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ABSTRACT BOOK

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ORAL POSTER ABSTRACT PRESENTATIONS

OS-37
The First-in-Asian Double-blind Randomized Trial to Assess the Efficacy and Safety of Insulin Sensitizer in Non-alcoholic Steatohepatitis

Jee-Fu Huang[1 2], Chia-Yen Dai[1], Chung-Feng Huang[1], Ming-Lun Yeh[1], Ming-Lung Yu[1], Wan-Long Chuang[1]

[1]Kaohsiung Medical University Hospital, Hepatobiliary Division, Department of Internal Medicine, Taiwan, *[2]Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Department of Internal Medicine, Taiwan*
Email: jfliver@kmu.edu.tw

Background and aims: The efficacy and safety of insulin sensitizer in Asians with non-alcoholic steatohepatitis (NASH) remain elusive. We conducted a double-blind, randomized, placebo-controlled trial of insulin sensitizer in Taiwanese NASH patients. The primary end point was the efficacy of pioglitazone in reducing inflammation and liver fat at end-of-treatment (EOT). NASH resolution/improvement without fibrosis worsening were also evaluated.

Method: A total of 90 eligible Taiwanese NASH patients (66 males, age = 44.1 ± 12.7 years) were recruited from April 2009 to August 2019. They were prospectively randomized into oral pioglitazone 30 mg/day (Arm A) or placebo (Arm B) for 24 weeks. They received paired biopsies and MRI-PDFF examinations before randomization and at EOT.

Results: Diabetes, dyslipidemia, and metabolic syndrome were found in 21 (23.3%), 56 (62.2%), and 52 (57.8%) of the patients, respectively. The mean fat content on MRI-PDFF was 21.2 ± 8.4%, whereas the mean NAS was 4.3 ± 1.3. The pre-treatment mean ALT level was 90.0 ± 39.4 IU/L in 41 Arm A patients, and it significantly decreased to 45.7 ± 35.8 IU/L at EOT ($p = 0.003$). By contrast, there were no significant changes of ALT level (90.3 ± 39.0 IU/L to 79.8 ± 48.0 IU/L) in 46 patients of Arm B. In an intention-to-treat analysis, 66 patients who received at least one dose and completed paired biopsies were recruited into further analysis. The NAFLD activity score (NAS) of 30 Arm A patients significantly decreased from 4.27 ± 1.14 at baseline to 2.53 ± 1.63 at EOT ($p < 0.0001$), whereas there was no significant change in patients of Arm B (3.94 ± 1.41 vs 3.94 ± 1.51, $P = 1.0$). Liver fat content reduced (20.2 ± 9.0 to 14.3 ± 6.9%, $P < 0.0001$) on MRI-PDFF in Arm A compared to their counterparts. NASH improvement without worsening of fibrosis was achieved in 46.7% (14/30) patients in Arm A, compared to 11.1% (4/36) patients in Arm B ($p = 0.002$). No significant difference of adverse events occurred between groups.

Conclusion: A 24-weeks pioglitazone treatment was well-tolerated and effective in improving liver histology and reducing liver steatosis in Asian NASH patients. (ClinicalTrials.gov number: NCT01068444)

OS-42
Hepatocyte-specific cholinergic receptor nicotine alpha 4 (Chrna4) promotes non-alcoholic steatohepatitis

Chuyue Pan[1], Yingsheng Gao[1], Jun Liu[1], Song Yang[2], Lirui Wang[1]

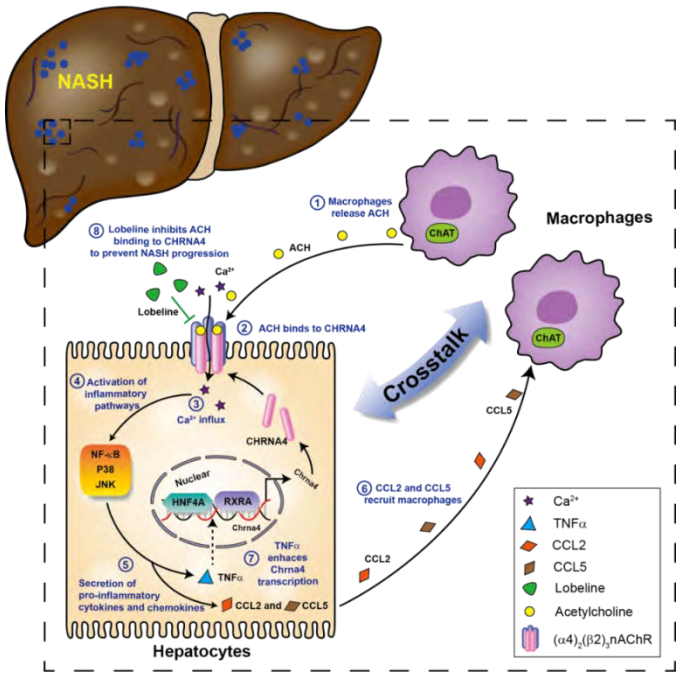
[1]*China Pharmaceutical University, The School of Basic medicine and clinical pharmacy, Nanjing, China*, [2]*Beijing Ditan Hospital, Capital Medical University, Department of Hepatology, Beijing, China*
Email: wanglirui@cpu.edu.cn

Background and aims: Non-alcoholic steatohepatitis (NASH) is a leading risk factor for liver cirrhosis and hepatocellular carcinoma, while the current treatment is very limited. Here, we found that CHRNA4, a subunit of nicotine acetylcholine receptors (nAChRs), was upregulated in mice and patients with NASH. We aimed to investigate the role of *Chrna4* in NASH pathogenesis and the underlying mechanism.

Method: The mechanistic study involves overexpression, hepatocyte-specific deletion and antagonist-based inhibition of CHRNA4 in mice. RNA sequencing, calcium imaging, trans-well migration assay and histological staining were integrated to delineate the function of *Chrna4* in NASH pathogenesis in cultured hepatocytes, mice and patients with NASH.

Results: We demonstrated that elevated CHRNA4 levels were positively correlated with the severity of NASH. Notably, we found that TNF-alpha could significantly upregulate CHRNA4 expression in hepatocytes possibly via the transcription factor HNF4A or RXRA. Further, during the progression of NASH, CHRNA4 could be activated by macrophage-derived acetylcholine and functioned as a calcium channel. The influx of calcium activated the inflammatory signaling pathways and increased the production of inflammatory cytokines and chemokines, such as TNF-alpha, CCL2 and CCL5. CCL2 and CCL5 subsequently recruited macrophages, which might further facilitate their communication with nearby hepatocytes via acetylcholine-CHRNA4 axis, while TNF-alpha induced in turn the increase of CHRNA4 expression in hepatocytes. Importantly, overexpression of CHRNA4 accelerated the development of NASH in HFD-induced and *ob/ob* mice, whereas the hepatocyte-specific deletion of *Chrna4* alleviated NASH-associated phenotypes. Lobeline, a small molecular antagonist of CHRNA4, effectively prevented NASH progression in HFFD and CDAA-induced mice with NASH.

Conclusion: Targeting CHRNA4 might be a novel strategy for the therapeutic intervention of NASH.
Figure:



OS-59
Ketohexokinase inhibition as a strategy for reducing steatosis and fibrogenesis in non-alcoholic steatohepatitis

Emma Shepherd[1], Raquel Saborano[2], Ellie Northall[1], Kae Matsuda[3], Hitomi Ogino[3], Hiroaki Yashiro[4], Jason Pickens[4], Banu Cole[5], Nobuhiro Nishigaki[3], Ulrich Gunther[2], Gideon Hirschfield[6], Derek Erion[4], Patricia Lalor[1]

[1]University of Birmingham, UK, Centre for Liver and Gastroenterology Research, Birmingham, United Kingdom, *[2]University of Birmingham, UK, Institute of Cancer and Genomic Sciences, Birmingham, United Kingdom*, *[3]Takeda Pharmaceuticals, Cardiovascular and Metabolic Drug Discovery Unit, Kanagawa, Japan*, *[4]Takeda Pharmaceuticals, Gastroenterology Drug Discovery Unit, Cambridge, United States*, *[5]Hemoshear Therapeutics, Charlottesville Virginia, United States*, *[6]University of Toronto, Toronto Centre for Liver Disease, Toronto General Hospital, Toronto, Canada*
Email: p.f.lalor@bham.ac.uk

Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD) may affect up to 25% of the adult population worldwide presenting a growing mortality risk for those affected and a huge challenge for the healthcare community. Thus, it is vital that we improve our knowledge of pathogenic mechanisms to develop novel therapeutic tools and improve outcome. Increasing evidence highlights dietary fructose as a major mechanistic driver. Most fructose is cleared by enzymatic phosphorylation to fructose-1-phosphate via ketohexokinase (*KHK*) enzyme on first pass through the hepatic circulation. This causes unregulated *de novo* lipogenesis and thus may present a tractable therapeutic target. The aim of this study was to investigate the effects of KHK inhibition in human liver tissue and a preclinical rodent model of NAFLD.

Method: We utilised a murine dietary model of NAFLD, human multicellular coculture systems and a novel whole tissue perfusion assay to understand the hepatocellular consequences of fructose administration. We also performed a detailed NMR-based metabolic tracing of the fate of isotopically-labelled fructose upon administration to the human liver.

Results: We confirm expression of *KHK* isoforms in multiple human hepatic cell types, with a predominance in hepatocytes. Administration of high fat/high fructose diet to mice increased steatosis, inflammation and early fibrogenesis, and these along with serum transaminase level were reduced in KHK-deficient animals. Similarly, cocultures of human hepatocytes and non-parenchymal cells exhibited steatosis and activation of lipogenic and fibrogenic gene expression upon fructose administration. These were reduced by pharmacological inhibition of *KHK* activity. Analysis of human liver wedges exposed to ^{13}C-labelled fructose showed that steatosis occurred due to accumulation of lipogenic precursors such as glycerol and enhanced glycolytic activity. All of these were dose-dependently reduced by administration of KHK inhibitor.

Conclusion: We have provided pre-clinical evidence using human livers to support use of KHK inhibition to improve steatosis and fibrosis in the context of NAFLD.

OS-93
RIPK3-dependent signalling modulates mitochondrial bioenergetics and lipid droplet dynamics in experimental NAFLD

Tawhidul Islam[1], Marta B. Afonso[1], Ricardo Amorim[2], Veronique Lenoir[3], Rui F. Simões[2], Liana C. Silva[1], Vlad Ratziu[4 5 6], Paulo J. Oliveira[2], Rui E. Castro[1], Carina PRIP-BUUS[3], Jérémie Gautheron[6 7], Cecília M. P. Rodrigues[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, *[2]Center for Neuroscience and Cell Biology (CNC), UC Biotech Building, Universidade de Coimbra, Coimbra, Portugal*, *[3]Institut Cochin, Inserm, CNRS UMR8104, Université Paris Descartes, Paris, France*, *[4]Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière Hospital, Department of Hepatology, Paris, France*, *[5]Sorbonne Université, Inserm, Centre de Recherche des Cordeliers (CRC), Paris, France*, *[6]Institute of Cardiometabolism and Nutrition (ICAN), Paris, France*, *[7]Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine (CRSA), Paris, France*
Email: mbafonso@ff.ulisboa.pt

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a plainly metabolic associated fatty liver disease. Receptor-interacting protein kinase 3 (RIPK3) is emerging as a pivotal mediator of NAFLD progression and a metabolic regulator, but its contribution to mitochondria dysfunction remains under debate. Here, we aimed to evaluate the role of RIPK3 in modulating mitochondria bioenergetics and function, coupled with lipid droplet architecture and dynamics in NAFLD.

Method: Functional studies evaluating mitochondria bioenergetics and stress and lipid droplet biology were performed in wild-type (WT) and *Ripk3*$^{-/-}$ mice fed a choline-deficient, amino acid-defined (CDAA) diet for 32 and 66 weeks and in CRIPSR-Cas9 *Ripk3*-null fat-loaded immortalized hepatocytes. The association between hepatic perilipin 1 (PLIN1) and 5 (PLIN5), RIPK3 and disease severity was also addressed in a cohort of NAFLD patients.

Results: *Ripk3* deficiency rescued mitochondrial biogenesis, bioenergetics and function in experimental NASH both in CDAA diet-fed mice and fat-loaded hepatocytes. Further, *Ripk3* deficiency was accompanied by a strong upregulation of antioxidant defence mechanisms, leading to diminished oxidative stress upon fat loading both *in vivo* and *in vitro*. Strikingly, *Ripk3*$^{-/-}$ hepatocytes displayed more and smaller lipid droplets than WT cells upon free fatty acid exposure, while *Ripk3* deficiency upregulated hepatic levels of the lipid droplet-associated proteins PLIN1 and PLIN5, in experimental NAFLD and in patients. In particular, PLIN1 upregulation in *Ripk3*$^{-/-}$ hepatocytes controlled lipid droplet structure and diminished mitochondrial stress upon free fatty overload and was associated with diminished disease severity in human NAFLD

Conclusion: Overall, impaired mitochondria respiratory chain complex activity correlates with mitochondrial dysfunction, reactive oxygen species overproduction and altered mitochondrial metabolism in experimental NAFLD. RIPK3 deficiency restores mitochondria bioenergetics and function, while also impacting on lipid droplet dynamics, thus halting NAFLD.
Funding support provided by EU H2020 Marie Sklodowska-Curie (722619), FEDER (LISBOA-01-0145-FEDER-029097), FCT (PTDC/MED-FAR/29097/2017; UIDB/04539/2020), Fondation pour la Recherche Médicale (ARF20170938613 and EQU202003010517), and Mairie de Paris (Emergences-R18139DD).

OS-118
Metabolic dysfunction associated fatty liver disease improves detection of high liver stiffness: the Rotterdam study

Laurens van Kleef[1], Ibrahim Ayada[1], Louise Alferink[1], Qiuwei Pan[1], Robert De Knegt[1]
[1]*Erasmus MC, University Medical Center, Gastroenterology and Hepatology*
Email: laurensvkleef@gmail.com

Background and aims: Recently a transition from non-alcoholic fatty liver disease (NAFLD) to metabolic dysfunction associated fatty liver disease (MAFLD) has been proposed to shift the focus to metabolic health, one of the main drivers of fatty liver disease (FLD). The novel definition requires metabolic dysfunction (overweight, diabetes or ≥2 minor criteria) together with steatosis. We investigated the application of the MAFLD criteria compared to the conventional NAFLD criteria.

Method: We performed a cross-sectional analysis within The Rotterdam Study, a large prospective population-based cohort. All participants between the years 2008 and 2014 who attended the abdominal ultrasound and transient elastography program were eligible for inclusion. Subsequently, individuals with viral hepatitis, alcohol intake >60 grams/day, missing alcohol data and/or missing body mass index (BMI) were excluded. According to their NAFLD and MAFLD status based on metadata and ultrasound, participants were allocated in overlap fatty liver disease (FLD), NAFLD-only, MAFLD-only or no-FLD. Fibrosis was defined as liver stiffness ≥8.0 kilopascal. Multivariable analyses were adjusted for age, gender, alcohol, smoking and education level.

Results: In our analysis, 5.445 participants were included, 1.866 (34.3%) had MAFLD and 1.623 (29.8%) NAFLD. This resulted in 1.566 (28.8%) individuals with overlap-FLD, 300 (5.5%) with MAFLD-only, 57 (1.0%) with NAFLD-only, and 3.522 (64.7%) with no-FLD. MAFLD-only was strongly associated with fibrosis (adjusted OR 5.27, $p < 0.001$) and log-transformed liver stiffness (adjusted beta 0.116, $p < 0.001$), opposing NAFLD-only in which no cases of fibrosis were identified and no association with liver stiffness (adjusted beta 0.006, $p = 0.90$) was found. Furthermore, among participants with MAFLD, there was a significant association with fibrosis for meeting all MAFLD inclusion criteria (aOR 2.42, $p <0.001$) or having the metabolic syndrome (aOR 1.86, $p = 0.004$). The associations were consistent among individuals with MAFLD regardless of exclusion criteria.

Conclusion: FLD is highly prevalent in the general population. However, not NAFLD-only, but MAFLD-only was associated with fibrosis and higher liver stiffness, which are important predictors for hepatic complications. Hence, the usage of the new MAFLD definition does not seem to lead to exclusion of patients with FLD at risk for fibrosis. We believe using the novel MAFLD criteria will help improve the identification and treatment of FLD patients at risk for fibrosis.

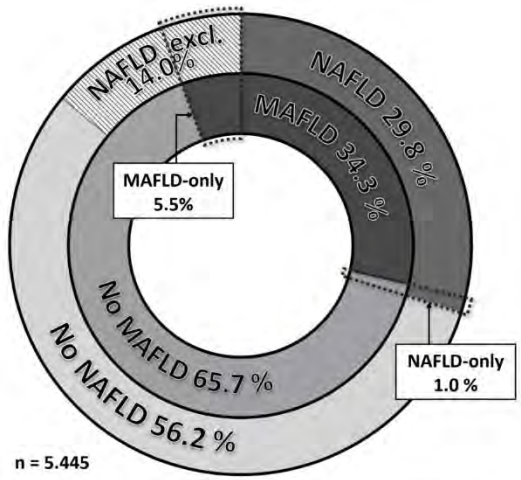


Figure: **Figure:** NAFLD and MAFLD distribution in participants that were eligible for both analyses (n = 5445). The inner circle represents MAFLD and outer circle NAFLD diagnosis.

