano Romeo is Professor in Molecular and Clinical Medicine at the Institute of Medicine at the University of Gothenburg, Sweden and Senior Consultant in Endocrinology and Metabolic Diseases at the Sahlgrenska University Hospital.He is adjunct Professor at University Magna Graecia of Catanzaro in Italy. Dr Romeo's research focus is the role of genetic variants in metabolic disorders such as fatty liver disease (FLD) and dyslipidemia. His research field ranges from genetic association studies in large cohorts to molecular genetics and characterization of human mutations. In the field of FLD: Dr Romeo identified genetic risk and protective determinants in the *PNPLA3, MBOAT7, GPAM, APOE and PSD3* genes; generated an easy-to-use multilineage 3D *in vitro* model of FLD. In the field of CVD, Dr Romeo recently generated an algorithm, empowered by machine learning, to easily perform diagnosis of familial hypercholesterolemia (FH); identified and characterized new mutations responsible for a severe hypertriglyceridemia in the *LPL* gene increasing the risk for pancreatitis.

What is the role of genetic testing in NAFLD?

Prof.dr. Charlotte Louise Scott

VIB-UGent Center for inflammation Research

Gent, Belgium

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Date of Birth: 14th November 1987
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Researcher ID: K-3563-2014
Google Scholar: https://scholar.google.com/citations?user=TXecfhYAAAAJ&hl=en&oi=ao
Qualifications: BSc (Hons); MRes (Distinction), PhD

Current Position: Associate Professor Ghent University Oct 2019-
VIB Group Leader Jan 2020-

Previous Positions and Education:
Ghent University 2017-2019
Assistant Professor
Ghent University/VIB
PostDoc 2013-2017
Supervisor: Prof. Martin Guilliams.
University of Glasgow
PhD 'Characterisation of Dendritic cells in the intestine' 2010-2014
Supervisor Prof. Allan Mowat, graduated April 2014.
University of Glasgow
MRes in Molecular Functions in Disease (Distinction) 2009-2010
University of Limerick
BSc. in Industrial Biochemistry (Hons; #1 University Class of 2009) 2005-2009

Fellowships and Awards

- EFIS-ACTERIA Early Career Prize in Immunology 2021
- EASL Emerging Leader Award 2021
- EMDS Young Investigator Award 2019
- ERC Starting Grant 2019: MyeFattyLiver
- Sir Henry Wellcome Postdoctoral Fellowship Ghent/Glasgow May 2017-September 2019
- FWO Travel Grant, April 2017 – Keystone Congress, USA
- BSI Travel Grant, September 2016 – International Congress of Immunology, Australia
- Marie Curie Intra-European Fellowship Ghent May 2015-April 2017

My lab is focused on understanding the functional heterogeneity of myeloid cells in the diseased liver. In recent years we have invested in establishing state of the art technologies including CITE-seq and spatial transcriptomics to allow us to identify the specific populations of myeloid cells present in different settings and to address how these different cells respond in different disease contexts across species.

deLIVERing a spatial cell atlas of the healthy and obese liver across species

The liver is the largest solid organ in the body, yet it remains incompletely characterized. Here, we present a spatial proteogenomic atlas of the healthy and obese human and murine liver combining single-cell CITE-seq, single-nuclei sequencing, spatial transcriptomics and spatial proteomics. By integrating these multi-omic datasets, we provide validated strategies to reliably discriminate and localize all hepatic cells, including a population of lipid-associated macrophages (LAMs) at the bile-ducts. We then align this atlas across seven species, revealing the conserved program of *bona fide* Kupffer cells and LAMs. We also uncover the respective spatially-resolved cellular niches of these macrophages and the microenvironmental circuits driving their unique transcriptomic identities. Specifically, we demonstrate that LAMs are induced by local lipid exposure, leading to their induction in steatotic regions of the murine and human liver. The profile, location and increased abundance of LAMs in NAFLD has made these cells a key target for our ongoing functional studies aimed at unravelling the role played by these cells in NAFLD pathogenesis.

Prof.dr. Emmanuel Tsochatzis

UCL Institute for Liver & Digestive Health

London, UK

Professor Emmanuel Tsochatzis (MD, MSc, FEBTM, FRCP, PhD) is a Professor of Hepatology and Consultant Hepatologist at the UCL Institute for Liver and Digestive Health, Royal Free Hospital in London. He is Head of the Centre for Metabolic Liver Disease in ILDH, chair of the EASL Scientific Committee and member of the EASL Governing Board. He is a member of the Baveno steering committee for portal hypertension. His main research interests include non-alcoholic fatty liver disease, cirrhosis and portal hypertension and non-invasive assessment of liver fibrosis.

Professor Tsochatzis finished his specialty training and PhD in Hippokration General Hospital in Greece, before moving to the Royal Free Hospital for his post-doc research under Professor Andy Burroughs. He is the recipient of the Rising Star in Gastroenterology prize by the UEG and the EASL Physician Scientist Fellowship.

His work on the cost-effectiveness of non-invasive fibrosis tests has informed the World Health Organization (WHO) guidelines on diagnosis and treatment of both HBV and HCV. He has designed and implemented a primary care pathway for NAFLD referrals to secondary care. He leads the specialist multidisciplinary service in NAFLD at the Royal Free Hospital and has an active research program in NAFLD. He has published more than 260 articles in peer-reviewed journals. He has received funding for his research from NIHR, EASL and EU Horizon 2020.

Referral pathways for patients with NAFLD

Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of deranged liver blood tests (LFTs) in primary care in Europe and North America, and has an estimated prevalence of 25-30% in the adult population. Only a minority of people with NAFLD (5%) develop clinically significant liver disease, but the burden is such that NAFLD is predicted to be the leading indication for liver transplantation within a decade.

The majority of patients with NAFLD are followed up in the community by general practitioners (GPs). Liver fibrosis severity is the key determinant of liver-related outcomes in NAFLD. However, identifying patients with significant fibrosis who might benefit from early specialist intervention is challenging. As clinical assessment is a poor discriminator of fibrosis, such patients progress silently until cirrhosis leads to complications. Accurate fibrosis assessment in primary care is limited by a reliance on LFTs, which correlate poorly with fibrosis and limited access to discriminatory fibrosis tests. Thus current management strategies are inefficient in identifying patients for specialist referral.

This talk will focus on referral pathways for patients with NAFLD, based on risk stratification using non-invasive fibrosis tests.

Prof. dr. Leo van Grunsven

Liver Cell Biology research group

Vrije Universiteit Brussel

Brussels, Belgium

Leo van Grunsven obtained his Biology degree in 1992 from the Utrecht University working on the identification of upstream regulatory regions of Cyclin genes. He went to Lyon (France) for a PhD on the regulation of NGF-dependent neural outgrowth in Pheochromocytoma cells and obtained his PhD in 1996 from the Ecole Normale Supérieure de Lyon. He had his postdoctoral training at the NINDS/NIH (Bethesda, USA) and the KU Leuven (Belgium) which focused on understanding the transcriptional regulation of neuronal differentiation. At the NIH the focus was on neuronal stem cells, while at the KU Leuven neuronal differentiation was studied during early neurogenesis in the mouse and frog with a strong emphasis on the transcriptional repressor SIP1/ZEB2. He joined the lab of the late Prof. Albert Geerts at the Vrije Universiteit Brussel (VUB, Belgium) in 2006 and became an assistant Professor in 2009 and heads the Liver Cell Biology research group since. His research group studies molecular mechanisms involved in liver -homeostasis, -fibrosis and –regeneration with a special focus on hepatic stellate cells. His group was the first to identify autophagy, AGE- and HIPPO-signaling as key mechanisms involved in hepatic stellate cell activation during liver fibrogenesis. His team pioneered in the establishment of an hepatocyte-injury dependent in vitro liver fibrosis model by using spheroid cultures of human hepatocytes and hepatic stellate cells (2016). The research efforts of his lab now also include induced pluripotent stem cell-derived hepatocytes and hepatic stellate cells as a cellular source for these spheroid cultures. The group continues to invest in further refinement of in vitro models of fibrosis and NAFLD based on primary mouse liver cells and investigates the regulation of stress pathways in hepatic stellate cells and sinudoidal liver cells during acute and chronic liver injury in mouse models.

More info: http://livr.vub.ac.be/ ResearchPortal VUB

Modeling fibrosis and NAFLD in 3D liver cultures

Liver fibrosis and cirrhosis can be caused by viral hepatitis, non-alcoholic fatty liver disease (NAFLD), alcoholic- (ASH) and non-alcoholic-steatohepatitis (NASH). Currently, no therapies are available in the clinic that can target fibrosis directly. Commonly used models for in vitro liver fibrosis consist of mono-layer cultures of rodent hepatic stellate cells (HSCs), thereby ignoring the role of hepatocyte injury, which usually triggers the switch of quiescent HSCs in a healthy liver to activated myofibroblastic HSCs in an injured liver. The presentation will give an overview of our progress on developing complex 3D liver cultures; from hepacotye-HSC cultures to primary- and iPSC-derived four cell-type cultures that can model fibrosis and NAFLD. Special attention will be given to a recenlty established multicellular in vitro spheroid culture model using primary mouse hepatocytes, HSCs, liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs). These liver spheroids maintain the independent liver cell functionalities for two weeks and demonstrate the capacity to mount a fibrotic response upon

drug-induced liver damage or fatty liver conditions. Importantly, the anti-steatotic and anti-fibrotic efficacy of anti-NAFLD compounds such as Elafibranor, Lanifibranor and Obeticholic acid as observed in mice, could be reproduced in these cultures. These culture models represent an important step forward towards in vitro compound testing for drug-induced liver fibrosis and NAFLD.

Abstracts

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ABSTRACT 1

Investigating microRNAs to explain the link between cholesterol metabolism and NAFLD in humans: a systematic review

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Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is characterized by free cholesterol accumulation in the liver. Moreover, there are clear indications that microRNAs (miRs) might be involved in NAFLD development. Therefore, we systematically reviewed the literature to examine the link between miRs and cholesterol metabolism in NAFLD.

Methods: Relevant studies (N=15) were retrieved by a systematic search of three databases in March 2020. From these papers, we evaluated 11 miRs and assessed associations between NAFLD and cholesterol metabolism. Additionally, their diagnostic potential was examined by evaluating the area under de Receiver Operating Curve.

Results: Four of the 11 miRs (miR 122, 34a, 132, and 21) were associated with cholesterol metabolism and markers for NAFLD. MiR122 was upregulated in serum of NAFLD patients, increased with disease severity, and correlated with HDL-C, TAG, VLDL-C, AST, ALT, ALP, lobular inflammation, hepatocellular ballooning, and NAFLD score. Serum and hepatic levels also correlated. Serum and hepatic miR34a levels were increased in NAFLD, and correlated with VLDL-C and TAG. Serum miR379 was also higher in NAFLD especially in early stages, while miR21 gave more ambiguous results. Their diagnostic properties were similar to those of existing biomarkers. However, serum miR122 levels appeared to be elevated before increases in ALT and AST are evident.

Conclusions: We postulate that miR122, miR34a, miR21 and miR132 may play a role in the development of NAFLD via a link in regulating cholesterol metabolism. Furthermore, miRs 122, 34a and 379 could be used as a panel of additional biomarkers in early detection of NAFLD.

ABSTRACT 2

A common variant in the ketohexokinase gene is associated with fructosuria and cardiometabolic outcomes

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BACKGROUND: There is an ongoing discussion on whether fructose per se is disadvantageous for cardiometabolic health. Recently, pharmacological inhibition of ketohexokinase (KHK), the first enzymatic step of fructolysis, was shown to reduce intrahepatic lipid content in humans. The study of individuals carrying functional variants in the KHK gene will provide more insight into the lifelong effects of inhibition of KHK, as these individuals have been exposed from birth to a KHK protein with less enzymatic activity. The aims of the present study were: 1) to study whether a common missense variant in KHK (rs2304681) is a functional variant that affects fructosuria (similar to pharmacological inhibition of KHK) and 2) to study the association between rs2304681 and cardiometabolic outcomes.

METHODS: First, linear regression analyses were performed to study the association between rs2304681 and 24h urinary fructose levels (quantified by tandem mass spectrometry), with adjustment for age, sex, and type 2 diabetes (T2D) in a population-based cohort (the Maastricht Study). Second, we used summary-level data on the association of rs2304681 with non-alcoholic fatty liver disease, T2D, hypertension, and myocardial infarction (from publicly available databases).

RESULTS: First, the rs2304681 minor A allele was associated with higher 24h urinary fructose levels (unstandardized beta: 0.064; 95%CI: 0.027-0.100; n=1,471). Second, the rs2304681 minor A allele protected from hepatic steatosis (OR: 0.972; 95% CI: 0.957-0.988; n=36,703; UK Biobank), T2D (OR: 0.985; 95%CI: 0.975-0.99; n=1,331,670; fixed-effects meta-analysis in the AGEN and the European DIAMANTE cohorts) and myocardial infarction (OR: 0.976; 95%CI: 0.961-0.992; n=583,191; fixed-effects meta-analysis in the CARIoGRAMplusC4D and the UK Biobank cohorts). Two studies both showed a protective effect on the risk of hypertension (OR: 0.988; 95%CI: 0.976-0.999; n=440,285; UK Biobank; and Z-score: -2.59; p=0.01; n=192,763; the combined CHD Exome+, ExomeBP, and GoT2D cohorts).

CONCLUSIONS: Lifelong impairment of KHK activity (reflected by rs2304681) is associated with fructosuria and protection from cardiometabolic disease. These findings suggest that fructose per se has harmful cardiometabolic effects, which may be mitigated by pharmacological inhibition of KHK.

ABSTRACT 3

Regulation of GDF-15 in obesity, type 2 diabetes and NAFLD

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Background: Growth differentiation factor 15 (GDF-15) is a cytokine from the transforming growth factor beta superfamily which is increased in blood of obese patients and positively associated with the development of type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD). GDF-15 was identified as a key regulator of food intake but it is still unclear how GDF-15 is regulated in obesity, T2D and NAFLD.

Methods: Mouse models of obesity, insulin resistance and NAFLD were used as well as human biopsies of adipose tissue (AT) and liver.

Results: Obesity increased plasmatic GDF-15 level and GDF15 expression in AT from high fat diet fed mice and obese patients. GDF15 expression in AT was positively associated with clinical parameters related to obesity and T2D. Classification of obese patients according to T2D status revealed that T2D patients displayed a higher GDF15 expression in AT. GDF15 expression in liver was not affected by obesity or T2D. However, a strong correlation was observed between GDF15 expression in liver and clinical parameters associated with liver function/disease. Classification of obese patients according to NAFLD spectrum revealed a significant upregulation of GDF15 expression in the liver from NASH patients and, to a lesser extent, from NAFL patients, suggesting that GDF15 expression in liver mainly follows the evolution of NAFLD rather than obesity or T2D. Supporting this finding, mice fed with CDAA diet developed a NASH-like disease dissociated from obesity and hyperglycemia and shown an increase of Gdf15 expression in liver, but not in AT, and plasma GDF-15 level. Mechanistically, macrophage infiltration regulated GDF-15 production in obesity and T2D while hepatocytes upregulated GDF15 expression in NAFLD.

Conclusions: AT is the main source of GDF-15 in obesity and T2D while NAFLD is an additional and independent factor driving a further GDF-15 production by liver. Both obesity/T2D and NAFLD regulates GDF-15 production following distinct and complementary mechanisms.

ABSTRACT 4

The role of the hepatokine fetuin B in glucose homeostasis and adipose tissue function

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Background: The liver plays a major role in maintaining whole-body glucose homeostasis, which is in part via the synthesis and secretion of proteins, i.e. hepatokines. The hepatokine secretion profile is altered in a fatty liver, and has been shown to cause muscle insulin resistance and inflammation. Fetuin B is identified as one of the steatosis-responsive hepatokines and has been shown to induce glucose intolerance in mice. The (1) tissue-specific gene and protein expression profile and (2) the underlying mechanism of action of fetuin B, however, are currently unknown.

Methods:(1) In male C57BL/6J mice, fetuin B gene expression and protein expression were measured in multiple organs. Fetuin B protein content was also measured 2 h after an intraperitoneal (IP) injection with fetuin B or bovine serum albumin control, as well as in high-fat diet-fed mice treated with adeno-associated virus containing a shRNA specific for fetuin B. (2) To investigate the link between white adipose tissue (WAT) fetuin B levels and indices of insulin sensitivity, mice underwent a hyperinsulinemic-euglycemic clamp 2 h after being treated with fetuin B. In 3T3-L1 adipocytes, RNA sequencing and PCR analysis was performed after 24 h incubation with or without fetuin B.

Results: In mice, fetuin B gene expression was high in liver, but nearly absent in other organs, including muscle, WAT, brown adipose tissue, and heart. In contrast, fetuin B protein expression was low in liver and muscle, but remarkably high in WAT (33.7-fold increase compared with liver). In mice, IP injection with fetuin B strongly increased fetuin B protein content in WAT (4.2-fold change compared with control, $p<0.05$). In the knockdown mouse model, fetuin B protein expression was reduced in plasma, liver, and heart, but most strongly reduced in WAT. Altogether, these findings suggest that fetuin B is produced and secreted by the liver, and taken up by WAT. To investigate whether fetuin B levels in WAT play a role in the regulation of glucose homeostasis, mice were treated with fetuin B and underwent a hyperinsulinemic-euglycemic clamp. There was a tendency towards a correlation between liver fetuin B levels and hepatic glucose output ($r^2=0.43$, $p=0.08$), but no correlation with glucose disposal rate (Rd) ($r^2=0.22$, $p=0.24$). WAT fetuin B levels also did not show a correlation with hepatic glucose output ($r^2=0.06$, $p=0.60$), but remarkably, there was a strong negative correlation between WAT fetuin B content and Rd ($r^2=0.95$, $p<0.001$). RNA sequencing and PCR analysis revealed that fetuin B treatment in 3T3-L1 adipocytes resulted in an increased inflammatory response.