OS-128
Protective association of Klotho rs495392 gene polymorphism against hepatic steatosis in non-alcoholic fatty liver disease patients

Wen-Yue Liu[1], Xiaofang Zhang[2], Gang Li[3], Liangjie Tang[3], Pei-Wu Zhu[4], Rafael Rios[3], Kenneth I. Zheng[3], Hong-Lei Ma[3], Xiaodong Wang[3], Qiuwei Pan[5], Robert De Knegt[5], Luca Valenti[6], Mohsen Ghanbari[2], Ming-Hua Zheng[3]

[1]the First Affiliated Hospital of Wenzhou Medical University, Department of Endocrinology, *[2]Erasmus University Medical Center, Rotterdam, the Netherlands, Department of Epidemiology*, *[3]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Department of Hepatology*, *[4]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Department of Laboratory Medicine*, *[5]Erasmus University Medical Center, Rotterdam, the Netherlands, Department of Gastroenterology and Hepatology*, *[6]Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy, Department of Transfusion Medicine and Hematology*
Email: liuwenyue@wmu.edu.cn

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is closely associated with metabolic dysfunction. Among the multiple factors, genetic variations act as important modifiers. Klotho, an enzyme encoded by the klotho (KL) gene in human, has been implicated in the pathogenesis of metabolic dysfunctions. However, the impact of KL variation in NAFLD remains poorly understood. The aim of this study was to investigate the impact of KL rs495392 C>A polymorphism on the histological severity of NAFLD.

Method: We evaluated the impact of the KL rs495392 polymorphism on liver histology in 531 Chinese with NAFLD and replicated that in the population-based Rotterdam Study cohort. Immunohistochemistry (IHC) staining was carried out on liver samples to observe the presence of Klotho protein. The interactions between the rs495392 and vitamin D and patatin-like phospholipase domain containing 3 (PNPLA3) rs738409 polymorphism were also analyzed.

Results: Carriage of the rs495392 A allele had a protective effect on steatosis severity (OR = 0.61, 95% CI: 0.42-0.89, p = 0.010) in Chinese patients. After adjustment for potential confounders, the A allele remained significant with a protective effect (OR = 0.66, 95% CI: 0.45-0.98, p = 0.040) (**Table 1**). The effect on hepatic steatosis was confirmed in the European Rotterdam Study cohort. The genotype AA was negatively associated with higher fatty liver index (OR = 0.36, 95%CI: 0.17-0.73, p = 0.006) in the Rotterdam Study. In IHC staining, a reduced expression of sKL seemed to present in the genotype AA group compared with the CC group. Additional analysis showed the association between serum vitamin D levels and NAFLD specifically in rs495392 A allele carriers, but not in non-carriers. Moreover, we found that the rs495392 A allele attenuated the detrimental impact of PNPLA3 rs738409 G allele on the risk of severe hepatic steatosis.

Conclusion: The KL rs495392 polymorphism had a protective effect against severe hepatic steatosis in Chinese and European patients with NAFLD. These data suggest that Klotho may represent a therapeutic target for the fatty liver disease that warrant further investigation.

Figure:

Table 1. Association between Klotho (*KL*) rs495392 polymorphism and liver histology features in patients with NAFLD.

	Severe steatosis	
	OR (95%CI)	**P value**
Crude model		
CC	Ref.	-
CA+AA	0.61 (0.42-0.89)	0.010
Adjusted model†		
CC	Ref.	-
CA+AA	0.66 (0.45-0.98)	0.040
	Severe ballooning	
Crude model		
CC	Ref.	-
CA+AA	0.73 (0.48-1.09)	0.126
Adjusted model†		
CC	Ref.	-
CA+AA	0.75 (0.50-1.13)	0.171
	Severe inflammation	
Crude model		
CC	Ref.	-
CA+AA	0.81 (0.51-1.29)	0.383
Adjusted model†		
CC	Ref.	-
CA+AA	0.85 (0.53-1.36)	0.493
	Significant fibrosis	
Crude model		
CC	Ref.	-
CA+AA	1.18 (0.77-1.81)	0.437
Adjusted model†		
CC	Ref.	-
CA+AA	1.17 (0.75-1.81)	0.493

† Adjusted for age, sex, BMI, presence of diabetes
Abbreviations: Ref, reference.

OS-133
Independent imaging biomarkers for steatosis and fibrosis and their temporal change in NAFLD evolution

Zheng Zhu[1], Alina Allen[2], Terry Therneau[3], Xin Lu[1], Jie Chen[1], Kevin Glaser[1], Jiahui Li[1], Jingbiao Chen[1], safa Hoodeshenas[1], Sudhakar VENKATESH[1], Armando Manduca[1 4], Richard L. Ehman[1], Meng Yin[1]
[1]Mayo Clinic, Radiology, Rochester, United States, *[2]Mayo Clinic, Gastroenterology and Hepatology, Rochester, United States*, *[3]Mayo Clinc, Biomedical Statistics and Informatics, Rochester, United States*, *[4]Mayo Clinic, Physiology and Biomedical Engineering, Rochester, United States*
Email: yin.meng@mayo.edu

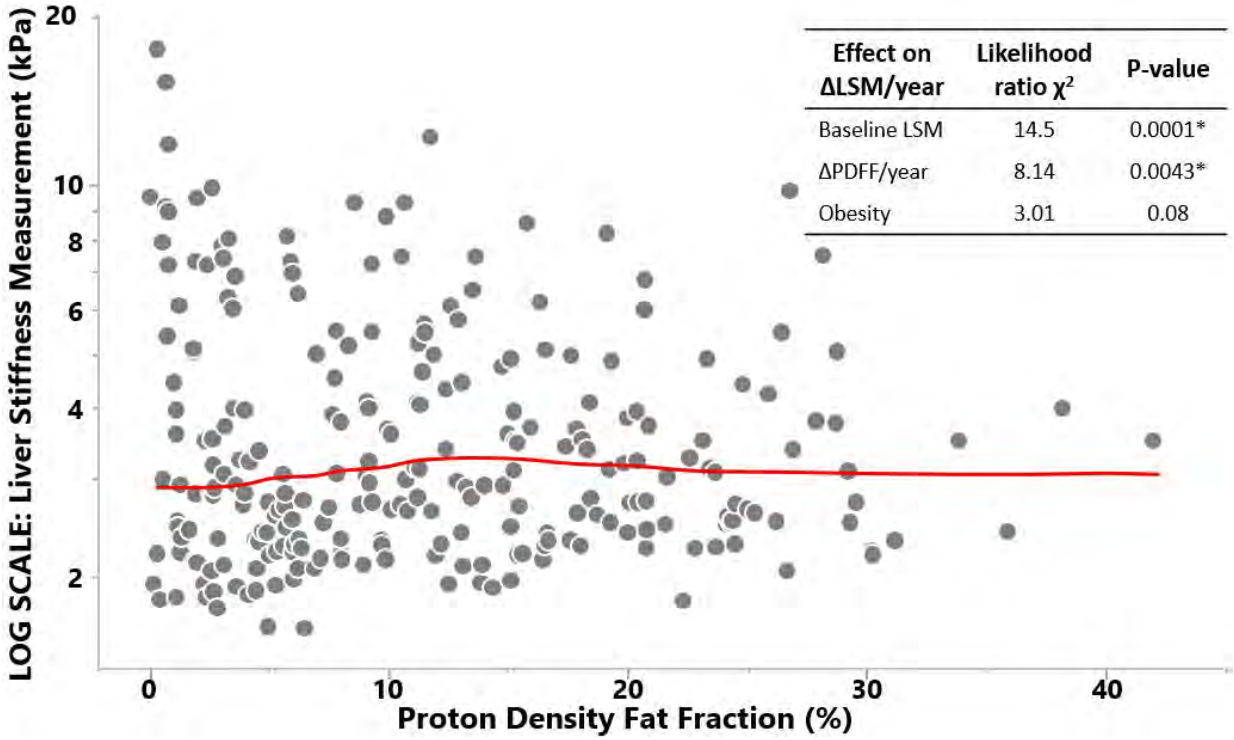
Background and aims: MRI-assessed proton density fat fraction (PDFF) and MR elastography (MRE)-assessed liver stiffness measurement (LSM) are two well-established imaging biomarkers for quantifying steatosis and fibrosis, respectively. However, their parameter independence and temporal evolution are unclear. Therefore, it is essential to evaluate the effect of steatosis on fibrosis quantification by MRE-LSM. Furthermore, as the primary driver for long-term outcomes and mortality, LSM change rate may identify patients at a high risk of rapid fibrosis progression. Therefore, this study aimed to evaluate the relationship between PDFF and LSM and explore their yearly change rates in clinical outcome prediction.

Method: We retrospectively identified 384 NAFLD patients with at least one multiparametric MRI/MRE exam. 256 of them had liver biopsy within one year. The impact of PDFF on LSM was assessed with linear regression after adjusting with identified influencing factors. 128 of them had serial MRI/MRE exams (N = 2-9). Significant LSM change was defined by a z-score≥1.96 with a sigma level of 0.10. The relative LSM and PDFF change rates per year (ΔLSM and ΔPDFF) and their relationships were analyzed by Spearman's correlation among groups regarding dichotomous classifications based on the presence of cirrhosis, type-2 diabetes, obesity, hyperlipidemia, hypertension, and obstructive sleep apnea. A generalized linear model was applied to predict ΔLSM with ΔPDFF and baseline LSM.

Results: No statistically significant relationship was found between the LSM and PDFF (Estimate = -0.02, P = 0.069) after adjusting fibrosis stages and age in patients with the presence of steatosis (i.e., PDFF>5%). 36 patients had significant LSM changes (56% women, mean age 59 years, BMI = 33.5 ± 4.9 kg/m^2, 50% with >2 MREs) with a median follow-up time of 4 years (range: 6 months-10 years). Among non-cirrhotic livers, low change rates and a positive correlation were shown between ΔLSM and ΔPDFF (ΔLSM = -0.19+0.071×ΔPDFF, r = 0.40). In cirrhotic livers, high change rates and a negative correlation were observed (ΔLSM = 0.51-0.10×ΔPDFF, r = 0.58). Baseline LSM and ΔPDFF are significant risk factors that could determine ΔLSM (p = 0.0001, 0.0043).

Conclusion: The severity of hepatic steatosis has no significant influence on LSM in NAFLDs, while the temporal change rate of PDFF and LSM show promise for identifying individuals at high risk of rapid clinical progression and improving patient management.

Figure:



Effect on ΔLSM/year	Likelihood ratio χ^2	P-value
Baseline LSM	14.5	0.0001*
ΔPDFF/year	8.14	0.0043*
Obesity	3.01	0.08

OS-185
ATG7 genetic variants predispose to severe fatty liver disease

Guido Alessandro Baselli[1 2], Oveis Jamialahmadi[3], Serena Pelusi[1 2], Ester Ciociola[3], Francesco Malvestiti[2], Paola Dongiovanni[4], Marco Maggioni[5], Cristiana Bianco[1], Federica Tavaglione[6], Annalisa Cespiati[4], Rosellina Mancina[6], Roberta D'Ambrosio[7], Salvatore Petta[8], Luca Miele[9], Umberto Vespasiani Gentilucci[10], Alessandro Federico[11], Jussi Pihlajamaki[12 13], Elisabetta Bugianesi[14], Anna Ludovica Fracanzani[2 4], Helen L. Reeves[15], Giorgio Soardo[16 17], Daniele Prati[1], Stefano Romeo[6 18 19], Luca Valenti[1 2]

[1]Translational Medicine-Department of Transfusion Medicine and Hematology-Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, Italy, *[2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy*, *[3]Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, Wallenberg Laboratory, University of Gothenburg; Gothenburg, Sweden, Sweden*, *[4]General Medicine and Metabolic Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy*, *[5]Pathology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy*, *[6]Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, Wallenberg Laboratory, University of Gothenburg; Gothenburg, Sweden*, *[7]Division of Gastroenterology and Hepatology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Milan, Italy*, *[8]Gastroenterology and Hepatology, PROMISE, Università di Palermo; Palermo, Italy*, *[9]Department of Internal Medicine, Fondazione Policlinico A. Gemelli, Università Cattolica di Roma; Rome, Italy*, *[10]Clinical Medicine and Hepatology Unit, Department of Internal Medicine and Geriatrics, Campus Bio-Medico University; Rome, Italy*, *[11]Division of Hepatogastroenterology, Department of Precision Medicine, Università della Campania "Luigi Vanvitelli"; Naples, Italy*, *[12]Institute of Public Health and Clinical Nutrition, University of Eastern Finland; Kuopio, Finland*, *[13]Department of Medicine, Endocrinology and Clinical Nutrition, Kuopio University Hospital; Kuopio, Finland*, *[14]Department of Medical Sciences, Division of Gastro-Hepatology, A.O. Città della Salute e della Scienza di Torino, Università di Torino; Turin, Italy*, *[15]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK; and Newcastle NIHR Biomedical Research Centre, Newcastle upon Tyne Hospitals NHS Foundation Trust; Newcastle upon Tyne, UK*, *[16]Clinic of Internal Medicine-Liver Unit, Department of Medical Area (DAME), Università degli Studi di Udine; Udine, Italy*, *[17]Italian Liver Foundation, Area Science Park, Basovizza Campus; Trieste, Italy*, *[18]Clinical Nutrition Unit, Department of Medical and Surgical Science, University Magna Graecia; Catanzaro, Italy*, *[19]Department of Cardiology, Sahlgrenska University Hospital; Gothenburg, Sweden*
Email: biancocristiana.md@gmail.com

Background and aims: Non-alcoholic fatty liver disease (NAFLD) has a strong inheritable component. The identification of its genetic determinants is leading to improvements in risk stratification and the identification of therapeutic target. The aim of this study was to identify new genetic variants involved in NAFLD pathogenesis.

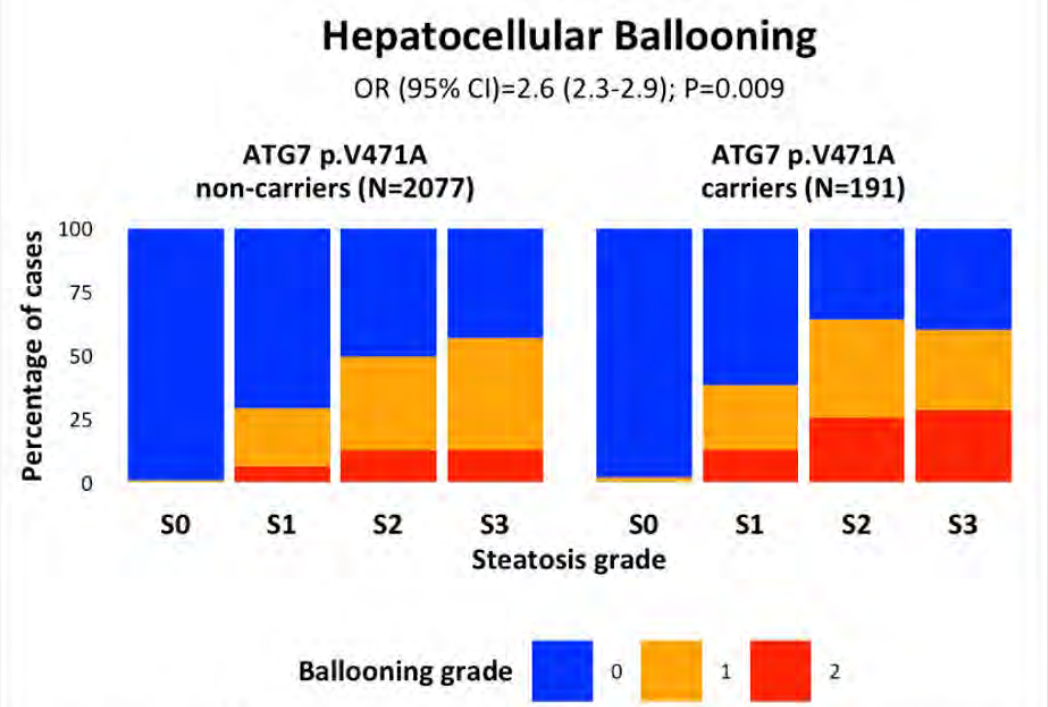
Method: We performed whole-exome sequencing in 301 European patients with NAFLD and advanced fibrosis and/or hepatocellular carcinoma, followed by variant prioritization based on *in silico* predictors, and identified variants enriched vs. the general population (GnomAD Non-Finnish Europeans and UK Biobank cohort (UKBB) without liver disease, n = 180, 391), followed by validation at gene level. We replicated the results in the European Liver Biopsy Cohort (LBC, n = 2268) and the UKBB, and we investigated the molecular mechanisms in a Liver Transcriptomic Cohort (LTC) of 125 obese patients.

Results: In the severe NAFLD cohort vs. the general population, we detected an enrichment of the p.P426L variant (OR = 5.26, 2.06-12.61; p = 0.003) of Autophagy Related Gene 7 (*ATG7*), involved in lipo-autophagy and steatohepatitis development in mice, which we characterized as a loss-function. We further observed a higher burden of rare (OR = 13.9, 1.9-611; p = 0.002) in the conserved C-terminal *ATG7* domain, and an increased frequency of the p.V471A variant (MAF = 0.06 vs 0.035, OR = 1.7, 1.2-2.5; p = 0.003). In the UKBB, loss-of-function *ATG7* variants increased the risk of cirrhosis and

hepatocellular carcinoma (OR = 3.30, 1.1-7.5 and OR = 12.30, 2.6-36, respectively; p <0.001 for both). In the UKBB, p.V471A was associated with liver injury (AST levels, p <0.001) and hepatocellular carcinoma in severely obese (p = 0.009). In the LBC, we confirmed p.V471A association with severe fibrosis, particularly in patients with severe steatosis (p = 0.002); p.V471A was an independent predictor of hepatocellular ballooning (p = 0.007). In the LTC *ATG7* correlated with suppression of the TNF-alpha pathway, which conversely was upregulated in carriers of p.V471A. ATG7 protein was expressed in Kupffer cells and predominantly periportal hepatocytes, where the staining was marked around lipid droplets, and more intense in presence of ballooning.

Conclusion: We identified a novel association of *ATG7* loss-of-function variants with severe NAFLD. The mechanism may involve impairment of autophagy and facilitation of hepatocellular ballooning and inflammation.

Figure:



Impact of p.V471A *ATG7* variant on hepatocellular ballooning grade according to liver steatosis severity in the overall cohort of LBC. Adjusted for sex, age, BMI, T2D, steatosis grade and PNPLA3 rs738409 C>G p.I148M, TM6SF2 rs58542926 C>T p.E167K, MBOAT7 rs641738 C>T, GCKR rs1260326 T>C p.P446L variant genotypes.

OS-204
Circulating hepatic proteins detect high disease activity in progressive Non-alcoholic fatty liver disease

Olivier Govaere[1], Megan Hasoon[2], Leigh Alexander[3], Dina Tiniakos[1 4], Jerome Boursier[5], Elisabetta Bugianesi[6], Vlad Ratziu[7], Ann K Daly[1], Quentin Anstee[1 8]

[1]Newcastle University, Translational and Clinical Research Institute, Newcastle upon Tyne, United Kingdom, *[2]Newcastle University, Bioinformatics Support Unit, Newcastle upon Tyne, United Kingdom*, *[3]SomaLogic, Inc., Boulder, United States*, *[4]National and Kapodistrian University of Athens, Dept of Pathology, Aretaieio Hospital, Athens, Greece*, *[5]Angers University Hospital, Hepatology department, Angers, France*, *[6]University of Turin, Department of Medical Sciences, Division of Gastro-Hepatology, Turin, Italy*, *[7]Sorbonne University, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié Salpêtrière, Paris, France*, *[8]Newcastle upon Tyne Hospitals NHS Trust, Newcastle NIHR Biomedical Research Centre, Newcastle upon Tyne, United Kingdom*
Email: olivier.govaere@ncl.ac.uk

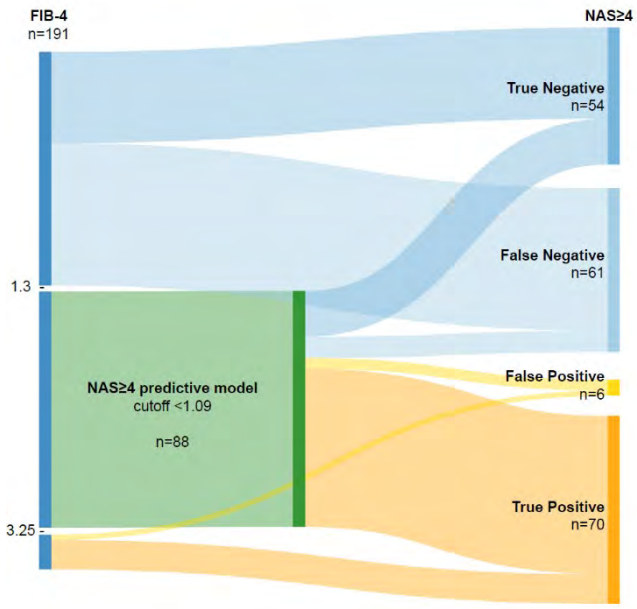
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of the metabolic syndrome and changes in circulating blood proteins have been associated with advanced NAFLD. Yet, it is still unclear which proteins originate from the liver and how these alter during disease progression.

Method: The cohort comprised 191 patients from the European NAFLD Registry with histologically proven NAFLD identified at four specialist centres. The histological semi-quantitative NASH CRN system was used to score the biopsies. Plasma samples were processed for proteomics analysis using the SomaScan™ platform. In a subset of 51 cases, snap-frozen liver biopsies underwent high-throughput RNA sequencing (RNAseq). Integrative analysis of these data with publicly available single-cell RNAseq data was used to identify cell of origin. Binary logistic modelling was implemented to predict disease activity.

Results: Comparing patients with advanced fibrosis (F3-4) to mild disease (F0-2) identified 156 differentially expressed circulating plasma proteins, while stratifying based on a NAFLD Activity Score (NAS) ≥4 identified 79 proteins. Of these, 34 proteins were common to both analyses, including AKR1B10, APOF, THBS2 and TREM2. To determinate which proteins originate from the liver, we performed a correlation analysis between plasma proteins identified by the F3-4/NAS≥4 analyses and mRNA within 51 patients, finding 40 proteins/mRNAs reaching the significance threshold. Deconvolution by single-cell RNAseq data indicated the different hepatic cellular changes during disease progression, such as *AKR1B10*, *APOF* and *GDF15* to originate from epithelial cells, *THBS2* from fibroblasts and *TREM2* from macrophages. Finally, to assess potential utility within a two-stage non-invasive diagnostic pathway to detect fibrosing-steatohepatitis, we performed logistic regression analysis in patients with an indeterminate FIB4 score (1.30-3.25; Figure 1). Backward elimination of variables identified a composite model that could predict a high disease activity (NAS≥4) with an Area Under the Curve of 0.925 based on markers including circulating TREM2.

Conclusion: We showed that circulating proteomic changes reflect grade of steatohepatitis and stafe of fibrosis that may be used to assess disease severity.

Figure:


FIB-4
n=191
NAS≥4
True Negative
n=54
False Negative
n=61
1.3
NAS≥4 predictive model
cutoff <1.09
n=88
False Positive
n=6
True Positive
n=70
3.25

OS-247

The Patatin-like phospholipase domain-containing-3 I148M mutation causes mitochondrial dysfunction and enhances the profibrogenic potential in human hepatic stellate cells

Elisabetta Caon[1], Maria Martins[2], Ana Levi[2], Dong Xia[3], Leo Ghemtio[4], Kessarin Thanapirom[2], Walid Al-Akkad[2], Jan-Willem Taanman[5], Michele Vacca[6 7], Giuseppe Mazza[2], Krista Rombouts[2], Massimo Pinzani[2]

[1]University College London, Insitute for Liver and Digestive Health, London, United Kingdom, *[1]University College London, Insitute for Liver and Digestive Health, London, United Kingdom*, *[3]University College London, Comparative Biomedical Sciences, Royal Veterinary College, London, United Kingdom*, *[4]University of Helsinki, Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy*, *[5]University College London, Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, United Kingdom*, *[6]University of Cambridge, Institute of Metabolic Sciences, London, United Kingdom*, *[7]University of Bari "Aldo Moro", Clinic Medica "Frugoni", Interdisciplinary Department of Medicine, Bari, Italy*
Email: elisabetta.caon@gmail.com

Background and aims: The I148M variant of the Patatin-like phospholipase domain-containing 3 (PNPLA3) protein is a well validated risk locus for the hHSC-driven fibrogenic progression of chronic liver diseases, particularly in NAFLD. Mitochondrial dysfunction has also been associated with NAFLD development. In this study we investigated the impact of *PNPLA3* I148M mutation on mitochondrial dysfunction in hHSCs in 2D and 3D culture models.

Method: Primary hHSC were isolated (n = 23 donors) and cultured in 2D, then genotyped for PNPLA3 (I148M) variants CG/GG. RNAseq data was analysed on 3 donors/genotype with Ingenuity pathway analysis (IPA). Cell behaviour of PNPLA3 (WT) hHSC and PNPLA3 (I148M) hHSC was evaluated in 3D decellularized scaffolds from human healthy and cirrhotic liver. Cells were cultured for 13 days and stimulated 3x48h with TGFbeta1. QRT-PCR, western blot and cytochrome-c-oxidase activity assay was performed.

Results: IPA associated the PNPLA3 (I148M) hHSC variant to the “NRF2 mediated oxidative stress response” and “Oxidative phosphorylation” signalling pathways. A possible derangement of intracellular anti-oxidant response was also suggested by qPCR on 3D cultured hHSC, with a significant decreased expression in VARS2, a mitochondrial enzyme, and GSTT1, a Glutathione-S-Transferase in PNPLA3 (I148M) hHSC compared to PNPLA3 (WT) hHSC with/without TGFB1 treatment ($p < 0.05$). This was further confirmed by protein expression of VARS2 and cytochrome-c-oxidase subunit MTCO1, which was significantly downregulated in PNPLA3 (I148M) hHSC compared to PNPLA3 (WT) hHSC ($p < 0.05$) and in PNPLA3 (WT/I148M) hHSC when cultured in healthy scaffolds compared to cirrhotic scaffolds ($p < 0.05$). The lower expression of MTCO1 protein also determined a significant reduction in enzymatic activity of the cytochrome-c-oxydase in PNPLA3 (I148M) hHSC compared to PNPLA3 (WT) hHSC when measured in 2D and healthy scaffolds ($p < 0.005$ and $p < 0.05$).

Conclusion: In this study, following IPA analysis on the genetic background of hHSC carrying different variants of the PNPLA3 I148M mutation, mitochondrial function of hHSC was investigated in a 3D model recapitulating the ECM microenvironment of normal and cirrhotic human liver. Results indicate that the PNPLA3 (I148M) variant is linked to a disrupted expression and activity of different mitochondrial proteins, including a key enzyme of the respiratory chain. This leads to a dysfunctional hHSC mitochondrial phenotype which is worsened by the fibrotic ECM.

OS-265

Icosabutate, a novel structurally engineered fatty acid receptor agonist, significantly reduces relevant biomarkers of NASH and fibrosis in 16 weeks: results of an interim analysis of the phase 2b ICONA trial

Stephen Harrison[1], Nadege T. Gunn[2], Muhammad Y Sheikh[3], Madhavi Rudraraju[1], Anita Kohli[4], David A. Fraser[5], Carine Beysen[5], Stephen Rossi[5], Arun Sanyal[6]
[1]Pinnacle Clinical Research, San Antonio, United States, *[2]Pinnacle Clinical Research, Austin, United States*, *[3]Fresno Clinical Research Center, Fresno, United States*, *[4]Arizona Liver Health, Glendale, United States*, *[5]Northsea Therapeutics, Amsterdam, Netherlands*, *[6]Virginia Commonwealth University, Richmond, United States*
Email: stephen.rossi@northseatherapeutics.com

Background and aims: Icosabutate (ICOSA) is a novel, oral, once-daily, liver-targeted, engineered eicosapentaenoic acid derivative with potent anti-inflammatory and antifibrotic effects, acting primarily through the G-coupled protein receptor (GPR120) and arachidonic acid signaling pathways. The ICONA trial is an ongoing 52-week, multicenter, placebo-controlled, phase 2b study enrolling 264 subjects with biopsy confirmed NASH. We present the results of a prespecified interim analysis evaluating multiple non-invasive biomarkers relevant for NASH, fibrosis, metabolic syndrome, lipid metabolism and cardiovascular risk.

Method: Ninety subjects were randomized (1:1:1) to ICO 300 mg or 600 mg or placebo and treated through Week 16. Histologic inclusion criteria included biopsy-proven NASH with an NAS ≥4 (1 point in each component), stage 1-3 fibrosis and ≥10% liver fat content by MRI-PDFF. Biomarkers of liver injury, inflammation, fibrogenesis, glycemic control and lipid metabolism were assessed through week 16. Liver fat content by MRI-PDFF was measured at Screening and Week 16.

Results: Rapid, sustained, and significant dose-dependent decreases were seen in ALT, AST, GGT, and ALP at levels predictive of histologic improvement (Table 1). Both doses showed significant reductions in PRO-C3 and ELF score (both total score and individual components) supporting an effect on fibrogenesis. High sensitivity-CRP (hs-CRP) significantly decreased by 52% with 600 mg in conjunction with improvements in glycemic control and key atherogenic lipoproteins. There were no changes in weight or BMI, suggesting a treatment effect independent of weight loss. Liver fat content was unchanged with both doses, consistent with ICO mechanism of action. Treatment was well tolerated with no evidence of hepatoxicity, cardiovascular events or other safety concerns as confirmed by an independent DSMB.

Conclusion: Treatment of NASH patients with ICO for 16 weeks has dose-dependent activity through multiple relevant biologic pathways. These broad and potent effects showed decreases in markers of liver injury, inflammation and fibrogenesis along with improvements in glycemic control and atherogenic lipids. These data, combined with a favorable safety profile, support a potential for impacting liver histology at 52 weeks as well as improving common comorbid conditions seen in NASH patients.

Table 1: Placebo adjusted absolute changes in non-invasive biomarkers

Parameter	ICO 300 mg	ICO 600mg
ALT (U/L)	-19*	-25.4*
AST (U/L)	-9.4#	-13.5#
GGT (U/L)	-16.9#	-28.6*
ALP (U/L)	-12.7*	-19.6*
Bilirubin (mg/dL)	-0.0	-014#
PRO-C3 (ng/ml)	-4.5*	-4.6*
ELF	-0.4#	-0.6*
hs-CRP (mg/L)	-1.2	-2.3#
HbA1c (%)	0.0	-0.3
HOMA-IR	-1.5	-2.1
LDL-C (mg/dL)	5.5	-3.9
HDL-C (mg/dL)	3.2#	2.3
Remnant-C (mg/dL)	-6.1#	-8.0*
ApoC3 (mg/dL)	-1.6#	-2.7*
TG (mg/dL)	-27.1#	-34.0#

*p< 0.001 #p<0.05

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OS-270
Suppression of insulin-induced gene 1 (INSIG1) function restrains NASH progression by promoting hepatic lipid remodelling

Vian Azzu[1 1 2], Michele Vacca[3 4 5], Ioannis Kamzolas[4 6], Zoe Hall[7 8], Jack Leslie[9], Stefania Carobbio[4], Samuel Virtue[4], Susan Davies[10], Agnes Lukasic[4], Martin Dale[4], Mohammad Bohlooly-Y[11], Animesh Acharjee[12 13], Daniel Lindén[14 15], Guillaume Bidault[4], Evangelia Petsalaki[6], Julian Griffin[7 16], Fiona Oakley[9], Antonio Vidal-Puig[2 4 17], Michael Allison[18]

[1]Norfolk and Norwich University Hospital, *[2]University of Cambridge, Wellcome Trust/MRC Institute of Metabolic Science, [3]University of Bari Aldo Moro Clinica Medica Cesare Frugoni, Department of Interdisciplinary Medicine, [4]University of Cambridge Wellcome Trust/MRC Institute of Metabolic Science*, *[5]University of Bari Aldo Moro, Clinica Medica Cesare Frugoni, Department of Interdisciplinary Medicine, [6]European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI)*, *[7]University of Cambridge Department of Biochemistry and Cambridge Systems Biology Centre*, *[8] Imperial College London Biomolecular Medicine, Systems Medicine, Department of Metabolism, Digestion and Reproduction*, *[9]Newcastle University Newcastle Fibrosis Research Group, Biosciences Institute, Faculty of Medical Sciences, [10]Cambridge University Hospitals NHS Foundation Trust Pathology*, *[11]AstraZeneca Translational Genomics, Discovery Sciences, BioPharmaceuticals RandD*, *[12] University of Birmingham College of Medical and Dental Sciences, Institute of Cancer and Genomic Sciences, Centre for Computational Biology*, *[13] University of Cambridge Department of Biochemistry and Cambridge Systems Biology Centre*, *[14]AstraZeneca, Bioscience Metabolism, Research and Early Development Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals RandD*, *[15]University of Gothenburg Division of Endocrinology, Department of Neuroscience and Physiology*, *[16]Imperial College London Biomolecular Medicine, Systems Medicine, Department of Metabolism, Digestion and Reproduction*, *[17]Wellcome Trust Sanger Institute, [18]Cambridge University Hospitals NHS Foundation Trust Hepatology*
Email: michael.allison@addenbrookes.nhs.uk

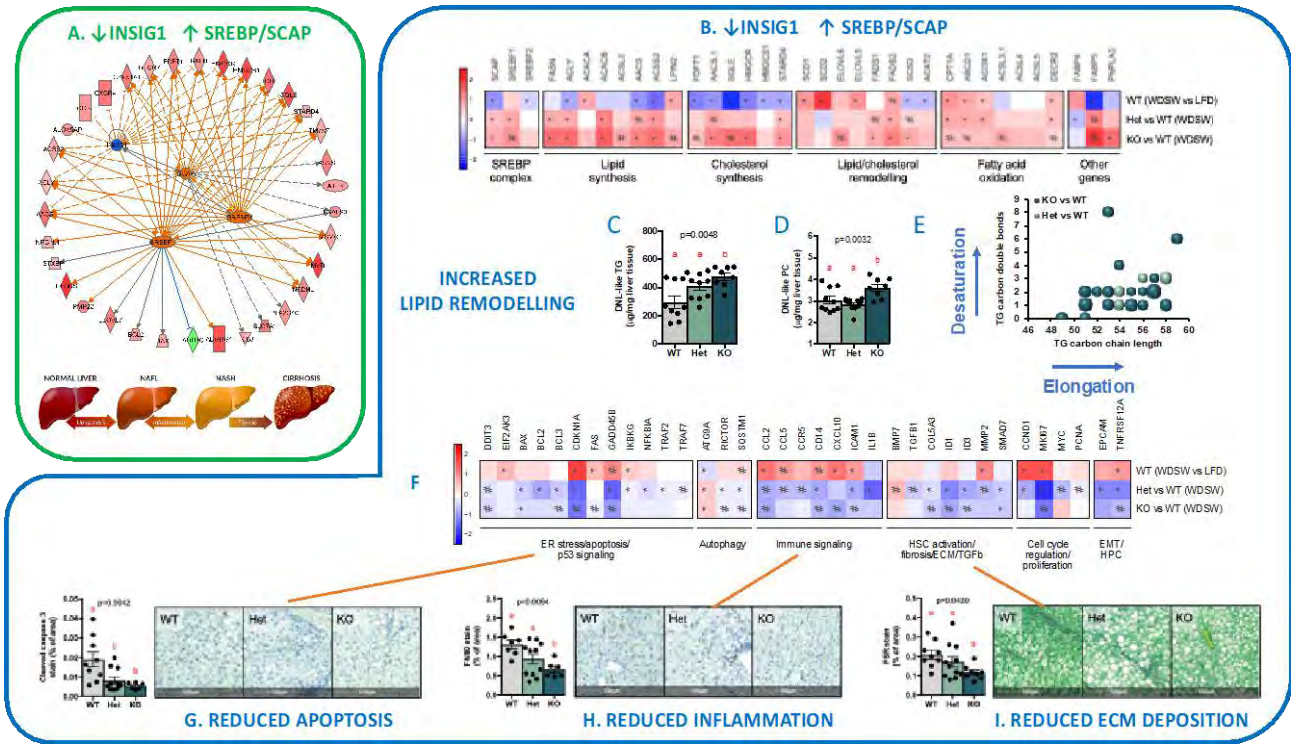
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a silent pandemic associated with obesity and the metabolic syndrome, and is linked to increased cardiovascular- and cirrhosis- related morbidity and mortality. It remains debated whether activation of Sterol Regulatory Element-Binding Proteins (SREBPs) act as a pathogenic drivers of lipotoxicity in non-alcoholic steatohepatitis (NASH), or promote the biosynthesis of protective lipids that buffer excessive lipid accumulation, thus preventing inflammation and fibrosis. We aimed to better understand these adaptive compensatory metabolic programs that modulate NAFLD pathophysiology and NASH progression.