Conclusions: The results of our experiments suggest that fetuin B may affect glucose homeostasis, at least partly, via changes in WAT.

ABSTRACT 5

Human hepatic in vitro models identify ANGPTL4, PDK4 and PLIN2 as potential pro-steatogenic mediators induced by elafibranor

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Background: Earlier, we showed the possibility of potency classification of promising peroxisome proliferator-activated receptor (PPAR)-agonists for anti-non-alcoholic steatohepatitis (NASH) treatment using human-derived hepatic in vitro disease models including primary human hepatocytes (PHH), human skin stem cell-derived hepatic progenitors (hSKP-HPC) and cancer cell lines (HepaRG, HepG2). Among those PPAR-agonists, elafibranor, followed by saroglitazar and pioglitazone showed the strongest anti-NASH potencies in vitro. Elafibranor, however, recently failed to show sufficient efficacy in a phase III clinical trial interim analysis and its development was discontinued. Therefore, we aimed to investigate possible molecular mechanisms that could lay at the basis of this failure.

Methods: PHH, hSKP-HPC and HepaRG cultures were exposed to NASH-inducing triggers (insulin, glucose, sodium oleate, palmitic acid, TNF-α, IL-1β and TGF-β) with or without elafibranor for 24h. Hereafter, whole-genome microarrays (Affymetrix) were performed and used for pathway analysis. Publicly-available transcriptomic data of clinical samples of NASH patients and patients with resolved NASH after bariatric surgery served as benchmarks.

Results: Hepatic 'NASH-induced' cultures showed up to 35% overlap with transcriptional signatures present in samples of NASH patients, indicating the relevance of the in vitro models. In all in vitro models, elafibranor restricted NASH-specific cellular processes through the activation and inhibition of processes also seen in pre- and post- bariatric surgery samples of NASH patients. PPARGC1A, PPARA and SIRT1 were identified as common upstream regulators in the three in vitro models exposed to elafibranor. Elafibranor induced upregulation of PLIN2, PDK4 and ANGPTL4, which have been earlier linked to liver steatosis, under regulation of the common upstream regulators. These pro-steatogenic mediators were, however, not increased in liver samples of patients with resolved NASH.

Conclusions: Modulations of ANGPTL4, PDK4 and PLIN2 deserve further attention in the preclinical investigation of PPAR-agonists for the treatment of NASH.

ABSTRACT 6

FAT10 in the senescence of hepatocytes and NASH development

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Background: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease, characterized by steatosis and inflammation, occurring with age and obesity. During NASH, inflammation and oxidative stress induce notably a premature senescence of hepatocytes, contributing to the worsening of the pathology, up to liver cancer (HCC). Senescence is normally a protective phenomenon, characterized by a cell proliferation arrest with a maintained metabolic activity, but the accumulation of senescent cells is deleterious. Our research team aims to identify the physiopathological mechanisms linking senescence to NASH.

Methods: A transcriptomic analysis was performed ex vivo on livers from NASH patients and in vivo on isolated primary hepatocytes from mice submitted to a NASH diet (choline deficient, high cholesterol, sucrose and fructose). A promising protein target connecting NASH and senescence was highlighted, so its association to senescence pathways was studied in vitro by a transcriptomic analysis on human HCC cells (HepG2) made senescent by a 10 gray irradiation, and the effect of its downregulation by a siRNA in this model was measured by: senescence-associated beta-galactosidase activity staining, cumulative population doubling, p21 protein expression in Western Blot, immunofluorescent co-staining with γH2AX.

Results: In NASH patients, a positive correlation is observed between senescence-related genes and the gene expression of FAT10, an ubiquitin-like protein, as well as a positive correlation between FAT10 expression and NASH severity. These positive correlations are confirmed in NASH mice in vivo, and are specific of hepatocytes. In vitro, FAT10 expression is increased in senescent HepG2, and correlates positively with the cell proliferation arrest and the DNA damage response pathways. But interestingly, the senescence parameters are increased by the downregulation of FAT10 expression.

Conclusions: This results suggest that FAT10 could limit the induction and spread of senescence in hepatocytes, which could act on NASH progression up to HCC.

ABSTRACT 7

Characterization of hepatic parenchymal hypoxia in a mouse model of nonalcoholic steatohepatitis

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Background: Knowledge of the pathogenesis of nonalcoholic steatohepatitis (NASH) and its progression is at present incomplete. We previously demonstrated the presence of increased intrahepatic vascular resistance and altered microvasculature in steatotic livers, both in humans and preclinical models. This could lead to reduced hepatic perfusion and consequently parenchymal ischaemia. We aimed at demonstrating that reduced oxygen levels are indeed present in NAFLD livers by direct measurement of hepatic parenchymal oxygen tension.

Methods: Male C57BL/6J mice (Janvier Labs, France) were fed the choline-deficient L-amino acid-defined high-fat diet (CDAHFD) or standard diet (SD) and subjected to hepatic parenchymal oxygen measurements (in mmHg) with a Clark-type microelectrode (OX-25 oxygen microsensor, Unisense, Denmark) prior to sacrifice after 1, 2, 3, 4 and 6 weeks on their respective diets (n=6 per timepoint). RNA was isolated from snap-frozen liver tissue to study expression levels of hypoxia-related genes with reverse transcription polymerase chain reaction (RT-PCR). Data are presented as means with standard error of the mean (SEM); means of groups were compared with Mann-Whitney test.

Results: CDAHFD induced steatosis after 1 week with the histological features of NASH after 2 weeks, compared to normal liver histology in SD group. Mean hepatic parenchymal oxygen pressures were non-significantly different in the first weeks, but became significantly lower in NASH compared to healthy livers after 6 weeks of dietary intervention (Fig. 1, $p<0.05$). Moreover, expression levels of hypoxia-related genes, i.e. hypoxia-inducible factor 1-alpha (HIF1α) and glucose transporter 1 (GLUT1), were higher in NAFLD livers compared to controls (Fig. 2A and 2B, respectively), being both significant at week 2 and 6.

Conclusions: The mean hepatic parenchymal oxygen levels were significantly lower in dietary induced NASH compared to healthy control livers. In accordance with this finding, the expression levels of two hypoxia-related genes were increased. Further research is warranted to elucidate the precise role of hepatic hypoxia and its downstream effects in the pathogenesis or progression of NAFLD.

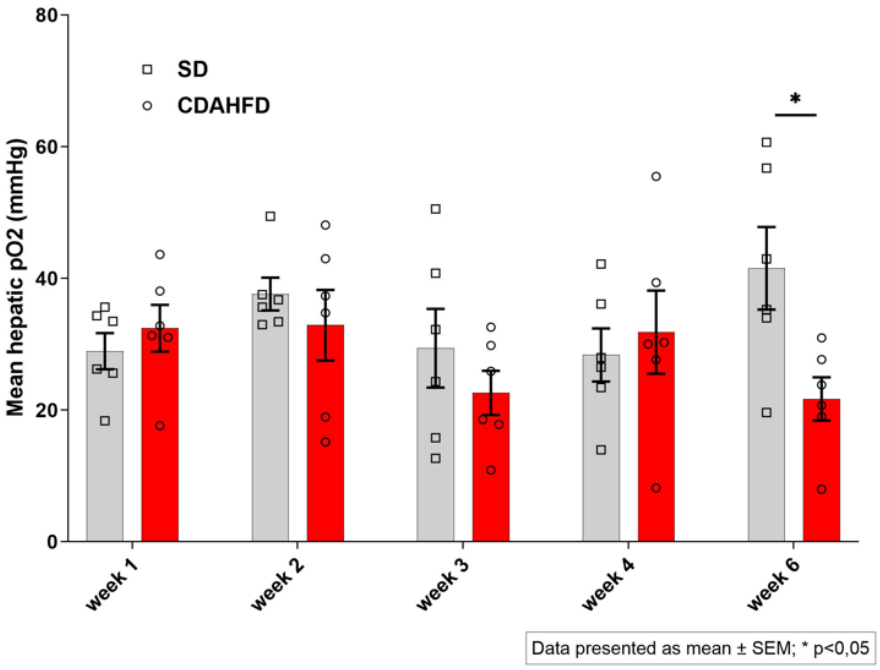

Figure 1: C57L/6J mice fed the choline-deficient L-amino acid-defined high-fat diet (CDAHFD) for six weeks display decreased hepatic oxygen pressures compared to standard diet (SD). Groups of male C57BL/6J mice on CDAHFD or SD were sacrificed after 1, 2, 3, 4 or 6 weeks. Prior to sacrifice hepatic parenchymal oxygen pressures (in mmHg) were measured with the Unisense® oxygen microsensor under general anesthesia with isoflurane. The mean hepatic oxygen level was calculated per subject (n=6/group).