Method: To understand the role of lipid/cholesterol synthesis/remodelling pathways in NASH progression, we employed transcriptomic analysis of NAFLD patients' liver biopsies coupled with reverse translation in INSIG1 deficient mice (which display hyperefficient SREBPs activation) challenged with a preclinical murine model of mild NASH (Western Diet Sugar Water, WDSW-12 weeks). Mice were phenotyped with a multiomics systems biology approach.

Results: Clustering the data against NASH progression, Ingenuity Pathway Analysis of hepatic transcriptome showed substantial rewiring of metabolic pathways: SREBPs transcriptional networks and *de novo* lipid/cholesterol synthesis and remodelling were predicted to be activated (Fig.1A). In WDSW, *Insig1* KO mice had similar systemic metabolism and insulin sensitivity to Het/WT littermates; however, the hyperefficient SREBPs activity in KO mice led to enhanced lipid/cholesterol biosynthesis (Fig.1B-D) and remodelling (Fig.1B, E) and restrained NASH progression as a result of decreased apoptotic hepatocellular damage and inflammation, resulting in decreased extracellular matrix deposition (Fig.1F-I).

Conclusion: Our results suggest that the SCAP/SREBP/INSIG1 trio governs transcriptional programs aimed at protecting the liver from lipotoxic insults in NASH and provides knowledge

Figure: Upregulation of SREBP metabolic pathways is seen in patients with advanced NASH and mediates hepatic protection from diet induced NASH in mice**.** (A) Network-like representation of IPA Upstream Regulator Analysis of human liver transcriptome shows strong SREBP/SCAP pathway upregulation that is associated with predicted INSIG1 downregulation in advancing NASH. (B-H) Mice treated with WDSW: *Insig1* ablation results in upregulation of lipid/cholesterol synthesis (B), shown by *Fasn*-mediated increase in DNL-TG (C) and DNL-PC (D), as well as and lipid remodelling shown by desaturation and elongation of carbon chains (E). These lipidome changes correlate with reduced ER stress, apoptosis, inflammation and HSC activation/collagen deposition (F: NGS; G-H: IHC; I: PSR).



POSTER
ABSTRACT
PRESENTATION

BASIC SCIENCE

PO-12
Metabolic, biochemical, histopathological, and transcriptomic effects of resmetirom (MGL-3196) in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

Michael Feigh[1], Jacob Nøhr-Meldgaard[1], Sanne Veidal[1], Martin Rønn Madsen[1], Henrik B. Hansen[1]
[1]*Gubra, Hørsholm, Denmark*
Email: mfe@gubra.dk

Background and aims: The thyroid receptor β-selective agonist resmetirom (MGL-3196) has in a recent phase 2 clinical trial been reported to have beneficial effects on key metabolic and histological end points in NASH patients, as demonstrated by a significantly greater proportion with at least 2-point improvement in NAFLD Activity Score (NAS) compared to placebo, although without significant improvement in fibrosis stage (Harrison et al., Lancet, 2019). The present study aimed to evaluate the metabolic, biochemical, histopathological, and transcriptomic effects of resmetirom treatment in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH with hepatic fibrosis.

Method: Male C57BL/6J mice were fed the GAN diet high in fat, fructose and cholesterol for 37 weeks prior to study start. A liver biopsy was sampled 4-weeks prior to study start. Only animals with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage ≥F1) were included and stratified into treatment groups. DIO-NASH mice received (PO, QD) vehicle (n = 16) or resmetirom (1 mg/kg, n = 16) for 12 weeks. Vehicle-dosed chow-fed C57BL/6J mice (n = 10) served as lean healthy controls. Pre-to-post liver biopsy histology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and Fibrosis Stage. Terminal quantitative liver histology, liver whole-transcriptome analysis, blood and liver biochemistry were assessed.

Results: Compared to vehicle-dosed DIO-NASH mice, resmetirom reduced hepatomegaly without influencing body weight. In addition, plasma liver transaminases, plasma and liver lipids (total cholesterol, triglycerides) were also reduced by resmetirom treatment. Notably, resmetirom demonstrated ≥2 point significant improvement in NAS. Therapeutic effects of resmetirom were supported by reduced quantitative histological markers of steatosis (lipids, hepatocytes with lipid droplets) without influencing inflammation markers (number of inflammatory foci, galectin-3). While resmetirom did not influence fibrosis stage or histomorphometry markers of fibrosis (PSR, collagen 1a1), a histological marker for activated stellate cells (α-SMA) was significantly reduced suggesting attenuation of fibrogenesis activity. Finally, resmetirom improved NASH-associated transcriptome signatures, including lowered expression of hepatic genes associated with inflammation and fibrogenesis in DIO-NASH mice.

Conclusion: Resmetirom treatment improved metabolic, biochemical, histological, and transcriptomic markers of steatohepatitis. Consistent with clinical findings, resmetirom was weight-neutral and improved NAS without reducing fibrosis stage. These findings further validate clinical translatability of the GAN DIO-NASH mouse model.

PO-27
The NIF mouse-a unique way to distinguish between immunological and metabolic effects of NASH drug candidates

Julia Nilsson[1 2], Maria Hörnberg[2], Kajsa Linde[2], Dan Holmberg[1 2], Sofia Mayans[2]
[1]*Department of Experimental Medical Science, Lund University, Malmö, Sweden*, [2]*InfiCure Bio AB, Umeå, Sweden*
Email: sofia.mayans@inficurebio.com

Background and aims: Many candidate drugs for treatment of NAFLD/NASH affect both metabolic and immunological phases of the disease. Most preclinical models used to understand the mode of action of new drugs targeting NASH do not distinguish between metabolic and immunological effects. InfiCure Bio has developed a new and unique preclinical model, the NIF mouse, which can be used to separate immunological and metabolic effects of NASH drug candidates.

Method: The NIF mouse is an NKT cell transgene on the NOD background and the model spontaneously develops chronic inflammation and fibrosis in the liver. Development of chronic inflammation is evident already at 3-4 weeks of age and liver fibrosis is established already at 6 weeks of age. The inflammatory phenotype reaches peak levels at 8-10 weeks of age followed by a decline, but the inflammation is not resolved. Levels of liver fibrosis peak at 10 weeks of age, with an ISHAK score of 3-4, and stays at this level until at least 40 weeks of age. The phenotype of the model is not induced by metabolic stress, but by the population of transgenic NKT cells, and both the inflammatory and the fibrotic phenotype is treatable. An example of treatment of the fibrotic phenotype is shown in the figure below.

Results: The transgenic NKT cells in the NIF mouse initially drive a type 1 inflammatory response involving the activation of the NLRP3 inflammasome. The transgenic cells do also promote a switch to a predominantly reparative/profibrotic response through the production of type 2 cytokines. Together, this results in the development of an inflammatory/fibrotic phenotype in the NIF mouse that show large similarities with human NASH.
The NIF mouse model provides a new and unique tool to test the effect of new drugs for treatment of inflammation and/or fibrosis, and to separate treatment effects mediated by immunological versus metabolic pathways.

Conclusion: The robust fibrotic component, early on-set, spontaneous nature, 100% reproducibility, similarity to human NASH and treatable nature of the phenotype makes the NIF model a unique tool for efficacy tests of new anti-fibrotic drugs. The model offers a new possibility to separate immunological and metabolic effects of NASH drug candidates.

Figure:

Picro Sirius Red stained livers from NIF mice. A) 4-week-old NIF B) 10-week-old NIF treated with vehicle for 4 weeks C) 10-week-old NIF treated with blocking TGFβ1 antibody, 1D11, for 4 weeks.

PO-30
Anti-oxidant, anti-inflammatory, and anti-fibrotic properties of triterpenic acids and phenylpropanoids on in vitro models of non-alcoholic steatohepatitis

Noel Salvoza[1 2], Natalia Rosso[1], Claudio Tiribelli[3], Andrea Saccani[4], Chiara Bedin[4]
[1]Fondazione Italiana Fegato Onlus, Basovizza, Italy, *[2]Philippine Council for Health Research and Development, Taguig, Philippines*, *[1]Fondazione Italiana Fegato Onlus, Basovizza, Italy*, *[4]ABRESEARCH srl, Brendola, Italy*
Email: noel.salvoza@fegato.it

Background and aims: There is no approved pharmacological treatment for NASH. Preliminary data showed hepatoprotective properties of triterpenic acids ABRTA22 (TA) and phenylpropanoids ABRPP09 (PP) in animal models of steatosis but the molecular mechanisms underlying the beneficial effects are still elusive. We assessed the effects of both compounds in a well-established *in vitro* models of steatosis and early-stage NASH.

Method: Monocultures of human hepatocytes (HuH7) and simultaneous co-cultures (SCC) of human hepatoytes (HuH7) and hepatic stellate cells (LX2) were exposed to free fatty acid (FFA) alone or in combination with TA (10 nM; 50 nM; 100 nM) and PP (0.1 µM; 10 µM; 40 µM). Intracellular FFA accummulation was assessed by flow cytometry using Nile Red assay; the expression of genes involved in inflammation and fibrogenesis were quantified by RT-PCR. The generation of reactive oxygen species (ROS) and collagen deposition were also determined.

Results: Exposure of HuH7 cells to FFA induced a 40% ($p < 0.001$) increase in ROS generation and up-regulation of pro-inflammatory genes, IL-8 (1.7-fold, $p < 0.05$) and TNF-alpha (2.5-fold, $p < 0.01$). The exposure of SCC to FFA significantly increased both COL1A1 gene expression and extracellular collagen deposition. Co-treatment of either TA and PP and FFA resulted in a reduction of ROS generation but only PP showed a potent antioxidant effect at all concentrations ($p < 0.05$; $p < 0.01$ vs FFA). Addition of TA resulted in a non-significant reduction in IL-6 and IL-8 expressions but a significant downregulation of TNF-alpha at 10nM and 50nM ($p < 0.05$). Likewise, PP reduced the expression of all the cytokines, with significant downregulation in TNF-alpha at 0.1µM and 10µM ($p < 0.05$). Both compounds decreased COL1A1 gene expression (1.7-folds, $p < 0.05$ vs FFA) and extracellular collagen depositionn (50%, $p < 0.05$ vs FFA) at the lowest concentration. In both models, treatment of TA or PP in the absence of FFA did not induce any effects in the extent of steatosis, inflammation, ROS generation, and collagen deposition.

Conclusion: Either triterpenic acids ABRTA22 (TA) or phenylpropanoids ABRPP09 (PP) reduces the FFA-related inflammation and ROS generation. Of notice, both compounds reduce collagen deposition suggesting their possible use in a clinical setting.

PO-52
Metabolic, biochemical, histopathological, and transcriptomic effects of dietary intervention in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

Michael Feigh[1], Kirstine Tølbøl[1], Sanne Veidal[1], Martin Rønn Madsen[1], Larissa Pfisterer[2], Eric Simon[2], Henrik B. Hansen[1], Andre Broermann[2]
[1]Gubra, Hørsholm, [2]Boehringer Ingelheim Pharma GmbH and Co. KG, Biberach an der Riss, Germany
Email: mfe@gubra.dk

Background and aims: The recommended standard of care for NASH is lifestyle modification, notably dietary intervention, aiming at promoting weight loss to promote regression or resolution of NASH and hepatic fibrosis. The present study aimed to evaluate the effect of long-term chow dietary intervention in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) and biopsy-confirmed mouse model of NASH with hepatic fibrosis.

Methods: Male C57BL/6J mice were fed the GAN diet high in fat, fructose and cholesterol for 32 weeks prior to study start. A liver biopsy was sampled 4-weeks prior to study start. Only animals with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage ≥F1) were included and stratified into treatment groups. DIO-NASH mice received vehicle (PO, QD) plus GAN diet-maintenance (n = 14) or vehicle plus dietary intervention by shifting to chow feeding (n = 14) for 8 and 12 weeks. Vehicle-dosed chow-fed C57BL/6J mice (n = 6) served as lean healthy controls. Pre-post liver biopsy histology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and Fibrosis Stage. Terminal quantitative liver histology, liver whole-transcriptome analysis, blood and liver biochemistry were assessed.

Results: Compared to baseline, chow dietary intervention in DIO-NASH mice promoted a substantial weight loss of approx. 20% concurrent with reversal of hepatomegaly, and normalization of plasma markers of liver injury (transaminases) as well as plasma/liver lipids (total cholesterol, triglycerides). Notably, all DIO-NASH mice demonstrated ≥2 point significant improvement in NAS following dietary intervention for 8 weeks. Therapeutic effects of dietary intervention were supported by quantitative histological markers of steatosis (lipids, hepatocytes with lipid droplets) and inflammation (number of inflammatory cells/foci, galectin-3). In contrast, dietary intervention did not improve Fibrosis Stage, although histological markers for fibrosis (PSR, collagen 1a1) and activated stellate cells (α-SMA) were significantly reduced after 12 weeks of treatment, suggesting anti-fibrotic efficacy. Accordingly, dietary intervention improved NASH-associated transcriptome signatures, including lowered expression of gene markers of inflammation and fibrogenesis.

Conclusion: Dietary intervention improved metabolic, biochemical and liver histological markers of steatosis, inflammation and fibrosis in biopsy-confirmed DIO-NASH mice. Importantly, dietary intervention induced clinically relevant improvements in NAS. These findings highlight the beneficial effect of dietary intervention to promote regression of NASH. In combination with dietary intervention, emerging pharmacotherapies may therefore have the potential to further attenuate disease activity in the GAN DIO-NASH mouse.

PO-60
Evaluating use of small molecule inhibitors of integrin function in NAFLD

Emma Shepherd[1], Deepika Balakrishna[2], Ellie Northall[1], Mark Sabat[3], Daniel Carney[3], Ron De Jong[4], Hiroaki Yashiro[5], Derek Erion[5], Rohan Manohar[5], Patricia Lalor[1]
[1]University of Birmingham, UK, Centre for Liver and Gastroenterology Research, Birmingham, United Kingdom, *[2]Takeda Pharmaceuticals, Biological Sciences, Gastroenterology Drug Discovery Unit, San Diego, United States*, *[3]Takeda Pharmaceuticals, Medicinal Chemistry, Gastroenterology Drug Discovery Unit, San Diego, United States*, *[4]Ron de Jong Consulting, San Diego, United States*, *[5]Takeda Pharmaceuticals, Gastroenterology Drug Discovery Unit, Cambridge, United States*
Email: p.f.lalor@bham.ac.uk

Background and aims: Inhibition of TGF-β is a promising therapeutic approach to reduce organ fibrosis but is associated with safety concerns. Arg-Glu-Asp (RGD) integrins are known to be the principal mediators of TGF-β activation in chronic fibrosis, and integrin inhibition allows for localized and therapeutically safe inhibition of TGF-β. Of the many integrin heterodimers, more than half contain either alphaV or beta1 chains. Recent data suggest that the RGD integrin alphaVbeta1 plays a role in liver fibrosis but there remains limited data for alphaVbeta1 (αVβ1) inhibition in human NASH tissue and preclinical NASH models. The aim of this study was to investigate the effect of 2 αVβ1 inhibitory small molecules in human NASH tissue and a diet induced preclinical NASH model.

Method: Expression of αVβ1 heterodimers and phosphorylation of SMAD3 (pSMAD3) was determined using Meso Scale Discovery (MSD) analysis. Adhesion to LAP was measured by xCELLigence impedance assay and adhesion assays. The effect of αVβ1 inhibition *in vivo* was assessed in rats fed a Choline Deficient High Fat Diet (CDHFD) by MSD, qRT-PCR and histology. Effects of αVβ1 inhibitors in perfused donor and cirrhotic human liver wedges was assessed by histology and qRT-PCR.

Results: We report that αVβ1 integrin expression is up regulated in cirrhotic NASH tissue and was expressed on human hepatic stellate cells (HSCs) at higher levels than other primary liver cells. *In vitro* pharmacological inhibition of αVβ1 resulted in decreased pSMAD3 in an overexpressing cell line, and abrogated human HSC binding to LAP but did not affect binding to other extracellular matrix ligands including fibronectin. Inhibition of αVβ1 *in vivo* in CDHFD fed rats reduced pSMAD3, fibrogenic gene expression and histological liver fibrosis. Finally, perfusion of human liver wedges with an αVβ1 inhibitor resulted in decreased integrin expression and gene expression associated with fibrosis (for e.g., LOX, Col1A1, TGF-b1, PGDFRb, TIMP1 and CTGF).

Conclusion: In this study we have shown that 2 αVβ1 inhibitors caused ligand-specific blockade of HSC function *in vitro* and reduced liver fibrosis *in vivo*. Importantly, our data demonstrate that these inhibitors can be delivered to cirrhotic human liver tissue *ex vivo* and still mediate phenotype regulation. Taken together, these data suggest that integrin inhibition in the context of chronic human fibrosis may be beneficial as a therapeutic strategy.

PO-70
Design and evaluation of autophagy-inducing particles: towards a treatment of fatty liver disease?

Stavroula Zagkou[1 2]
[1]*LBTI-UMR5305, Lyon, France*, [2]*Adjuvatis, Lyon, France*
Email: stavzagkou@gmail.com

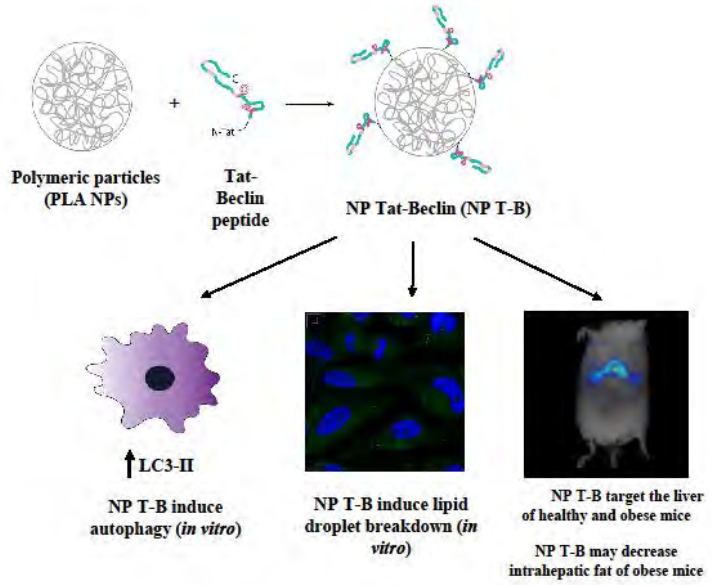
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome (MetS), a cluster of symptoms which include hyperlipidemia, hypertension and insulin resistance. MetS has become the major health hazard of the developed world and is a risk factor of cardiovascular disease, the major cause of death worldwide. NAFLD is characterized by an efflux of free fatty acids in the liver, where steatosis and lipotoxicity contribute to disease progression to steatohepatitis (NASH). Recently, a novel role of autophagy in the degradation of hepatic lipid droplets has come to light (Singh *et al.*, 2009). Various molecules have shown great promise in preclinical studies in the context of NAFLD or NASH, partially owing to their autophagy-inducing properties (Kim *et al.*, 2017; Lim *et al.*, 2018; Li *et al.*, 2019;). In this study, we aimed to improve the bioavailability and targeting of a specific autophagy inducer (Shoji-Kawata et al., 2013), the tat-beclin peptide, using polymeric particles for targeted delivery in the liver.

Method: A reproducible and stable formulation of tat-beclin (T-B) was developed by adsorbing it on biodegradable, biocompatible particles, made of poly- (lactic acid). Tat-beclin particles (NP T-B) and tat-scrambled control particles (NP T-S) were produced and characterized for their size, surface charge and adsorption efficiency. Autophagy modulation by tat-beclin and tat-scrambled, either in free form or in particles, was examined by western blot of LC3-II (marker of autophagosomes) in a model cell line (HeLa) and in hepatoma HepG2 cells. An *in vitro* model of steatosis was induced by incubating HeLa with a combination of oleic and palmitic acid and the ability of T-B or NP T-B to improve steatosis was examined by quantification of lipid droplets. Next, the ability of fluorescently labelled NP T-B to target the liver was examined in SKH1 (healthy) and genetically obese mice. Quantification of intrahepatic lipid in obese mice having received repeated injections of NP T-B was examined in liver tissue.

Results: NP T-B were more efficient in inducing autophagy compared to the T-B peptide, at a lower dose and autophagy induction was longer-lasting. When tested in an *in vitro* model of steatosis, NP T-B were as efficient in inducing lipid droplet (LD) breakdown as the free peptide, at a lower dose. Liver targeting of fluorescent tat-beclin particles was confirmed in SKH1 mice as well as in obese mice. Preliminary experiments suggest that NP T-B reduce the intrahapatic lipid of obese mice.

Conclusion: Given the beneficial effects of autophagy inducing particles *in vitro* and *in vivo*, and their capacity to target the liver of normal and obese mice, NP T-B could be a promising therapeutic tool in the context of NAFL or other liver disease, requiring further mechanistic investigation.

Figure:


Polymeric particles (PLA NPs)
Tat-Beclin peptide
NP Tat-Beclin (NP T-B)
LC3-II
NP T-B induce autophagy (*in vitro*)
NP T-B induce lipid droplet breakdown (*in vitro*)
NP T-B target the liver of healthy and obese mice
NP T-B may decrease intrahepatic fat of obese mice

PO-75
Crosstalk between ileal macrophage polarization and bacterial translocation in NASH: Role of PPAR alpha/delta dual activation

Andrew Hakeem[1], Yasmeen Attia[1], Aiman El-Khatib[2], Olfat Hammam[3]
[1]The British University in Egypt, Pharmacology and Biochemistry, Cairo, Egypt, *[2]Faculty Of Pharmacy Cairo University, Pharmacology and Toxicology, Cairo, Egypt*, *[3]Theodor Bilharz Research Institute, Pathology, Cairo, Egypt*
Email: andrew.hakeem@bue.edu.eg

Background and aims: A complex network of interrelated extrahepatic metabolic and inflammatory insults appear to converge towards the progression of non-alcoholic steatohepatitis (NASH). Congruent lines of evidence point out that metabolic rewiring of macrophages lie at the center of their functional plasticity. As such, peroxisome proliferator-activated receptor (PPAR) activation has been previously addressed in modulating macrophage polarization (MP) in different tissues. However, the role of PPAR activation on ileal MP and whether a likely impact on intestinal integrity and bacterial translocation (BT) in a NASH setting exists, has not been deciphered yet. We herein investigate whether dual stimulation of PPAR alpha/delta using elafibranor (ELA) would restore gut barrier function and mitigate BT in NASH by skewing intestinal MP and whether such alterations would be reflected onto the histological landscape of the liver.

Method: Eight-week old C57/BL6 male mice were fed a high fat diet for 12 weeks with concurrent administration of dextran sodium sulfate (DSS) in cycles to induce gut barrier disruption. Each DSS cycle consisted of a 7-day interval of DSS in drinking water (0.5%) followed by a 10-day interval of DSS-free water. Treatment with ELA (30 mg/kg/day, p.o.) was initiated starting week 8 of induction for a duration of 4 weeks. Gut permeability was assessed using fluorescein isothiocyanate (FITC)-dextran assay in serum samples. Histological analyses were carried out for liver, ileal and colonic sections. The ileal expression of toll-like receptor 4 (TLR4) and tight junctional claudin-1 as well as MP markers, inducible nitric oxide synthase (iNOS) and arginase1 (Arg1), was analyzed by immunohistochemistry.

Results: Macrovesicular steatosis and hepatocyte ballooning observed in the control group were ameliorated after treatment with ELA alongside restoration of normal hepatic architecture. Moreover, ELA-treated group demonstrated almost normal colonic mucosa and villous architecture. Increased intestinal permeability was also curbed by ELA, as evidenced by decreased FITC-dextran serum concentrations. Modulation of MP markers, on the other hand, was observed with ELA manifested in an increase in Arg1 expression paralleled with a decrease in iNOS expression in ileal epithelial lining cells of villi and crypt (Fig. 1). Ileal immunoreactivity of TLR-4 was also curtailed after ELA treatment. Additionally, the ileal expression of claudin-1, a key tight junction protein, surged with ELA treatment.

Conclusion: PPAR alpha/delta stimulation appeared to circumvent bacterial translocation and intestinal barrier dysfunction in NASH by driving macrophage polarization towards the M2 phenotype in ileum. These findings may suggest the use of dual PPAR alpha/delta agonists in large cohorts of NASH patients with concomitant intestinal barrier dysfunction.

Figure:

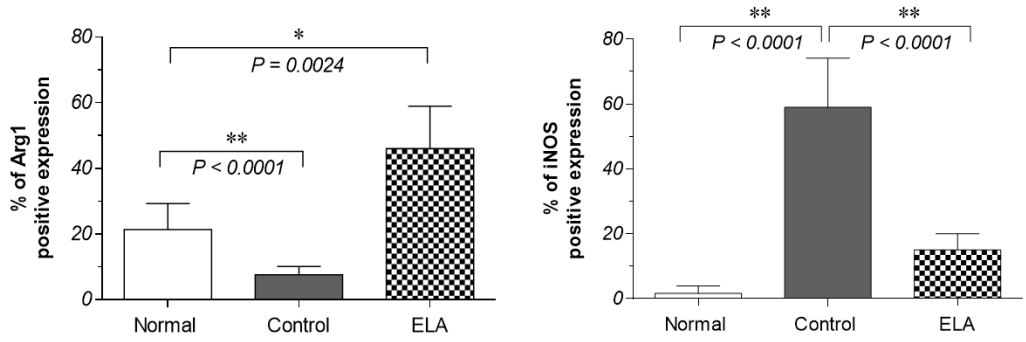


Fig. 1. Immunohistochemical analysis against Arg1 and iNOS in ileum sections of mice from normal, positive control, and elafibranor (ELA)-treated groups expressed as means ± S.D. of the percentages of positively stained cells. Statistical significance at **P<0.01 and **P<0.001* was determined using one-way ANOVA followed by Tukey's *post hoc* test.

PO-79
Receptor Activity-Modifying Protein (RAMP2) alters glucagon trafficking in hepatocytes with functional effects on signalling

Emma Rose McGlone[1], Yusman Manchanda[1], Ben Jones[1], Phil Pickford[1], Asuka Inoue[2], Dave Carling[3], Stephen Bloom[1], Tricia Tan[1], Alejandra Tomas-Catala[1]
[1]Imperial College London, Metabolism, Digestion and Reproduction, London, United Kingdom, *[2]Tohoku University, Graduate School of Pharmaceutical Sciences, Sendai, Japan*, *[3]Imperial College London, MRC London Institute of Medical Sciences, London, United Kingdom*
Email: e.mcglone@ic.ac.uk

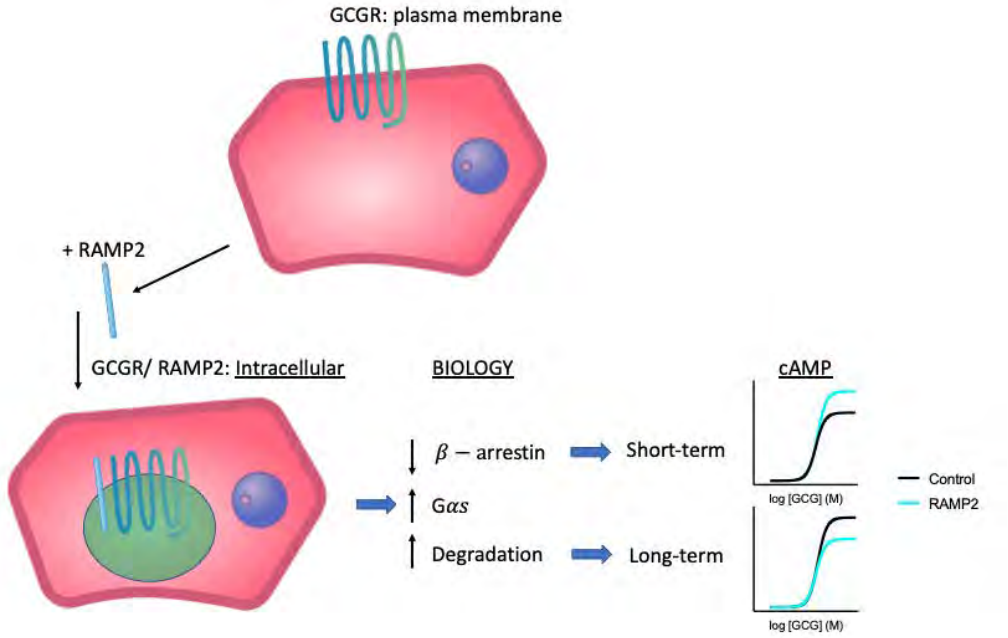
Background and aims: Glucagon decreases fat storage in the liver and manipulation of its signalling is a strategy for treating non-alcoholic fatty liver disease (NAFLD). Receptor Activity-Modifying Protein 2 (RAMP2) is an endogenous chaperone protein which interacts with the glucagon receptor (GCGR). The aims of this study were to investigate the effects of RAMP2 on GCGR trafficking and signalling in the liver.

Method: Subcellular localisation of GCGR in the presence and absence of RAMP2 was investigated using confocal microscopy, trafficking assays and radioligand binding assays in human embryonic kidney (HEK293T) and human hepatoma (Huh7) cells. Mouse embryonic fibroblasts (MEFs) lacking Wiskott Aldrich Syndrome protein and scar homologue (WASH) complex were used to investigate the effect of a halt in recycling of internalised proteins on GCGR signalling in the absence of RAMP2. NanoBiT complementation and cyclic AMP assays were used to study the functional effect of RAMP2 on recruitment and activation of GCGR signalling mediators. Response to hepatic RAMP2 up-regulation in lean and obese adult mice using a bespoke adeno-associated viral vector was also studied.

Results: GCGR is predominantly localised at the plasma membrane in the absence of RAMP2 and exhibits slow internalisation in response to agonist stimulation. Rapid intracellular retention of glucagon-stimulated GCGR in cells lacking WASH complex indicates that activated GCGRs undergo continuous cycles of internalisation and recycling despite apparent GCGR plasma membrane localisation up to 40 minutes post-stimulation. Co-expression of RAMP2 induces GCGR internalisation both basally and in response to agonist-stimulation. The intracellular retention of GCGR in the presence of RAMP2 confers a bias away from β-arrestin-2 recruitment coupled to increased activation of $G_{\alpha s}$ proteins at endosomes. This is associated with increased short-term efficacy for glucagon-stimulated cAMP production, although prolonged stimulation dampens signalling by increased receptor lysosomal targeting for degradation. Despite these signalling effects, only minor disturbance of carbohydrate metabolism was observed in mice with up-regulated hepatic RAMP2.

Conclusion: By retaining GCGR intracellularly, RAMP2 acutely increases GCGR signalling. Further exploration of the effects of RAMP2 on GCGR *in vivo* is warranted.

Figure:


GCGR: plasma membrane
+ RAMP2
GCGR/ RAMP2: Intracellular
BIOLOGY
cAMP
β − arrestin
Short-term
Gαs
Degradation
Long-term
log [GCG] (M)
log [GCG] (M)
Control
RAMP2

PO-87
Rescuing the brain from non-alcoholic fatty liver disease: role of the monocarboxylate transporter-1

Anna Hadjihambi[1 2], Christos Konstantinou[2], Jan Klohs[3], Luc Pellerin[1]
[1]*UNIL, Département des Sciences biomédicales, Lausanne, Switzerland*, [2]*The Institute of Hepatology, London, United Kingdom*, [3]*Institute for Biomedical Engineering, Zürich, Switzerland*
Email: ucbthad@ucl.ac.uk

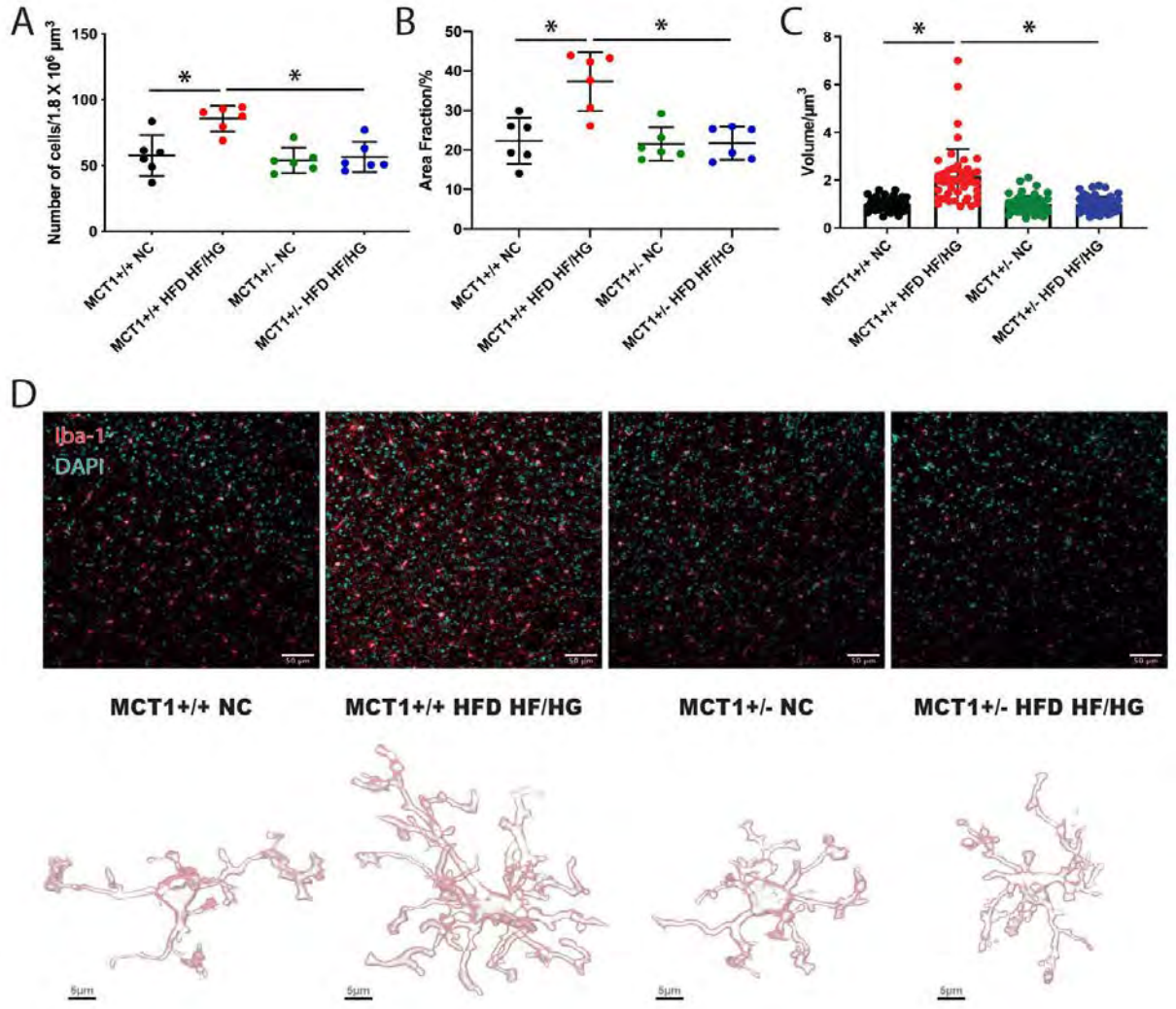
Background and aims: Non-alcoholic fatty liver disease (NAFLD) has recently been associated with mild cerebral dysfunction and cognitive decline, although the exact pathophysiological mechanism remains ambiguous. Using a model of NAFLD (high fat diet+high fructose/glucose in water [HFDHF/HG]) and monocarboxylate transporter-1 haploinsufficient mice ($MCT1^{\pm}$), which resist HFD-induced hepatic steatosis, we investigated alterations in cerebral physiology and cognition, as well as the role of MCT1.