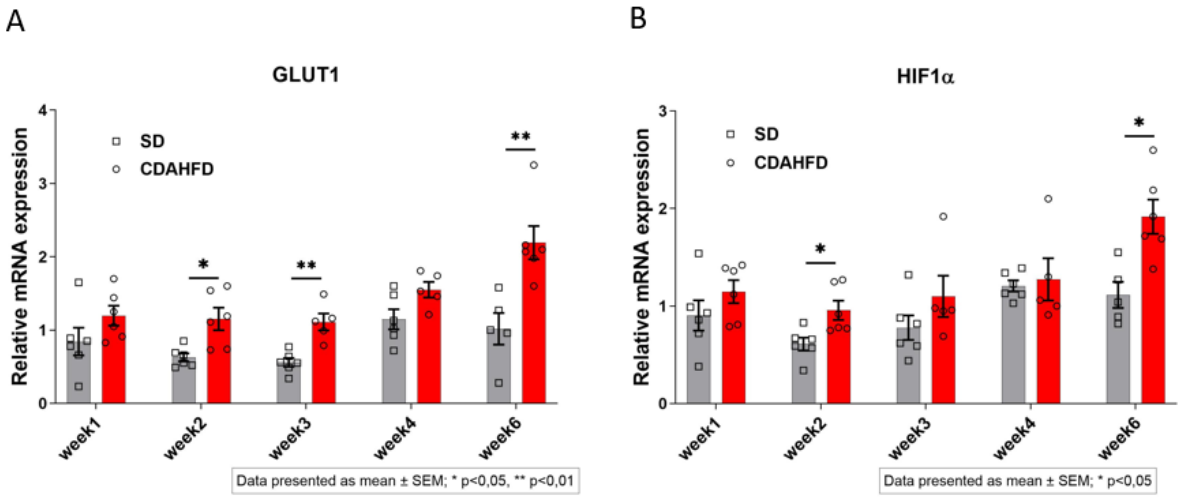

Figure 2: Upregulated expression of hypoxia-related genes in dietary-induced nonalcoholic steatohepatitis. (A) Relative RNA expression of glucose transporter-1 (GLUT1), a marker of chronic hypoxia, in liver tissue after 1, 2, 3, 4 and 6 weeks of CDAHFD, compared to SD group. (B) Increased hepatic relative RNA expression of hypoxia-inducible factor-1 alpha (HIF1α) after 2 and 6 weeks of CDAHFD compared to SD. Expression levels of target genes were normalized to the expression of housekeeping genes GAPDH and HPRT1 (M 0.60, CV 0.21). Data are presented as means ± standard error of mean (n=6/group). *p<0.05; **p<0.01. Mann-Whitney U tests.

ABSTRACT 8

Investigating cDC involvement in the pathogenesis of non-alcoholic fatty liver disease

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Background: The rise in obesity and insulin resistance and therefore the accumulation of triglycerides and free fatty acids in the liver has resulted in a growing epidemic of non-alcoholic fatty liver disease (NAFLD). NAFLD is a progressive disease, characterised by an initial, often asymptomatic build-up of fat in the liver, which can lead to the development of fibrosis, cirrhosis and liver cancer in the advanced stages of disease. While only a fraction of patients (~30%) progress through the entire disease spectrum, NAFLD is predicted to become the leading cause for liver transplantation in the West. Despite this, the mechanisms behind this progression remain unclear resulting in a lack of therapeutic strategies for these patients. Our understanding of hepatic DC function in NAFLD remains limited, as studies have been hampered by inconsistencies defining DC subsets and an inability to distinguish bona fide cDCs from monocytes and macrophages.

Methods: Harnessing a combination of single cell technologies including single cell transcriptomics and flow cytometry, we have been able to fully characterize hepatic DCs in both health and disease.

Results: We observed a significant increase in cDC1s and cDC2s as well as migratory CCR7+ cDCs in NAFLD, a finding conserved across distinct diet induced models. Despite obesity being a systemic disease, this expansion of cDC subsets was specific to the liver and liver draining lymph nodes. Interestingly, this increase in cDCs preceded an increase in hepatic cytotoxic T lymphocytes and other phenotypic changes in the T cell pool.

Conclusions: Utilising single cell technologies we identified significant changes in the cDC and T cell pool that was specific to the liver and liver draining lymph nodes. With these findings in mind, we are currently examining how cDCs regulate T cell fate and function in NAFLD including investigating the specific antigens involved. Finally, by manipulating the DC populations, we aim to examine the exact mechanisms by which cDCs contribute to NAFLD pathogenesis.

ABSTRACT 9

Changes of non-invasive tests for liver steatosis and fibrosis by dual PPAR-α/γ agonism in T2DM and coronary artery disease - Post-hoc analysis of the AleCardio RCT

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Background: Peroxisome proliferator-activated receptor (PPAR) agonists have been suggested to have favourable outcomes in patients NAFLD, and dual agonists of PPAR-α/γ have been suggested to reduce inflammation in patients with type 2 diabetes mellitus (T2DM). NAFLD has close bidirectional relations with T2DM, yet the effect of PPAR agonists on NAFLD has been understudied in clinical trials. This study serves as proof of concept of the PPAR agonist pathway to treat NAFLD/NASH by re-analyzing data from a large trial, to determine if a dual PPAR-α/γ agonist (aleglitazar) improves indirect markers for steatosis and fibrosis in over 7000 patients with T2DM and coronary artery disease.

Methods: a post-hoc analysis of a large randomized, double-blind, placebo-controlled, multi-centre trial including 7226 patients with T2DM and recent coronary syndrome (AleCardio trial). Eligible patients were randomized to receive aleglitazar or matching placebo added to standard medical care for two years. Main outcomes were changes from baseline of indicators of NAFLD: the Liver Fat Score (LFS), Liver Accumulation Product (LAP), Fibrosis-4 (FIB-4), and NAFLD Fibrosis Score (NFS).

Results: All indicators for steatosis and fibrosis (LFS, LAP, FIB-4, NFS) showed a steep decrease in the treatment group at 3-6 months, followed by a slow gradual increase over time. However, all indicators remained to be significantly lower in the treatment group throughout follow-up (24 months), whereas in the placebo group they remained the same or increased ($P < 0.001$). In the treatment group more participants showed improvement by shifting to a lower FIB-4 and NFS category compared to the placebo group (FIB-4: 22% vs. 11% and NFS: 12% vs. 8% for aleglitazar vs. placebo ($P<0.001$)).

Conclusions: This analysis in patients with T2DM and a recent coronary syndrome showed improvement of liver steatosis and fibrosis proxies after the start of dual PPAR-α/γ agonist treatment compared to placebo, adding evidence from a large trial that this pathway has potential for NAFLD/NASH treatment.

ABSTRACT 10

Effect of bile acids on CD1d-restricted antigen presentation to NKT cells

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Background: Bile acids (BA), synthetized in the liver from cholesterol facilitate intestinal lipid absorption and act as signalling molecules via their binding to receptors such as TGR5, FXR and VDR. These regulate lipid, glucose and BA metabolism, which are dyregulated in pathological contexts such as obesity, insulin resistance and T2D, and represent risk factors for Non Alcoholic Steato-Hepatitis (NASH) development characterized by a strong immunoinflammation. Inflammatory pathways are also upregulated in cholestatic diseases where intrahepatic BA accumulation results in progressive hepatotoxicity. Mouse but not human hepatocytes express the CYP2C70 enzyme converting the Chenodeoxycholic acid (CDCA) into α,β-muricholic acids (MCA) impairing the translation of experimental results to human pathology. Hence we used Cyp2c70-deficient(-/-) mouse, which exhibit a "humanized" BA profile and cholestasis evolving to fibrosis to characterize the hepatic immune compartment.

Methods: Cyp2c70-/- and Wild-Type (WT) littermates liver immune cells were analyzed by flow cytometry at basal state. Cytokine production by Kupffer Cells (KC) and Natural Killer T cells (NKT) was analysed upon in vitro stimulation. Comparative bulk transcriptome of sorted NKT was performed by RNAseq.

Results: Both myeloid and lymphoid lineages were altered in Cyp2c70-/- livers. Compared to their WT counterparts, Cyp2c70-/- KC express lower levels of CD1d, involved in glycolipid antigen presentation, secrete more IL-1β and less TNFα. NKT, activated in response to lipid antigen presentation by CD1d, are two-fold less abundant and produce significantly more IL-17A and IFNγ upon stimulation. In line, transcriptomic analysis confirmed a basal pro-inflammatory phenotype.

Conclusions: These results suggest that mouse or humanized BA profile differentially affects immune compartment. Understanding the underlying molecular mechanisms is paramount for translating the results from BA-related studies performed in mouse models to a human context.