Method: Behavioural tests were performed in mice following 16 weeks of control diet (NC; $MCT1^{+/+}$/$MCT1^{\pm}$+NC) or HFDHF/HG ($MCT1^{+/+}$/$MCT1^{\pm}$+HFDHF/HG). Neuroinflammation was evaluated via microglial immunofluorescence (Iba-1) and cortical mitochondrial oxygen consumption was measured *ex vivo* by high-resolution respirometry. Neuronal activity was assessed by *in vivo* electrophysiology. Cerebral blood volume (CBV), cortical partial pressure of oxygen (PO_2) at baseline and in response to systemic hypercapnia (10% CO_2) were monitored under anaesthesia by optoacoustic tomography and a fluorescence method. Cerebral blood flow (CBF) was measured via MRI and the presence of liver steatosis was confirmed by histology.

Results: $MCT1^{+/+}$+HFDHF/HG mice with NAFLD exhibited:
an anxiety and depression-related behaviour, but preserved learning and memory
cortical microglial activation (increased number, volume and area fraction)
lower CBV and baseline PO_2
preserved cerebrovascular reactivity, CBF and neuronal activity
compared to $MCT1^{+/+}$+NC controls. Interestingly, $MCT1^{\pm}$ mice on HFDHF/HG were resistant to all of the above brain alterations, indicating normal behaviour and cerebral physiology.

Conclusion: Our results suggest that NAFLD is associated with cerebral hemodynamic, metabolic and inflammatory events causing cognitive alterations. Haploinsufficient MCT1 mice are protected from these effects, thus suggesting a putative therapeutic target.

Figure:



<u>Figure 1:</u> NAFLD-induced microglial activation. Summary data illustrating **A)** number of Iba-1-microglia cells per 1.8 x 10^6 µm^3, **B)** % area fraction of Iba-1-microglia cells and **C)** volume of Iba-1-microglia cells in the somatosensory cortex of MCT1$^{+/+}$ and MCT1$^{\pm}$ mice on NC and HFDHF/HG. **D)** Representative confocal images (upper panel) and 3D reconstructions (lower panel) of Iba-1-microglia cells in the somatosensory cortex of MCT1$^{+/+}$ and MCT1$^{\pm}$ mice on NC and HFDHF/HG. *p <0.05

PO-88
Silencing Cyclin M4 induces hepatic magnesium accumulation to resolve NASH

Jorge Simón[1], Naroa Goikoetxea[2], Marina Serrano-Macia[2], David Fernández Ramos[3], Diego Saenz de Urturi[4], Jessica Gruskos[5], Pablo Fernández-Tussy[2], Sofia Lachiondo-Ortega[2], Irene González-Recio[2], Virginia Gutiérrez de Juan[6], Begoña Rodriguez Iruretagoyena[2], Marta Varela-Rey[2], Paula Gimenez-Mascarell[2], Maria Mercado-Gómez[2], Beatriz Gómez Santos[4], Carmen Fernández-Rodríguez[2], Fernando Lopitz Otsoa[6], Maider Bizkarguenaga[6], Sybille Dames[7], Ute Schaeper[7], Franz Martin-Bermudo[8], Guadalupe Sabio[9], Paula Iruzubieta[10], Javier Crespo[10], Patricia Aspichueta[11], Kevan H Chu[5], Daniela Buccella[5], Cesar Augusto Martín[12], Luis Alfonso Martinez-Cruz[2], María Luz Martínez-Chantar[1]
[1]CIC bioGUNE, Liver Disease Laboratory, CIBERehd, *[2]CIC bioGUNE, Liver Disease Laboratory*, *[3]CIC bioGUNE, Liver Disease Laboratory, Precision Medicine and Metabolism Laboratory, CIBERehd*, *[4]UPV/EHU, Department of Physiology*, *[5]New York University, Department of Chemistry*, *[6]CIC bioGUNE, Liver Disease Laboratory, Precision Medicine and Metabolism Laboratory*, *[7]Silence Therapeutics GmbH*, *[8]CABIMER, UPO, CSIC, CIBERdem*, *[9]CNIC*, *[10]Marqués de Valdecilla University Hospital, IDIVAL, Gastroenterology and Hepatology Department, Clinical and Traslational Digestive Research Group*, *[11]UPV/EHU, Biocruces Health Research Institute, CIBERehd, Department of Physiology*, *[12]UPV/EHU, Biofisika, Department of Biochemistry*
Email: jsimon@cicbiogune.es

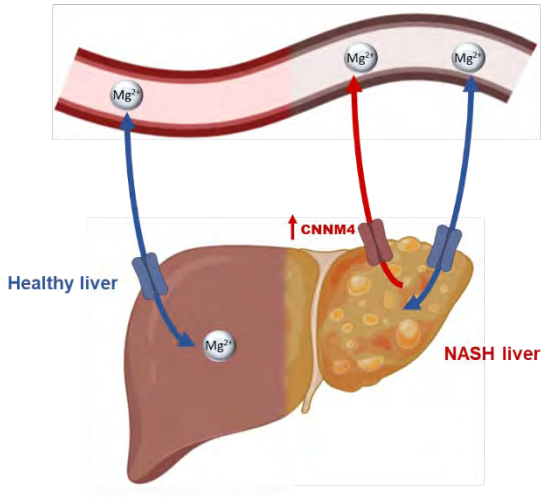
Background and aims: Perturbations of intracellular magnesium (Mg^{2+}) homeostasis have implications for cell physiology. In particular, deficiencies of the cation have been identified in cirrhosis and liver cancer, whereas the supplementation of the cation has proved to reduce mortality derived from liver diseases. Among all magnesiotropic proteins, the cyclin M family, CNNM, perform key functions in the transport of Mg2+ across cell membranes. Although they have been previously characterized to interact with phosphatases of regenerating liver (PRLs) involved in tumour development, the role of CNNMs in the liver remains poorly understood.

Method: Clinical characterization of serum Mg^{2+} levels and hepatic CNNM4 expression. Primary hepatocytes cultured under methionine and choline deprivation. In vivo rodent NASH models: 0.1% methionine and choline-deficient and choline-deficient high-fat diets. Cnnm4 was silenced using siRNA, in vitro with DharmaFECT and in vivo with Invivofectamine or siRNA conjugated to N-acetylgalactosamine.

Results: Patients with NASH showed hepatic CNNM4 overexpression and dysregulated Mg^{2+} levels in the serum. Correlated to this, CNNM4 expression was also upregulated in rodent NASH models. *Cnnm4* silencing ameliorated lipid accumulation *in vitro* and hepatic lipid accumulation, inflammation and fibrosis in the rodent NASH models. Mechanistically, CNNM4 knockdown in hepatocytes induced cellular Mg^{2+} accumulation, which reduced the production of reactive oxygen species (ROS) and the appearance of endoplasmic reticulum (ER) stress. The reduced ER stress might lead to an increased microsomal triglyceride transfer activity, which promoted hepatic lipid clearance by increasing the secretion of very-low-density lipoprotein (VLDL). The magnesium restoration in secreted VLDL might promote lypolisis and beta-oxidation in the adipose tissue preventing the appearance of atherogenic secondary effects. The conjugation of siRNA with GalNAc allows a stable and effective delivery to the liver, leading to an improvement of NASH.

Conclusion: Hepatic CNNM4 is a valuable therapeutic target for treating NASH.

Figure:



PO-95
GTX-011 improves portal hypertension, liver fibrosis and endothelial dysfunction in pre-clinical non-alcoholic steatohepatitis

Maria Andres-Rozas[1], Zoe Boyer-Diaz[1], Eugenia Ruiz-Canovas[2], Peio Aristu[1], Noemi Garcia-Delgado[2], Jaume Mercade[2], Jaime Bosch[3], Jordi Gracia-Sancho[4]
[1]Barcelona Liver Bioservices-BLB, *[2]GAT Therapeutics*, *[3]Hepatology-Inselspital*, *[4]Liver Vascular Biology Lab, IDIBAPS Biomedical Research Institute*
Email: jordi.gracia@idibaps.org

Background and aims: Non-alcoholic steatohepatitis (NASH) is a hepatic metabolic disease that can lead to cirrhosis and the need for liver transplantation in more advanced stages. The prevalence of the disease is increasing, thus intensifying the need to find an effective treatment. Considering that liver fibrosis and portal hypertension are the strongest prognostic markers in advanced NASH, the present study evaluated the effects of a new small molecule with strong anti-fibrotic properties (GTX-011) on hepatic hemodynamics and fibrosis, as well as its underlying mechanisms, in pre-clinical NASH.

Method: Male Wistar rats with NASH, induced by 10-week high-fat high-cholesterol diet combined with CCl_4 and phenobarbital, were randomly assigned to receive two different concentrations of GTX-011 (1 or 10 mg/kg/day p.o.) or vehicle, for 14 days (n = 15/group). In vivo systemic and hepatic hemodynamics (mean arterial pressure; portal pressure, PP; portal blood flow, PBF), fibrosis (Sirius red staining), hepatic stellate cell activation (alpha-SMA), liver sinusoidal endothelial cell (LSEC) de-differentiation (p-eNOS/eNOS, adhesion molecules), biochemical and metabolic parameters were analyzed. A validation pre-clinical model of advanced cirrhosis (chronic thioacetamide, TAA) was included.

Results: NASH rats receiving GTX showed significantly lower PP compared with vehicle-treated animals, in a dose-dependent manner (-8% p = 0.05 and -12% p = 0.005 for GTX 1 and 10 mg/kg, respectively) without changes in PBF or in systemic hemodynamics. Furthermore, they exhibited a reduction of hepatic fibrosis (-20% p = 0.08 and -28% p = 0.009), suggesting that the observed hemodynamic effects might respond to a reduced intrahepatic vascular resistance. At the cellular level, GTX-treated rats showed an improvement of LSEC phenotype, evidenced by an increased p-eNOS/eNOS ratio (+34% p = 0.13 and +80% p = 0.009) and a decreased expression of adhesion molecules (ICAM1, -30% p = 0.03 and -36% p = 0.01; vCAM1, -38% p = 0.08; and E-Selectin, -57% p = 0.03 and -33% p = 0.1). Treated animals also showed an improved metabolic profile, with reduced serum FFA (-30% p = 0.01 and -18% p = 0.18) and insulin resistance (glucose tolerance test, p = 0.001), along with a reduction trend in alpha-SMA (-23% p = 0.18 and -28% p = 0.09). Altogether, these results suggest that the mechanisms mediating GTX beneficial effects might heavily rely on endothelial phenotype restoration. Lastly, beneficial effects of GTX on hepatic hemodynamics were confirmed in the TAA rat model of cirrhosis.

Conclusion: This study shows for the first time the beneficial effects of GTX-011 on portal hypertension and liver fibrosis in pre-clinical NASH, encouraging its clinical evaluation as a possible new treatment for this disease.

PO-100
Tocotrienol rich fraction activates farnesoid-x receptor and modulates metabolites profile of non-alcoholic fatty liver disease in mice model

Mohd Danial Mohd Efendy Goon[1 2], Siti Hamimah Sheikh Abdul Kadir[2 3], Sharaniza Ab Rahim[3], Musalmah Mazlan[3], Mardiana Abdul Aziz[4], Norizal Mohd Noor[4]

[1]Institute For Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA (UiTM), Cawangan Selangor, Sungai Buloh, Malaysia, *[2]Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForm), Universiti Teknologi MARA (UiTM), Cawangan Selangor, Sungai Buloh, Malaysia*, *[3]Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Sungai Buloh, Malaysia*, *[4]Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Sungai Buloh, Malaysia*
Email: sitih587@uitm.edu.my

Background and aims: The use of antioxidant such as vitamin E or its isomer in non-alcoholic fatty liver disease (NAFLD) is recommended, despite its unclear mechanism of action. Thus far, knowledge on its modulation on metabolites of NAFLD remained limited. This study was conducted to investigate whether tocotrienol rich fraction (TRF) supplementation is able to reduce NAFLD characteristics in mice model by modulating the serum metabolites and liver key nuclear receptor, the farsenoid-x receptor (FXR) expression.

Method: Twenty-one B6-CgLepOb/J male mice at seven weeks old were randomly divided into three groups; high-fat diet (HFD; 60% kcal fat) only, HFD supplemented with palm-kernel oil (PKO; TRF vehicle) and HFD supplemented with TRF (TRF). Supplements were given at 200 mg/kg daily for 6 weeks. Body weight and food intake were recorded weekly. At the end of the study, abdominal circumference and random blood glucose were measured. All livers were collected for histological analysis and FXR immunohistochemistry staining. Meanwhile, sera were collected for untargeted metabolomics analysis.

Results: No significant changes on food intake, body weight, abdominal circumference and random blood glucose were observed in all groups ($p > 0.05$). Smaller liver span was measured in TRF supplemented group in comparison to HFD group ($p < 0.05$). HFD and PKO groups showed pancinar steatosis with substantial fibrosis and inflammation. TRF group showed only Zone 3 liver steatosis with minimal fibrosis and inflammation. Metabolites profiled demonstrated downregulation of primary and secondary bile acids in TRF group in comparison with other two groups. Significant increase in FXR translocation was observed in TRF group compared to both control groups ($p < 0.05$). Downregulation of anti-inflammatory metabolites such sphingolipids ($p < 0.05$) and allantoic acid ($p < 0.05$) were also seen in TRF group. Beta-oxidation was most likely improved with TRF as upregulation of N6, N6, N6-Trimethyl-L-lysine and downregulation of L-acetylcarnitine ($p < 0.05$) were observed.

Conclusion: TRF may have no effect on NAFLD physical parameters and blood glucose level, yet it improves liver histology and limiting liver enlargement which could be mediated by its ability to regulate FXR, bile acids metabolism, anti-inflammatory pathways, and beta-oxidation. These findings contribute to the understanding of TRF in alleviating NAFLD with its relation to FXR expression and associated metabolites.

PO-101
Characterization of disease progression and pharmacological intervention in the GAN diet-induced obese mouse model of NASH with advanced fibrosis and hepatocellular carcinoma

Michael Feigh[1], Mathias Møllerhøj[1], Sanne Veidal[1], Martin Rønn Madsen[1], Henrik B. Hansen[1]
[1]*Gubra*
Email: mfe@gubra.dk

Background and aims: Obesity-associated non-alcoholic steatohepatitis (NASH) predisposes to the development of severe fibrosis and hepatocellular carcinoma (HCC), thus pharmacological interventions targeting metabolic NASH and fibrosis might also affect HCC. The present study aimed to characterize disease progression and evaluate treatment response for the late-stage clinical candidate elafibranor (peroxisome proliferator-activated receptor α/δ agonist) in the extended GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH.

Method: Male C57Bl/6J mice were fed the GAN diet (40 kcal-% fat, 22% fructose, 10% sucrose, 2% cholesterol) for up to 88 weeks (n = 12-15/group). Disease progression in DIO-NASH mice was evaluated by liver histopathological NAFLD Activity Score (NAS) and Fibrosis Stage, histomorphometry, macroscopic tumor evaluation and liver transcriptome analysis. DIO-NASH mice with extended GAN diet-induction (60 weeks) and biopsy-confirmed steatosis (score ≥2), advanced fibrosis (stage F3) and HCC (DIO-NASH-HCC) received treatment (PO, QD) with vehicle (n = 16), elafibranor (30 mg/kg, n = 14) for 12 weeks. Age-matched lean control animals (n = 10) received vehicle.

Results: DIO-NASH animals demonstrated progressive NASH with consistent development of liver fibrosis from 28 weeks on diet. Advanced bridging fibrosis (stage F3) and tumour development including HCC was consistently observed from ≥58 weeks on diet. Hepatic transcriptome signatures were consistent with severe disease progression in DIO-NASH mice. In DIO-NASH-HCC mice, elafibranor treatment demonstrated ≥2-point significant improvement in NAFLD Activity Score and promoted a 1-stage significant improvement in Fibrosis Stage, compared to vehicle group. Therapeutic effects of elafibranor were supported by quantitative histological markers of steatosis (lipids, hepatocytes with lipid droplets), inflammation (number of inflammatory cells/foci, galectin-3), fibrogenesis (α-SMA) and fibrosis (PSR, collagen 1a1). Elafibranor had no effect on macroscopically evaluated tumor numbers and size, although improved HCC-associated transcriptomic signatures including lowered expression of hepatic genes involved in apoptosis, inflammation, cell cycle and proliferation.

Conclusion: DIO-NASH mice show progressive advanced fibrosis and high HCC incidence. Elafibranor treatment improved hepatic steatosis, inflammation and fibrosis in DIO-NASH-HCC mice with advanced fibrosis, but had no effect on HCC burden. The extended GAN DIO-NASH-HCC mouse model is suitable for profiling novel drug therapies for advanced fibrosing NASH and HCC.