ABSTRACT 11

Resmetirom reduces lipid load, restores THRB expression and prevents cell damage in a human stem cell based in vitro MAFLD model

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Background: Metabolic dysfunction-associated fatty liver disease (MAFLD) distinguishes metabolic-, gene-tic- and environmental disease drivers. In this study, we aim to develop an in vitro model based on human skin-derived precursors (hSKPs) differentiated to hepatic cells (hSKP-HPC) for environmentally driven MAFLD. This model will be used to assess the efficacy of the thyroid hormone receptor beta (THRB)-selective agonist resmetirom (MGL-3196, Madrigal Pharmaceuticals).

Methods: hSKP-HPCs are sequentially exposed for 24h to MAFLD-inducing triggers (fatty acids, fructose and ethanol) followed by an additional 24h-exposure to lipopolysaccharide (LPS) and resmetirom. Then, the cells are fixed with paraformaldehyde and stained with DAPI (nuclei) and BODIPY™ (neutral lipids) for fluorescence microscopy quantification of lipids using ImageJ. Gene expression analysis using RT-qPCR is performed for APOB, CD36, FASN, THRB, SCD and SREBF1. Intracellular ATP levels are measured using the CellTiter Glo® Luminescent Cell Viability Assay.

Results: After 24h, hSKP-HPCs significantly accumulate intracellular lipids. Subsequent exposure to LPS potentiates this effect, along with reduced intracellular ATP content and expression of THRB, which is in line with clinical data. Expression of APOB potently decreases after 48h, indicating diminished lipid export by very low-density lipoproteins (VLDL). Expression of CD36 (fatty acid import) and SREBF1 and its downstream targets SCD and FASN (de novo lipogenesis) also decreases in this condition. Exposure to resmetirom reduces the increased lipid load and restores THRB expression as well as ATP levels back to baseline. Resmetirom also induces the expression of CD36, FASN, SCD and SREBF1, suggesting increased energy turnover by thyroid signaling. In addition, resmetirom potently induces APOB expression, indicating VLDL-mediated triglyceride clearance.

Conclusions: hSKP-HPCs triggered with environmental MAFLD factors exhibit key MAFLD characteristics, including increased lipid load, reduced ATP levels and reduced THRB expression. Resmetirom reduces the lipid load and restores ATP and THRB levels. Therefore, hSKP-HPCs could represent a promising tool for the development of thyromimetic anti-MAFLD drugs.

ABSTRACT 12

Run for your live(r): Exercise training at different times of day differentially modulates hepatic inflammation in early NAFLD

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Background: Exercise effectively prevents obesity-related disorders, but it is unclear whether the beneficial health effects of exercise are restricted to unique circadian windows. We previously showed that late, but not early, exercise training over four weeks reduces atherosclerosis (-40%) and body fat mass (+0.43 g with early vs. -0.49 g with late training) in mice, suggesting a greater improvement of hyperlipidemic and inflammatory diseases with late training. Therefore, we now aimed to study whether timing of exercise training also differentially modulates NAFLD development and progression.

Methods: We endurance-trained high fat-high cholesterol fed NAFLD-prone male APOE*3-Leiden.CETP mice for eight weeks (5x per week, 1 hour) either in the early (ZT13) or in the late (ZT22) active phase and assessed the NAFLD score (histology) and hepatic inflammation (FACS) compared to sedentary mice.

Results: Exercise training prevented an increase in body fat mass (+1.13 g with early, +1.06 g with late vs. +3.67 g with no training) and fasting plasma glucose (-0.7 mM with early, -0.8 mM with late training) as expected, but neither early nor late training affected liver triglyceride or cholesterol content compared to sedentary mice, likely due to a very early stage of hepatic steatosis. In line, hepatic expression of de novo lipogenesis genes (e.g., Fasn, Srebp1c) was similarly downregulated by early and late training. However, exercise had a distinct time-dependent effect on hepatic inflammation, as only early training promoted an influx of neutrophils and monocytes into the liver paired with increased expression of the pro-inflammatory cytokines (e.g. Tnfa, Il1b).

Conclusions: Timing of exercise is a critical factor for the positive effect in obesity and cardiometabolic disease management. We currently investigate the effect of timed training on advanced NAFLD.

ABSTRACT 13

NAFLD and cardiometabolic health: Importance of visceral obesity and cardiorespiratory fitness

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Background: Non-alcoholic fatty liver disease (NAFLD) is a common liver disease worldwide and is predicted to become a leading cause of liver transplantation in the coming years. Individuals with NAFLD are also at increased risk of cardiovascular disease (CVD) because of its link with features of the metabolic syndrome, type 2 diabetes and hypertension. However, evidence from large cardiometabolic imaging studies suggest that NAFLD may be closely associated with a specific adiposity phenotype: visceral obesity.

Methods: We expand on the relationship between visceral obesity and liver fat by presenting 1-studies using either computed tomography or magnetic resonance imaging exploring the relationships between body fat distribution, liver fat and cardiometabolic outcomes, and 2-lifestyle intervention studies quantifying associations between physical activity/cardiorespiratory fitness and liver fat.

Results: Our imaging studies have shown that individuals who do not have the ability to store excess energy in subcutaneous adipose tissue are characterized by a selective deposition in visceral adipose tissue (VAT). Excess VAT is also often accompanied by an unwanted accumulation of lipids in the heart, liver and skeletal muscle, a phenomenon described as ectopic fat deposition. Subcutaneous obesity, in the absence of excess visceral adiposity, is not associated with NAFLD. Whereas imaging studies have shown that both excess visceral adiposity and liver fat are independently related to type 2 diabetes, excess liver fat does not seem to predict CVD after control for visceral adiposity; therefore, the specific contribution of liver fat to CVD risk remains uncertain. These studies also reveal that the prevalence of the adiposity phenotype characterized by excessive liver fat accumulation in the absence of visceral obesity is quite low. Lifestyle intervention studies using endurance exercise have been shown to reduce visceral adiposity and liver fat, even in the absence of major weight loss. At any BMI value, individuals with high levels of cardiorespiratory fitness are characterized by low levels of both visceral adiposity and liver fat compared to poorly fit subjects.

Conclusion: These results highlight the importance of targeting excess visceral adiposity and low cardiorespiratory fitness to manage individuals with NAFLD.

ABSTRACT 14

The Fibrosis-4 cut-off value for significant fibrosis is dependent on the type of non-alcoholic fatty liver disease patients

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Background: Non-alcoholic fatty liver disease (NAFLD) has become the most frequent cause of chronic liver disease. The fibrosis stage has been identified as the most important predictor of prognosis. The Fibrosis-4 (FIB-4) is an easy-to-use non-invasive score to predict fibrosis based on routine blood parameters. However, we previously showed that the 1.3 FIB-4 cut-off value had a low performance for detecting significant fibrosis (≥F2) in a primary care (PC) population. We aimed to determine whether NAFLD risk-group-specific optimal FIB-4 cut-off values can be determined to identify patients with a high likelihood of ≥F2, considered an at-risk population to be referred for further management.

Methods: In a prospective study in the Belgian regions of Limburg, East-Flanders, and Antwerp, patients were screened in seven PC practices. Type 2 diabetes mellitus patients (T2DM) were screened in the hospital of Ziekenhuis Oost-Limburg. As a proxy of the fibrosis stage, liver stiffness was determined using Vibration Controlled Transient Elastography (VCTE) by FibroScan® (Echosens, France) and was used as the reference method. The FIB-4 was calculated based on recent laboratory data from the electronic patient file. The Youden Index (J) was used to determine the optimal cut-off value for detecting ≥F2 (>7.9kPa for the M-probe and >7.2kPa for the XL-probe) in three populations: PC (unselected all-comers), T2DM screened in PC (T2DM-PC, subgroup of the PC group), T2DM screened in a hospital (T2DM-H).

Results: Of the 816 (424 (52.0%) PC, 103 (12.6%) T2DM PC, and 289 (35.4%) T2DM-H) screened patients, 534 (65.4%) had FIB-4 values and no other causes of steatosis but NAFLD. Of these, 275 (51.5%) were male, median age was 63 (26-71) years, median BMI 29.8 (26.2-33.46) kg/m2, waist circumference 104.0 (97.0-113.0) cm for men and 98.0 (86.1-110.0) cm for women, and 276 (51.9%) had T2DM. The number of patients with ≥F2 was 30 (11.8%), 20 (30.3%), and 96 (45.7%) for the PC, T2DM-PC, and T2DM-H cohorts. Using a logistic regression model, both FIB-4 ($p<0.05$) and the type of NAFLD-group significantly ($p<0.05$) contributed to ≥F2. The area under the operating curve (AUROC) of the model was 0.76 compared to an AUROC of 0.61 using only FIB-4. The maximum J was 0.40461 and corresponded to a sensitivity of 0.61 and specificity of 0.80. Applying the linear predictor, the optimal cut-off value for the different populations was 4.3 for the PC cohort, 2.3 for the T2DM-PC, and 0.66 for the T2DM-H cohort. The optimal cut-off value was not influenced by age or by the grade of steatosis as measured by controlled attenuation parameter (CAP).

Conclusions: The optimal cut-off value of the FIB-4 score for detecting NAFLD-associated significant fibrosis depends on the patient groups' characteristics, potentially resulting from the different prevalence of the condition in the respective risk groups. However, the cut-off values were not influenced by age or the severity of the steatosis.

ABSTRACT 15

Involvement of the alteration of the biological clock in the development of non-alcoholic fatty liver disease (NAFLD)

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Background: Steatohepatitis (NASH), which is charaterized by steatosis, inflammation and hepatic fibrosis, is a pathology whose prevalence has considerably increased. A western diet is commonly accepted as a risk factor. In addition, there is an association between disruption of the biological clock and the risk of developping metabolic diseases, such as diabetes and dyslipidemia. However, the underlying molecular mechanisms remain poorly understood.

Methods: The objective of this work is to study the impact of the alteration of the biological clock on the risk of developing NAFLD. To do this, C57BL/6J mice underwent chronic desynchronization of the day / night cycles over different periods of time and received either a diet rich in fat, sugar and cholesterol or a standard rodent chow.

Results: Our study shows that these two interventions were accompanied by liver damage with elevated ALT and AST levels and exacerbated fibrosis. In addition, a remodeling of hepatic immune populations in response to an alteration in the biological clock and / or a high calorie diet has been observed.

Conclusion : Clock alteration aggravates diet-induced NASH in mice. The modulation of the biological clock in shift workers might be an interesting target to fight against the development of metabolic diseases.

ABSTRACT 16

Deep phenotyping of high intensity interval training in patients with advanced stages of NAFLD

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Background: Although lifestyle modification is a cornerstone in the treatment for non-alcoholic fatty liver disease (NAFLD), the therapeutic effect of exercise on non-alcoholic steatohepatitis (NASH) and liver fibrosis has not been established. We performed deep multi-omic phenotyping of liver, muscle and fat tissue of patients with NAFLD/NASH upon exercise. Since NASH, physical fitness and gut microbiome composition may be interrelated, we also analyzed the gut microbial composition of the patients.

Methods: Fifteen patients with histologically characterized NAFLD participated in a 12-week personalized high intensity interval training (HIIT) program combined with home-based training following international guidelines. At baseline and upon completion of the training, NAFLD was assessed by histological readings as well as by multiparametric magnetic resonance imaging (MRI), completed with RNAseq of liver samples. Muscle and adipose tissue RNAseq, fecal shotgun metagenomics and plasma metabolite analysis were performed before and after the 12-week period.

Results: The 12-week exercise period significantly increased VO2max by 10.1%, underscoring the effect and compliance. We did not observe changes in liver histology since scoring for steatosis (Z=-1.0, p=0.32), inflammation (Z=-1.4, p=0.16), ballooning (Z=-0.45, p=0.66), NAS (Z=0.33, p=0.74) and fibrosis (Z=0.45, p=0.66) were not affected by the exercise. In line, MRI-determined hepatic fat content did not change (-0.88 %, 95% CI: -2.31, 4.07, df=12, p=0.14) while MRI-determined visceral fat volume decreased significantly by -11.5% without a change in total body weight. Analyses of the RNAseq data of liver, muscle and adipose tissue as well as gut microbiota and plasma metabolites are expected to be completed in Q3 2022.

Conclusions: HIIT improved cardiorespiratory fitness and decreased visceral fat in patients with NAFLD. Despite these metabolic improvements, our 12-week intervention period did not induce any reduction of NAFLD itself. This warrants larger trials with longer interventions to more firmly establish the place of treatment programs for NAFLD. Patterns of tissue crosstalk and gut microbiota are now being studied.

ABSTRACT 17

Liraglutide reduces hepatosteatosis in NAFLD and drug-induced fatty liver cell culture models through PPARγ signaling pathway

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Background: With the increasing prevalence of obesity, fatty liver injury, and drug use, appropriate treatment of non-alcoholic (NAFLD) and drug-induced fatty liver disease (DIFLD) is becoming more important. Numerous studies have proposed and tested a number of potential hepatoprotective agents. The aim of this study was to establish reliable cell culture models for NAFLD and DIFLD and to determine the effect of the GLP-1 agonist liraglutide in these models.

Methods: Huh7 cells were grown overnight to reach 90% confluency, followed by 24h of incubation according to further described protocols. Experiments were performed in triplicates, with the following cell subgroups: Huh7 cells incubated in medium only as a negative control, cells incubated with increasing concentrations of liraglutide (5nM, 10nM, 20nM), cells treated with 0,5 mM oleic acid (OA) (NAFLD model) , and/or cotreated with liraglutide, and cells treated with 20μM amiodarone (DIFLD model), and/or cotreated with liraglutide. The antiproliferative and cytotoxic effects were determined using a colorimetric MTT assay. Cells were stained with Oil-Red-O dye and Fluorescent mounting medium with DAPI, and visualized under a microscope. ImageJ software was used to count the cell nuclei and measure the integrated density relative to the cell count. After extraction of total RNA with NucleoZol, cDNA synthesis, PCR method and gel electrophoresis were performed, and finally the signals were analyzed by ImageJ software.

Results: Both oleic acid and amiodarone reduced cell viability by 30% ($p<0,001$, $p<0,05$) compared to the control. Cells cotreated with lower concentrations of liraglutide (5 and 10nM) showed a slight increase in viability ($p<0,05$), while higher concentration had an opposite effect. Both amiodarone and oleic acid significantly increased integrated density ($p<0,001$), while 5nM liraglutide decreased it in cells cotreated with amiodarone ($p<0,05$). PPARγ gene expression was decreased in both oleic acid and amiodarone treated cells, and 10nM liraglutide exerted greatest increase in PPARγ gene expression.

Conclusions: Hepatoprotective effect of liraglutide in both models explored in this study is mediated through PPARγ signaling pathway.

ABSTRACT 18

Evaluation of liver fibrosis using the Fibroscan™ in the bariatric workup: recommendations for clinical practice

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Background: The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing with the prevalence of obesity. According to new research, 75% of patients who have liver fibrosis before bariatric surgery still have it afterward. While liver biopsy remains the gold standard for NAFLD, the use of Fibroscan™ could improve the follow-up in this high-risk patient group. However, accepted criteria for Fibroscan measurements in these subjects with (severe) obesity are lacking.

Methods: A literature search using PubMed (MEDLINE) database including the terms “Fibroscan”, “bariatric surgery”, “transient elastography” and “obesity” was carried out. We focused on the recent reviews (<5 years) or studies published in English regarding adult humans with obesity and excluding papers about patients infected with HIV, HCV or B virus (HBV). We also focused on papers using an XL probe.

Results: European guidelines recommend to use the following cut off values for fibrosis: 7.2 kPa and 7.9 kPa for F3 and F4 respectively when using a XL probe. We identified 9 different studies which evaluated liver fibrosis with an XL probe in patients referred for bariatric surgery. Based on these studies, we propose to identify fibrosis stages as follows:

F0	F1	F2	F3	F4
<6,9 kPa	6,9 – 7,94 kPa	7,95 - 8,94 kPa	8,94 – 14,24 kPa	≥ 14,25 kPa

Conclusions: The cut-off values proposed in this paper differ from those mentioned by the European guidelines. Although these values are being supported by the literature, the number of included patients is still low, therefore larger studies with histology are necessary.

ABSTRACT 19

Relationship between non-alcoholic fatty liver disease and coronary artery disease: A Mendelian randomization study

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Background: There is an ongoing debate on whether non-alcoholic fatty liver disease (NAFLD) is an active contributor or an innocent bystander in the pathogenesis of coronary artery disease (CAD). The aim of the present study was to assess the causal relationship between NAFLD and CAD.

Methods: We performed two-sample Mendelian randomization (MR) analyses using summary-level data to assess the association between genetically predicted NAFLD (i.e. chronically-elevated serum alanine aminotransferase levels [cALT], imaging-based and biopsy-confirmed NAFLD) and risk of CAD. NAFLD is characterized by increased VLDL secretion, so analyses were repeated after exclusion of NAFLD susceptibility genes that are associated with impaired VLDL secretion.