PO-102
Subclinical liver damage detection by NMR spectroscopy

María Martín Grau[1], María de las Mercedes Pardo Tendero[1], José Manuel Morales Tatay[1], Vannina Gonzalez Marrachelli[2], Daniel Monleón Salvadó[1]

[1]University of Valencia, Pathology, Spain, *[2]University of Valencia, Physiology, Spain*
Email: maria.martin-grau@uv.es

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a complex disease in which there are different stages, being only the first step asymptomatic and reversible. Currently, many studies are focused on the discovery and development of non-invasive biomarkers for detecting NAFLD in this subclinical and early stage. Metabolomics is an omic technique based on the study of cell metabolism and therefore can help in the detection of early molecular alterations. The objective of this work is the identification of metabolomic biomarkers in the early stages of NAFLD development.

Method: Male and female Wistar rats 16 weeks old were randomly divided into different groups based on sex and diet, fed with a control diet (CTL group) or with a 45% high-fat diet (HFD group). After 12 weeks, the animals were sacrificed and the livers extracted. At the initial and end time, serum samples were obtained from all the animals. The serum samples were analyzed by Proton Nuclear Magnetic Resonance (^{1}H-NMR) and the liver samples by ^{1}H-NMR High-Resolution Magic Angle Spinning (HR-MAS). Additionally, a histological and biochemical study was carried out. Significant differences among groups (t-student, ANOVA) at the 95% confidence level ($p < 0.05$) were identified.

Results: We observed histological and biochemical differences associated with diet although many of them did not reach statistical significance. At the histological level, an accumulation of fat without a development of fibrosis was observed in HFD male and female groups. The biochemical analysis (glucose, triglycerides and cholesterol) revealed significant differences between CTL and HFD males but there were no differences between CTL and HFD females. Regarding metabolomics results, the Principal Component Analysis (PCA) divided the global data into different groups based on sex and diet. Not only there were diet-specific metabolites observed, but also there were sex-specific metabolites. The main altered metabolites (VIP score ≥1) in serum and liver were related to the TCA cycle and microbial metabolism.

Conclusion: Metabolomics allowed the detection of early metabolic changes in both sexes not yet observed with other techniques. This suggests a potential role of metabolomics for the search of new specific biomarkers in the NAFLD development.

PO-107
Hepatic steatosis contributes to the development of muscle atrophy via inter-organ crosstalk

Kenneth Pasmans[1], Michiel Adriaens[2], Peter Olinga[3], Ramon Langen[4], Sander Rensen[5], Frank Schaap[5] [6], Steven Olde Damink[5] [6], Florian Caiment[7], Luc van Loon[1], Ellen Blaak[1], Ruth Meex[1]

[1]Maastricht University, Department of Human Biology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht, Netherlands, *[2]Maastricht University, Maastricht Centre for Systems Biology, Maastricht, Netherlands*, *[3]University of Groningen, Department of Pharmaceutical Technology and Biopharmacy, Groningen Research Institute of Pharmacy, Groningen, Netherlands*, *[4]Maastricht University, Department of Respiratory Medicine, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht, Netherlands*, *[5]Maastricht University, Department of Surgery, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht, Netherlands*, *[6]RWTH University Hospital Aachen, Department of General, Visceral and Transplantation Surgery, Aachen, Germany*, *[7]Maastricht University, Department of Toxicogenomics, School of Oncology and Developmental Biology (GROW), Maastricht, Netherlands*
Email: k.pasmans@maastrichtuniversity.nl

Background and aims: Individuals with hepatic steatosis often display several metabolic abnormalities including insulin resistance and muscle atrophy. Previously, we found that hepatic steatosis results in an altered hepatokine secretion profile, thereby inducing skeletal muscle insulin resistance via inter-organ crosstalk. In this study, we aimed to investigate whether the altered secretion profile in the state of hepatic steatosis also induces skeletal muscle atrophy via effects on muscle protein turnover.

Method: Eight-week-old male C57BL/6J mice were fed a chow (4.5% fat) or a high-fat diet (HFD; 45% fat) for 12 weeks to induce hepatic steatosis, after which the livers were excised and cut into slices with a thickness of approximately 200 µm. Slices were cultured to collect secretion products (conditioned medium; CM). Differentiated L6-GLUT4myc myotubes were incubated with chow or HFD CM to measure glucose uptake. Differentiated C2C12 myotubes were incubated with chow or HFD CM to measure protein synthesis and breakdown, and gene expression via RNA sequencing. Furthermore, proteomics analysis was performed in chow and HFD CM.

Results: HFD CM caused insulin resistance in L6-GLUT4myc myotubes compared with chow CM, as indicated by a blunted insulin-stimulated increase in glucose uptake. Furthermore, protein breakdown was increased in C2C12 cells incubated with HFD CM, while there was no effect on protein synthesis. RNA profiling of C2C12 cells indicated that 197 genes were differentially expressed after incubation with HFD CM, compared with chow CM, and pathway analysis showed that pathways related to anatomical structure and function were enriched. Proteomic analysis of the CM showed that 32 proteins were differentially expressed in HFD CM compared with chow CM. Pathway enrichment analysis indicated that these proteins had important functions with respect to insulin-like growth factor transport and uptake, and that they affect post-translational processes, including protein folding, protein secretion and protein phosphorylation.

Conclusion: The results of this study support the hypothesis that secretion products from the liver contribute to the development of muscle atrophy in individuals with hepatic steatosis.

PO-110
Hepatocyte cholesterol content modulates glucagon receptor signalling

Emma Rose McGlone[1 1], Ben Jones[1], Cecilia Dunsterville[1], Alejandra Tomas-Catala[2], Dave Carling[2], Tricia Tan[1], Stephen Bloom[1]

[1]Imperial College London, Department of Metabolism, Digestion and Reproduction, London, United Kingdom, *[2]Imperial College London, MRC London Institute of Medical Sciences, London, United Kingdom*
Email: e.mcglone@ic.ac.uk

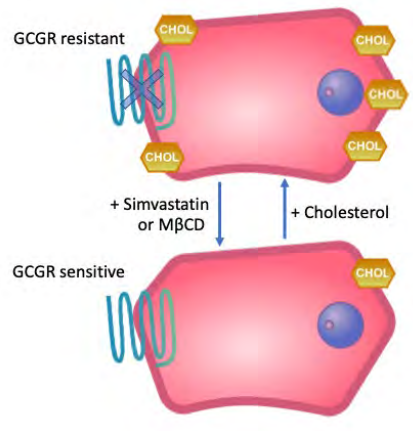
Background and aims: The glucagon receptor (GCGR) is a class B G protein-coupled receptor (GPCR), activation of which decreases lipid accumulation in hepatocytes. Glucagon resistance is a feature of non-alcoholic fatty liver disease (NAFLD), though the mechanism via which this occurs is unclear. There is increasing evidence that the local lipid environment regulates the functioning of plasma membrane GPCRs. The aim of this study was to evaluate the impact of experimentally modulating the cholesterol content of hepatocyte membranes on GCGR signalling, ligand uptake and glucose output.

Method: Human hepatoma cells (Huh7) and primary mouse hepatocytes were loaded or depleted of cholesterol acutely using methyl-β-cyclodextrin (MβCD) saturated or not with cholesterol, respectively; treatment with simvastatin (an HMG CoA reductase inhibitor) was also used to lower cholesterol content. Glucagon-stimulated cyclic AMP production, agonist-uptake and glucose output were measured. To manipulate hepatic cholesterol in vivo, mice were fed diets with variable cholesterol content with or without simvastatin for one week, and blood glucose responses to a combined glucagon/pyruvate challenge test were measured.

Results: Cholesterol depletion with MβCD in Huh7 cells enhanced glucagon-mediated cyclic AMP production and reduced uptake of fluorescent glucagon, with opposite effects observed following cholesterol loading. In primary mouse hepatocytes, cyclic AMP signalling was also decreased by cholesterol loading, while ex vivo glucose output was enhanced by overnight simvastatin treatment. Mice with increased hepatic cholesterol due to the high cholesterol diet showed a blunted hyperglycaemic response to the glucagon/pyruvate challenge test, which was partially restored by inclusion of simvastatin.

Conclusion: Using both *in vitro* and *in vivo* models, we show that hepatic cholesterol content is inversely related to glucagon sensitivity. Increased hepatic cholesterol could provide a mechanism via which glucagon resistance occurs in NAFLD. Our findings suggest that this effect can be lessened by inhibiting de novo cholesterol synthesis.

Figure:



PO-111
Transcriptomic signature of high fat diet murine models of NAFLD and its modulation by anti-steatotic treatments: a meta-analysis

Isabel Fuster-Martínez[1 2], José F. Català-Senent[3 4], Francisco J. Roig[3 5], Juan V. Esplugues[1 2 6], Nadezda Apostolova[1 2 6], Francisco García-García[3 5], Ana Blas-García[2 6 7]

[1]Universitat de València, Departamento de Farmacología, Valencia, Spain, *[2]FISABIO (Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana), Valencia, Spain*, *[3]Principe Felipe Research Center, Bioinformatics and Biostatistics Unit, Valencia, Spain*, *[4]ELIXIR-Spain (INB, ELIXIR-ES), Spanish National Bioinformatics Institute, Valencia, Spain*, *[5]Universidad San Jorge, Facultad de Ciencias de la Salud, Zaragoza, Spain*, *[6]CIBERehd (Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas), Spain*, *[7]Universitat de València, Departamento de Fisiología, Valencia, Spain*
Email: isabel.fuster-martinez@uv.es

Background and aims: High fat diet (HFD) murine models have been widely used as preclinical models of NAFLD. Hence, the identification of its complete hepatic transcriptomic signature, the characterization of the transcriptional impact exerted by different anti-steatotic treatments (AST) and a comparison with human NAFLD transcriptomic data would be relevant to understand the potential of these models in the development of effective therapies.

Method: We carried out a systematic review in GEO for transcriptomic studies that compare the hepatic gene expression profile of wild type C57BL6/J mice fed with HFD with that of control mice (normal diet), and with that of HFD mice receiving AST. After performing a differential gene expression analysis and biological processes enrichment analysis with Gene Ontology, we conducted a meta-analysis using a random-effects method to define the hepatic signature of HFD mice at gene expression and at functional level. Then, we evaluated the capacity of each AST to modulate this transcriptomic signature. We also identified the genes generally reversed or not by the AST. Finally, we compare our results with a transcriptomic signature of human NAFLD progression from the literature.

Results: In the systematic review, 62 full articles were assessed and 19 were selected with 22 different AST. We obtained a transcriptomic signature of HFD murine model of 2670 genes, 60 of which were part of the 218 genes associated with at least one trait of human NAFLD progression in a previous published meta-analysis. At the functional level, HFD showed 2567 biological processes altered, with predominance of those involved in inflammation, cellular stress, cell cycle, metabolism and extracellular matrix organization. Nevertheless, AST in general partially reversed the HFD transcriptomic signature, significantly normalizing the expression of 369 genes but not that of 538. Among the 60 genes with evidence to be altered in human NAFLD, 10 were of those generally reversed by AST and 12 of those that were not.

Conclusion: This work offers valuable data defining a robust hepatic transcriptomic signature of HFD-induced model of NAFLD and a group of genes that are consistently altered with the different anti-steatotic treatments. These results increase our knowledge of molecular mechanisms of NAFLD and could contribute to the discovery of new biomarkers and therapeutic targets.

PO-117
Myeloid-specific fatty acid transport protein 4 deficiency in mice induces a shift towards M2 macrophages that leads to aggravation of NASH after high-fat/high-cholesterol feeding

Deniz Göcebe[1], Chutima Jansakun[1], Simone Staffer[1], Sabine Tuma-Kellner[1], Sandro Altamura[2], Martina Muckenthaler[2], Uta Merle[1], Walee Chamulitrat[1]

[1]Heidelberg hospital university, internal medicine IV, Heidelberg, Germany, *[2]Heidelberg hospital university, pediatric oncology, hematology and immunology, Heidelberg, Germany*
Email: denizgoecebe@gmail.com

Background and aims: Mutations of FATP4 are associated with blood lipids and insulin resistance, and these patients exhibit not only skin hyperkeratosis but also M2/Th2-related allergies and eosinophilia. We hypothesized that FATP4 deficiency in macrophages may contribute to pathogenesis of NASH because this disease is also linked to type 2 immunity. We determined the effects of myeloid-specific Fatp4 deficiency on macrophage polarization and diet-induced NASH.

Method: LysM-Cre Fatp4-deficient mice (KO) with exon 3 deletion were generated. Complete blood counts from male and female control and KO mice were analysed. Cytokine release and gene expression were analysed in bone marrow-derived macrophages (BMDMs) from male mice with or without in vitro LPS for 24 h. Male and female control and KO mice were fed with high-fat (15%)/high-cholesterol (1.25%) (HFHC) diet for 16 weeks. Plasma cytokines, hepatic gene expression, and liver histology were analyzed.

Results: Male KO mice showed an increase in % monocytes and granulocytes while female KOs showed a decrease in % monocytes. BMDMs from male KO mice showed an increase in spontaneous release of M2/Th2 IL-4 and IL-13 as well as spontaneous and LPS-induced expression of MCP-1. This deficiency on the other hand attenuated LPS-induced expression and release of M1 TNF-a, IL-6, and IL-1b. After HFHC feeding, both male and female mutants showed a further elevation of plasma IL-4, IL-13, and MCP-1, while that of plasma IL-5 and IL-6 was observed only in female mutants. On the other hand, HFHC-induced elevation of plasma TNF-a was attenuated in both male and female mutants. Strikingly, HFHC feeding induced a marked increase of hepatic steatosis in male mutants but a massive hepatic infiltration of immune cells in female KOs. Both male and female KO mice showed an increase of HFHC-induced liver fibrosis and hepatic expression of MCP-1 protein. Correspondingly, male mutants showed increased mRNA expression of collagen 1a1, collagen 3a1, Timp1, Icam1, and Vcam1.

Conclusion: Myeloid-specific Fatp4 deficiency led to a shift from M1 towards M2 cytokines IL-4, IL-13 and MCP-1 at the level of BMDMs and HFHC-induced NASH. Such increase of Th2 immunity led to aggravated liver fibrosis in both male and female mutants. Hepatic steatosis and inflammatory IL-5/IL-6 was prominent in male and female mutants, respectively. Our findings may have some implications for patients with FATP4 mutations who consume HFHC-rich diets.

PO-124
Microbiome metabolites aggravate hepatic lipid deposition by decreasing mitochondrial function in an in vitro model of non-alcoholic fatty liver disease

Paula Boeira[1], Charles Affourtit[1], Daniel Felmlee[1], Matthew Cramp[1], David Sheridan[1]
[1]*University of Plymouth, Faculty of Health: Medicine, Dentistry and Human Sciences, Plymouth, United Kingdom*
Email: paula.boeira@plymouth.ac.uk

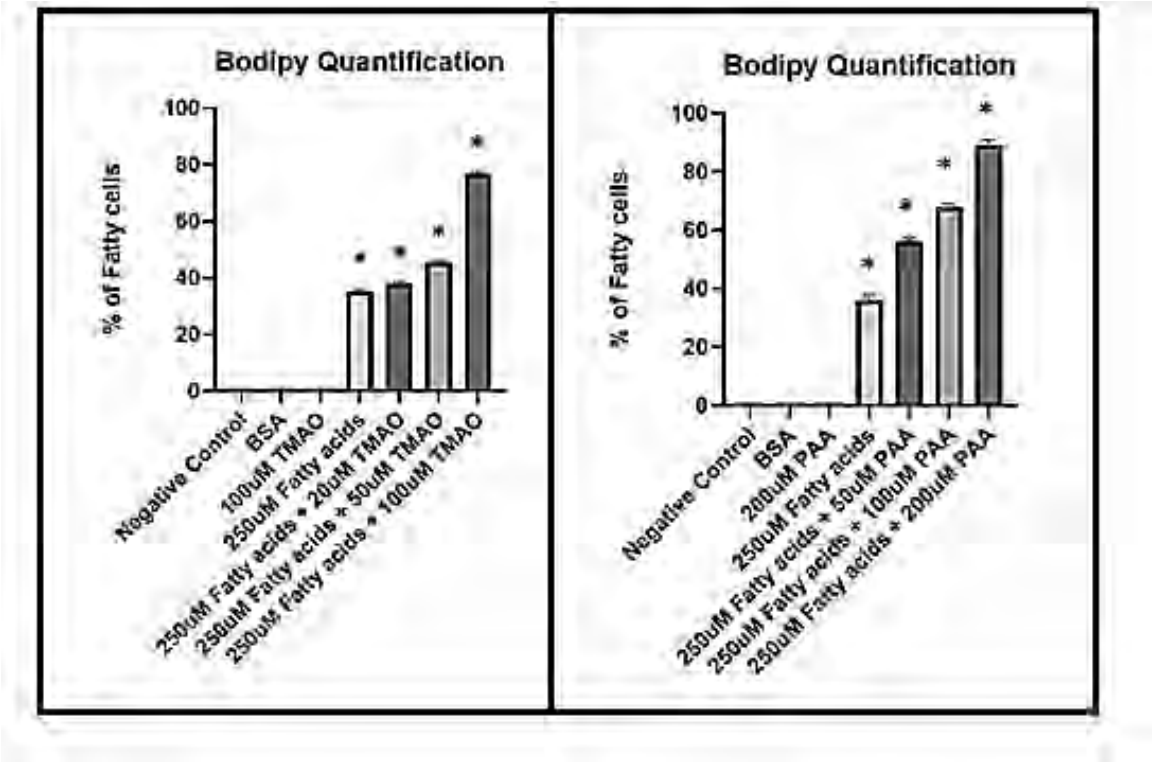
Background and aims: Several dietary metabolites produced by gut bacteria have been linked to disease including non-alcoholic fatty liver disease (NAFLD). Trimethylamine N-oxide (TMAO) and phenylacetic acid (PAA) are microbiome-derived metabolites that have been associated with early onset of NAFLD. Hypothesising that these metabolites contribute to lipid deposition in the liver by altering hepatic mitochondrial function, we assessed how TMAO and PAA affect hepatocyte bioenergetics in cell models of liver steatosis.