Results: Inverse-variance weighted (IVW) MR analyses showed a statistically significant association between genetically predicted cALT and risk of CAD (odds ratio [OR]:1.116, 95% confidence interval [CI]:1.039,1.199), but not for the other NAFLD-related traits (OR:1.046, 95%CI:0.764,1.433 and OR:1.014, 95%CI:0.968,1.062 for imaging-based and biopsy-confirmed NAFLD, respectively). MR Egger regression revealed a statistically significant intercept, indicative of directional pleiotropy, for all traits. Repeat analyses after exclusion of genes associated with impaired VLDL secretion, showed consistent associations between genetically predicted NAFLD and CAD for all traits, i.e. cALT (OR:1.203, 95%CI:1.113,1.300), imaging-based (OR:2.149, 95%CI:1.276,3.620) and biopsy-confirmed NAFLD (OR:1.113, 95%CI:1.041,1.189), which persisted when more stringent biopsy-confirmed NAFLD criteria were used (OR:1.154, 95%CI:1.043,1.278) or when more stringent MR methods were applied. MR Egger regression did not show a statistically significant intercept.

Conclusions: The two-sample MR analyses showed a robust association between genetically predicted NAFLD and CAD after exclusion of genetic variants that are implicated in impaired VLDL secretion.

ABSTRACT 20

Getting GRIP on NASH: Implementation of an International Transmural Screening Program

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Background: Fatty liver disease is considered one of the major challenges in contemporary clinical practice. Although the prevalence is as high as 25% of the world's population, with serious complications leading to the development of hepatic inflammation and fibrosis developing into NASH and ultimately cirrhosis, liver failure and hepatocellular carcinoma, the identification of these patients does not appear to be a priority among clinicians and policy makers. One of the reasons is the lack of knowledge about diagnosing and treating patients with fatty liver disease. In addition, these patients often have co-morbidities such as dyslipidemia, arterial hypertension, diabetes and obesity, which professionals tend to prioritize in their limited consulting time.

Methods: The current study will assist primary care physicians and clinicians to implement the patient care pathway as proposed by the EASL. Within 'GRIP on NASH' we aim to screen 10.000 high risk patients with type 2 diabetes mellitus, metabolic syndrome, obesity or arterial hypertension in 10 different European countries for the presence of fatty liver and fibrosis using 2 non-invasive measurements (FIB-4 and FibroScan®). Blood samples will be collected from all screened individuals. Liver biopsy material will be collected in F2-patients or higher following a predefined protocol.

(Expected) results: Prevalence estimates of NAFLD and NASH in European countries in patients at risk coming from primary care. In addition, genomic, metabolomic and lipidomic studies will be applied to gain a better understanding of the pathophysiology of NAFLD and NASH and to identify markers that will help to detect patients at risk.

Conclusions: Through the GRIP on NASH program we will promote awareness and implementation of NAFLD/NASH screening among primary care physicians and clinicians thereby improving clinical care.

ABSTRACT 21

Reduced handgrip strength is correlated with a higher FLI in NAFLD patients with type 2 diabetes and obesity

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Background: Non-alcoholic fatty liver disease (NAFLD) has become the most frequent cause of chronic liver disease. It is highly prevalent in type 2 diabetes mellitus (T2DM) and obesity. Insulin resistance is a major pathogenic driver affecting muscle metabolism leading to myosteatosis, with a potential impact on muscle function. Asian populations showed an inverse association between handgrip strength (HGS) and NAFLD prevalence. The evidence in Caucasian people is not clearly established. Therefore, we aimed to determine whether a decreased HGS is correlated with liver fibrosis or steatosis.

Method: In a prospective study in Belgium and The Netherlands, patients were screened in seven GP practices. Part of the T2DM were screened in the hospital Ziekenhuis Oost-Limburg (Genk, Belgium). The fibrosis and steatosis stages were determined using respectively Vibration Controlled Transient Elastography (VCTE) and Controlled Attenuation Parameter (CAP) by FibroScan® (Echosens, France). Cut-off values from the Belgian Association for the Study of the Liver (BASL) were used. Based on the most recent laboratory data from the electronic patient file and blood samples taken during the study visits, the following non-invasive scores were calculated: FIB-4, NFS, FAST score and the FLI. The HGS was measured with the analog JAMAR hydraulic hand dynamometer (SEHAN, Korea). Three measurements were taken for each patient for the dominant hand, the average strength (kg) was calculated. Based on the normal values accompanied by the device, a patient was rated as having a low, average, or high HGS.

Results: Of the 291 patients who were screened and had a HGS measurement, 53 (18.2%) were excluded due to incomplete HGS, VCTE IQR/MED >30%, or secondary causes of steatosis. Of the 238 included patients, 106 (44.4%) were male, the median age was 61 (54-68) years, the median BMI was 28.2 (24.2-31.7) kg/m², the median waist circumference was 100 (93-109.3) cm for men and 91.5 (80-104) cm for women, and 72 (30.1%) had T2DM. The median VCTE was 5.1 (3.9-7.1) kPa, and the median CAP was 265 (220-320.5) dB/m. No significant correlation was found between HGS of the dominant hand and VCTE, CAP, or any of the scores measured in the entire group. In 50 (21%) NAFLD (CAP>215 dB/m) patients with both T2DM and obesity, a significant inverse correlation ($p<0.05$) was found between HGS of the dominant hand and the FLI (-0.497). This association was not seen in the T2DM or obesity group alone.

Conclusion: There was no correlation between the HGS and liver fibrosis or steatosis as measured by VCTE or CAP in the total study population or subgroups (e.g., T2DM and obesity). However, we did find an inverse relationship between the FLI and HGS among diabetic and obese NAFLD patients. Future research is required to further clarify this correlation.

ABSTRACT 22

Hypoxia marker pimonidazole displays panlobular positivity in nonalcoholic steatohepatitis

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Background: The distribution of epitopes in different zones of the liver lobule can be studied with immunohistochemistry (IHC), but the latter is difficult to quantify. In nonalcoholic fatty liver disease (NAFLD), lipid vacuoles further hamper correct interpretation. We quantified IHC staining of hypoxia marker pimonidazole (Pimo) in different zones of the liver lobule of steatotic and normal livers.

Methods: After three weeks of choline-deficient L-amino acid-defined high-fat diet (CDAHFD) or standard diet (SD), C57BL/6J mice received an intraperitoneal injection of Pimo (100mg/kg) one hour prior to sacrifice (n=6). Serial sections of formalin-fixed paraffin-embedded liver tissue were stained for haematoxylin-eosin (H&E) and IHC for glutamine synthetase (GS) and Pimo adducts visualized with 3,3'-diaminobenzidine (DAB). Whole liver slides were digitalized with the Zeiss Axioscan Z1. A script for FIJI open-source image analysis was developed to measure Pimo positive area (based on autothreshold algorithm 'Percentile') with regards to the relative location within a liver lobule. Data were processed in R Studio using the additional package "dplyr".

Results: In normal livers in SD group Pimo positivity was present in the centrobular area, whereas CDAHFD induced nonalcoholic steatohepatitis (NASH) with panlobular hypoxia (Fig. 1A). Liver lobules follow the Voronoi principle wherein each lobule originates from a central vein identified by H&E and centrolobular GS staining (Fig. 1B). Central veins were manually annotated and the image analysis script calculated the Pimo positive area in different zones of the liver lobule. The percentage of Pimo positive area was significantly higher in the periportal zone in NASH livers compared to control livers ($p < 0.05$, Fig. 2) after exclusion of pixels belonging to lipid vacuoles.

Conclusions: By means of an in-house developed script we accurately quantified the panlobular spread of hypoxia in NASH vs normal livers. Owing to its intuitive design, this method should allow for objective zonal quantification of other IHC markers in livers affected by different pathologies as well.

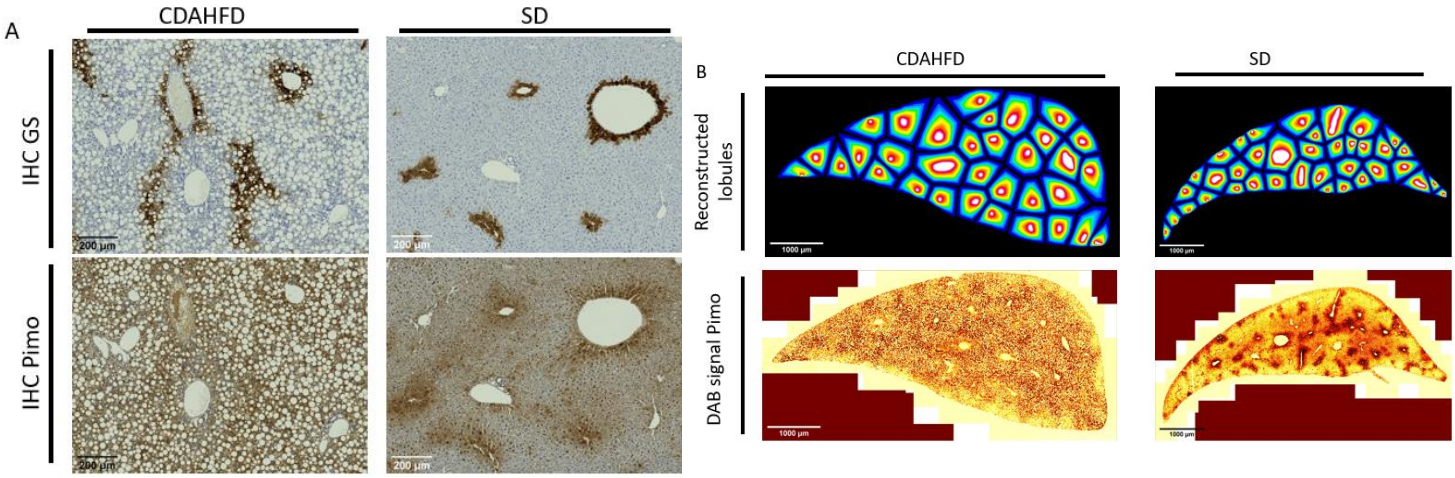

Figure 1: Representative pictures of immunohistochemistry (IHC) and reconstructed liver lobules in C57BL/6J mice fed the choline-deficient L-amino acid-defined high-fat diet (CDAHFD) or standard diet (control) for three weeks. (A) Representative images of IHC for glutamine synthetase (GS) and pimonidazole adducts (Pimo). The enzyme GS is expressed in centrolobular hepatocytes (upper panel). Pimonidazole is present around centrolobular veins in controls but displays panlobular distribution in CDAHFD (lower panel). (B) Representative images of reconstructed lobules in whole slide liver images with centrolobular veins displayed in white (upper panel). Next, the deconvoluted DAB signal for Pimo adducts, presented with the pseudocolor image 'Red Hot' (lower panel).

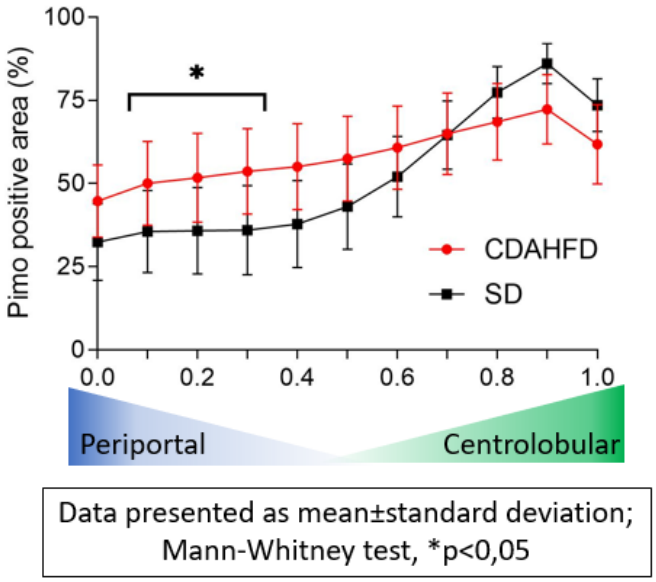

Figure 2: Zonated quantification of Pimonidazole immunohistochemistry in a mouse model of nonalcoholic steatohepatitis (NASH) vs. normal livers. *The percentage of Pimo positive area is calculated at eleven points of relative distance from centre to edge within each lobule on the x-axis. Lipid vacuoles were excluded from analysis. The graph represents the trend in Pimo IHC along the sinusoid in over 700 liver lobules from NASH vs controls (n=6). Two whole liver slides of Pimo IHC were generated per subject. Data presented as mean ± standard deviation. Mann-Whitney U test; *p<0.05. Scale bar represents 200µm or 1000µm.*

ABSTRACT 23

Metabolic-associated fatty liver disease in Turkish individuals living in and outside of Turkey: a shared burden at different locations?

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Background: With a prevalence of 25%, MAFLD is the leading liver disease worldwide. Highest rates are found in the Middle East of which Turkey has one of the highest with 48-60%. Research on genetic susceptibility, lifestyle and environment of its people could give insight on this public health problem. A comparison between the habitual population and the diaspora spread over the globe will be helpful to manifest shared factors, which is why we aim to comment on the similarities and differences to identify future perspectives for disease management of adult patients with MAFLD.

Methods: A systematic literature search using the electronic database PubMed was performed to identify articles based on obesity, diabetes mellitus and metabolic syndrome associated with MAFLD among Turkish individuals living in Turkey and Turkish migrants in Europe.

Results: Obesity rates between Turkey and its subgroup outside Turkey are similar to each other. When compared to native Europeans, the Turkish population across all countries scores significantly higher rates in obesity. Women are generally more susceptible to obesity, especially with increasing age. Furthermore, women and men alike live a more sedentary lifestyle when compared to their European counterparts. Metabolic syndrome seems to be similar in- and outside Turkey. Disturbed serum lipid levels and hypertension were significantly more often present within the population of Turkish descent when compared to European natives. T2DM and its risk is higher in the population of Turkish descent in comparison to their hosts however similar to Turkey.

Conclusions: The prevalence of obesity, the metabolic syndrome and T2DM within the Turkish population and its subpopulation outside Turkey is similar to each other, but significantly higher when compared to their European counterparts. Genetics, lifestyle and culture are the shared factors within the Turkish population in- and outside Turkey, being the possible underlying reason for these results. However more research as well as more awareness and education is needed on these factors in order to decrease the health burden and maximize health of individuals of this specific population.

ABSTRACT 24

The progression of NASH to hepatocellular carcinoma is influenced by extracellular cathepsins

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Background: Non-alcoholic steatohepatitis (NASH) is an increasingly important trigger for the development of hepatocellular carcinoma (HCC). Due to its limited therapeutic options, NASH-induced HCC (NASH-HCC) is among the most lethal cancers. Ample data points towards the involvement of cathepsins, lysosomal proteases, in many metabolic diseases, including many types of cancers. We previously determined that cathepsin D (CTSD), is increased in plasma of NASH patients and can serve as an early and sensitive marker for this condition. Furthermore, we have demonstrated that increased plasma (extracellular) CTSD contributes to metabolic dysregulation and low-grade chronic inflammation. However, information regarding the role of cathepsins in NASH-induced HCC (NASH-HCC) is lacking. In the current study, we investigate the role of these extracellular cathepsins in NASH-HCC.

Methods: Plasma CTSD levels were determined in healthy controls (N = 87), NASH (N = 63) and HCC (N = 15) patients. In addition, plasma cathepsin S (CTSS) levels were measured in healthy controls (N = 20), NASH (N = 20) and HCC (N = 16) patients. Furthermore, the effect of cathepsin B (CTSB) inhibition on cell viability and migration was studied in a set of cancer cells in vitro. Lastly, the effect of dietary and hormonal factors was determined on the CTSD activity in vitro and in (HCC) ASV-B mice.

Results: Plasma levels of CTSD and CTSS were significantly increased in HCC patients compared to healthy controls and to NASH patients. As expected, extracellular CTSB inhibition in cancer cells, led to a significantly lower viability and migration compared to intracellular CTSB inhibition and the control. In line with their function in metabolism, cortisol and glucose increased the activity of CTSD, whereas melatonin decreased the CTSD activity.

Conclusion: Our data demonstrate that dietary and hormonal factors affect NASH-HCC progression through extracellular cathepsins.

ABSTRACT 25

Pneumococcal immunization against oxLDL decreases tumor burden in NASH-derived hepatocellular carcinoma

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Background: Perturbed lipid metabolism, as observed in non-alcoholic steatohepatitis (NASH), has been linked to the development of hepatocellular carcinoma (HCC). In particular, increased production of oxidized low-density lipoprotein (oxLDL) has been associated with metabolic disturbances and inflammation. Previously, we have demonstrated that immunization with heat-inactivated pneumococci increased the production of anti-oxLDL antibodies, due to molecular mimicry with oxLDL, thereby leading to reduced hepatic inflammation in NASH. However, the effect of anti-oxLDL immunization on the occurrence and progression of NASH to HCC, is currently unexplored.

Methods: In this study, we used a non-alcoholic steatohepatitis (NASH) induced HCC mouse model, in which neonatal male mice were exposed to a low dose streptozotocin (STZ), followed by a high fat diet (HFD) after which all mice developed HCC. To test the effect of immunization against oxLDL on HCC occurrence and progression, mice (n=20) were split into 2 groups either receiving a subcutaneous injection with heat-inactivated *Streptococcus pneumonia* (108 CFU) or control-injection (0,9% NaCl). Plasma anti-oxLDL titers were measured and tumor growth rate and number of tumors were assessed through CT imaging. Additionally, apoptosis was measured.

Results: Immunization with heat-inactivated *Streptococcus pneumonia* resulted in increased plasma titers of anti-oxLDL IgM compared to control mice. Immunization reduced both the number of tumors and tumor growth rate compared to control injection. Hepatic TUNEL staining showed increased apoptosis in immunized versus control mice.

Conclusion: Immunization with heat-inactivated *Streptococcus pneumonia* could be a viable strategy to inhibit progression of NASH-derived HCC.

Sponsors

We would like to express our gratitude to our

Sponsors:

The EFLC2022 is also supported by:

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