Method: HepaRG cells were cultured under standard conditions, and steatosis was established by 48h exposure to oleate and palmitate (2:1 molar ratio). Lipid accumulation was assessed by BODIPY™ staining and quantified by CellProfiler software. To gain insight into HepaRG mitochondrial respiration, we profiled various bioenergetic parameters utilizing the Seahorse extracellular flux analyser in control cells and cells exposed to PAA (100uM and 200uM) or TMAO (20uM, 50uM, 100uM and 200uM).

Results: PAA and TMAO led to a significant increase in lipid deposition. With fatty acids alone 35% of cells were steatotic, while in the presence of 200uM PAA fat accumulation was observed in 89% of the cells and in 77% with 100uM TMAO. The same effects were seen in mitochondrial function. PAA and TMAO lowered spare respiratory capacity dose-dependently in HepaRG cells and mitochondrial changes preceded lipid accumulation.

Conclusion: Acquired data indicate that TMAO and PAA exacerbate hepatic lipid deposition by decreasing mitochondrial function suggesting a potential link between microbiome metabolite driven mitochondrial dysfunction and NAFLD onset. Agents targeting mitochondrial dysfunction are promising for NAFLD therapeutic intervention.

Figure:


Bodipy Quantification
% of Fatty cells
100
80
60
40
20
0
Negative Control
BSA
100uM TMAO
250uM Fatty acids
250uM Fatty acids + 20uM TMAO
250uM Fatty acids + 50uM TMAO
250uM Fatty acids + 100uM TMAO
Bodipy Quantification
% of Fatty cells
100
80
60
40
20
0
Negative Control
BSA
200uM PAA
250uM Fatty acids
250uM Fatty acids + 50uM PAA
250uM Fatty acids + 100uM PAA
250uM Fatty acids + 200uM PAA

PO-131
Sterol lipids are associated with the plasticity of adipose tissue and promote a different response in women and men with severe obesity

Gerard Baiges[1 2], Elisabet Rodríguez-Tomàs[1], Helena Castañé[1 2], Salvador Fernández-Arroyo[3], Andrea Jiménez-Franco[1], Julia Martí-Clofent[1], Jordi Camps[1 4], Jorge Joven[1 2 4]

[1]Rovira i Virgili University, Tarragona, Spain, *[2]Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain*, *[3]Eurecat-Centro Tecnológico de Cataluña, Reus, Spain*, *[4]Hospital Universitari Sant Joan de Reus, Reus, Spain*
Email: gerardbaiges93@gmail.com

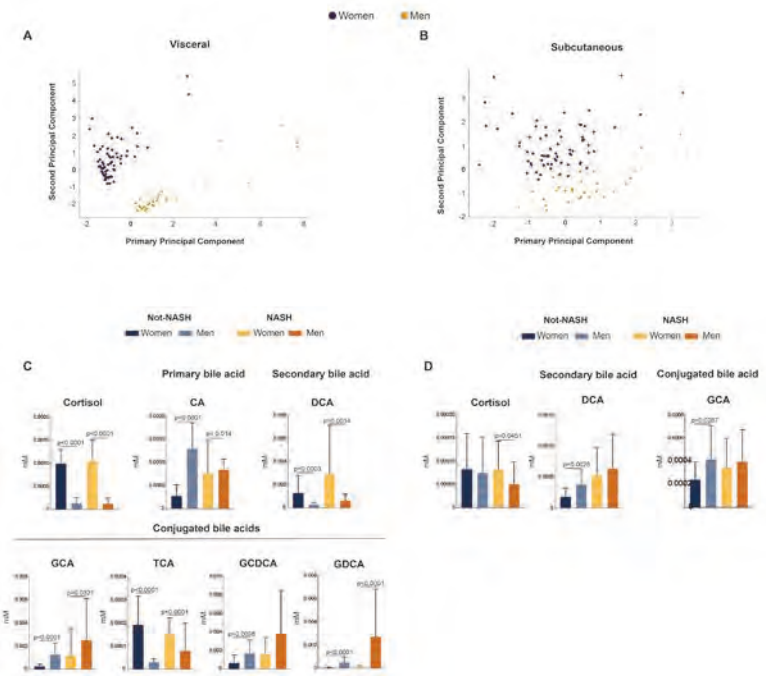
Background and aims: Obesity is a metabolic condition linked to metabolic associated fatty liver disease. However, when we put the focus on the sex variable a new paradigm is observed. The metabolic context in women tends to store the excess of energy in the subcutaneous adipose tissue likely to an innate capacity to promote the activation of adipogenesis and lipogenesis and as a consequence show less hepatic fat accumulation. In contrast, the metabolic context in men leads to an incapacity to storage the nutrient inputs in subcutaneous adipose tissue. Then, this excess tends to deposit in visceral adipose tissue, promoting a pro-inflammatory environment and favouring the hepatic fat accumulation. For this purpose, we aim is to explore the implication of lipids in the NASH transition taking into account the sex in a multi-organic analysis.

Method: Fifty-nine women and forty-three men with severe obesity candidates to undergo bariatric surgery were classified into the non-NASH (38 in women and 18 in men) and NASH (21 in women and 25 in men) groups. Samples of plasma, liver, omental visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) were used to perform a lipidomic analysis. The results obtained have been adjusted by FDR qvalue <0.05.

Results: Women with NASH showed alteration of sterol lipids species in all tissues. In contrast, men only display some alteration in the liver and plasma. To determine the magnitude of the results we perform a principal component analysis (PCA), and we observed a clear sex dimorphism of sterol lipids in VAT and SAT. The lipid species detected among sterol lipids were primary, secondary and conjugated bile acids and cortisol. We observed that cortisol levels of VAT were higher in women than men independently of the stage of the disease. By contrast, in SAT we observed a significant decrease in cortisol levels in men with NASH. Moreover, in VAT we observed that men showed a higher concentration of bile acids than women with the independence of the severity of the disease. On the other hand, in SAT only two bile acids species were higher in men than women.

Conclusion: The results presented herein propose that sterol lipids could be implicated in the metabolic plasticity of adipose tissue and promote a different response in women and men under the same phenotype.

Figure:


Women
Men
A
Visceral
Second Principal Component
Primary Principal Component
B
Subcutaneous
Second Principal Component
Primary Principal Component
Not-NASH
NASH
Women
Men
C
Primary bile acid
Secondary bile acid
Cortisol
CA
DCA
Conjugated bile acids
GCA
TCA
GCDCA
GDCA
D
Secondary bile acid
Conjugated bile acid
Cortisol
DCA
GCA

PO-132
A fast-induced model of fibrosing NASH: outcomes in different mice strains

Gabriel Bacil[1], Guilherme Romualdo[1], Bruno Cogliati[2], Luis Fernando Barbisan[1]

[1]São Paulo State University-UNESP, Department of Structural and Functional Biology, Botucatu, Brazil, *[2]School of Veterinary Medicine and Animal Science (FMVZ), Department of Pathology, São Paulo, Brazil*
Email: gabriel.bacil@unesp.br

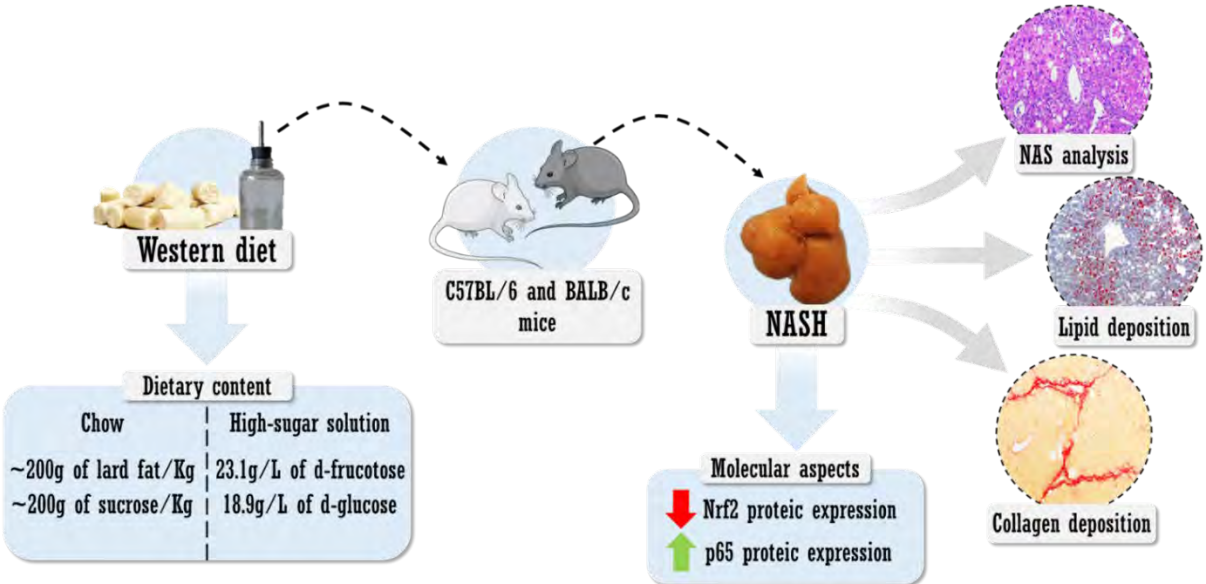
Background and aims: Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease and comprises a wide spectrum of severity, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and liver cancer. NASH-related mortality has recently increased, and it is projected to double by 2030. NAFLD pathogenesis is closely linked to the western lifestyle, which is characterized by the high intake of fat/sugar foods and sedentary habits. Although there are several efforts to unveil the NASH-related mechanisms, there is still no effective therapy available. Thus, we sought to establish a fast-induced murine model of NASH that resembles molecular/histopathological features of advanced human disease in two different mice strains.

Method: C57BL/6 and BALB/c mice were fed a western diet (WD) protocol [high-fat/sucrose chow (20% w/w), and a drinking high-sugar solution (55/45% of d-fructose/d-glucose)], and received, concomitantly, weekly-increased intraperitoneal injections of 10% oil-diluted carbon tetrachloride (CCl_4) solution [0.25µL/g of body weight (bw) to 1.50µL/g bw], for 8 weeks. Control mice received basal diet, tap water and vehicle. Mice were euthanized and hepatic samples were collected for histological [NAFLD activity score (NAS), collagen and lipid droplets morphometry in Sirius red and Oil-red stained sections, respectively)], immunohistochemistry [(alpha-smooth muscle-actin (a-SMA), CD68, Ki-67 and cleaved caspase-3)], and molecular analyses (Nrf2 and p65 protein levels by western blot).

Results: In both mice strains, the hybrid protocol (WD+CCl_4) demonstrated an increased NAS ($p = 0.0001$), featuring a microvesicular steatosis profile and a frequent occurrence of inflammatory cells foci. Likewise, mice presented higher levels of collagen deposition ($p < 0.0001$), a-SMA expression ($p < 0.0001$), and hepatocyte lipid deposition ($p < 0.0001$) in comparison to the control group. Furthermore, the WD+CCl_4 model induced a higher infiltration of $CD68^+$ macrophage ($p < 0.0001$), as well as an increased number of cleaved $caspase\text{-}3^+$ ($p < 0.0001$) and $Ki\text{-}67^+$ hepatocytes ($p < 0.0001$). At molecular level, the antioxidant Nrf2 was reduced in both mice strains ($p < 0.0001$), while pro-inflammatory p65 was increased only in C57BL/6 mice ($p = 0.005$).

Conclusion: We establish a fast-induced model of NASH in two different mice strains, featuring marked pro-inflammatory features and antioxidant impairment, resembling the corresponding human disease at an advantage stage.

Figure:



PO-140

New insights into the role of Mitochondia in the development of Non-alcoholic fatty liver disease

Inês C. M. Simões[1], Ricardo Amorim[2], José Teixeira[2], Agnieszka Karkucinska-Wieckowska[3], Paulo J. Oliveira[2], Yaiza Potes[1], Mariusz Więckowski[1]

[1]Nencki Institute of Experimental Biology, Laboratory of Mitochondrial Metabolism and Biology, Warsaw, Poland, *[2]CNC-Center for Neuroscience and Cell Biology, UC-Biotech Building, Biocant Park-Cantanhede, Cantanhede, Portugal*, *[3]The Children's Memorial Health Institute, Department of Pathology, Warsaw, Poland*
Email: i.simoes@nencki.edu.pl

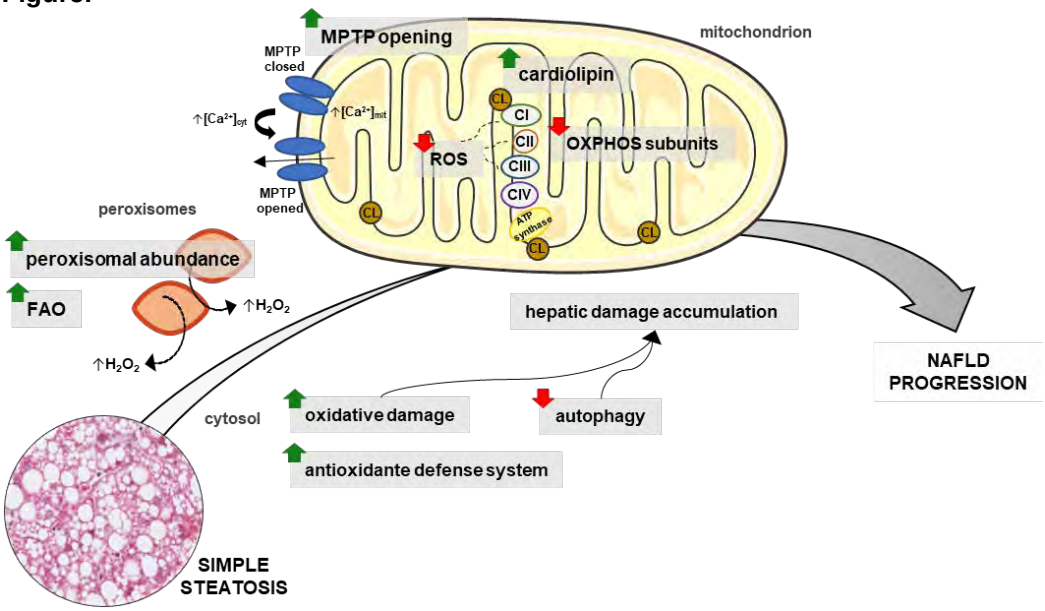
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized by the development of steatosis, which when progresses to more severe stages of the disease can ultimately compromise liver function. Disease progression implicates multiple mechanisms, chief of which is oxidative stress and mitochondrial dysfunction. However, the sequence of events underlying mitochondrial impairment is still poorly clarified.

Method: In this work, male C57BL/6J mice were fed with a high-fat plus high-sucrose (HFHS) diet for 16, 20, 22, and 24 weeks.

Results: HFHS diet caused simple steatosis with early signs of steatosis but no signs of inflammation up to 24th week of feeding. At mitochondrial level, an early mitochondrial remodelling with increased OXPHOS subunits levels and higher mitochondrial respiration occurred up to 20th week. Interestingly, a progressive loss of mitochondrial respiration along HFHS feeding was identified, accompanied by higher susceptibility to mitochondrial permeability transition pore opening. Importantly, our findings prove that mitochondrial alterations and subsequent impairment are independent of an excessive mitochondrial reactive oxygen species (ROS) generation, which was found to be progressively diminished along with disease progression. Instead, increased peroxisomal abundance and peroxisomal fatty acid oxidation-related pathway suggest that peroxisomes may contribute to hepatic ROS generation and oxidative damage. Accordingly, a de-regulation of antioxidant defense system was observed in cytosolic fraction of hepatocytes. Additionally, this early steatotic stage is also associated with autophagic flux impairment.

Conclusion: We show for the first time the sequential events of mitochondrial alterations involved in NAFL progression and demonstrate that mitochondrial ROS are not one of the first hits responsible for disease progression. Then, the accumulation of damaged/dysfunctional organelles could instigate hepatocyte injuries and NAFLD progression.

Figure:


MPTP opening
mitochondrion
MPTP closed
cardiolipin
↑[Ca²⁺]cyt
↑[Ca²⁺]mit
CI
CII
CIII
CIV
ATP synthase
ROS
OXPHOS subunits
MPTP opened
peroxisomes
peroxisomal abundance
FAO
↑H2O2
↑H2O2
hepatic damage accumulation
NAFLD PROGRESSION
cytosol
oxidative damage
autophagy
antioxidante defense system
SIMPLE STEATOSIS

PO-141
Discovery of a novel role for endogenous interleukin-22 in maintaining lipid homeostasis supports the development of liver targeted therapy for NAFLD/NASH

Haressh Sajiir[1], Sahar Keshvari[1], Kuan Yau Wong[1], Veronika Schreiber[1], Md Moniruzzaman[1], Ran Wang[1], Percival Wiid[1], Alexandra Mueller[1], Jack Lockett[1], Rohan Lourie[1], Grant Ramm[2], Katharine Irvine[1], Graeme Macdonald[3], John Prins[4], Michael McGuckin[4], Sumaira Hasnain[1 5 6]

[1]Mater Research Institute-The University of Queensland, Australia, *[2]QIMR Berghofer Medical Research Institute, Hepatic Fibrosis Group, Australia*, *[3]Princess Alexandra Hospital, Australia*, *[4]The University of Melbourne, Faculty of Medicine, Dentistry and Health Sciences, Australia*, *[5]Australian Infectious Diseases Research Centre, Australia*, *[6]Translational Research Institute, Australia*
Email: sumaira.hasnain@mater.uq.edu.au

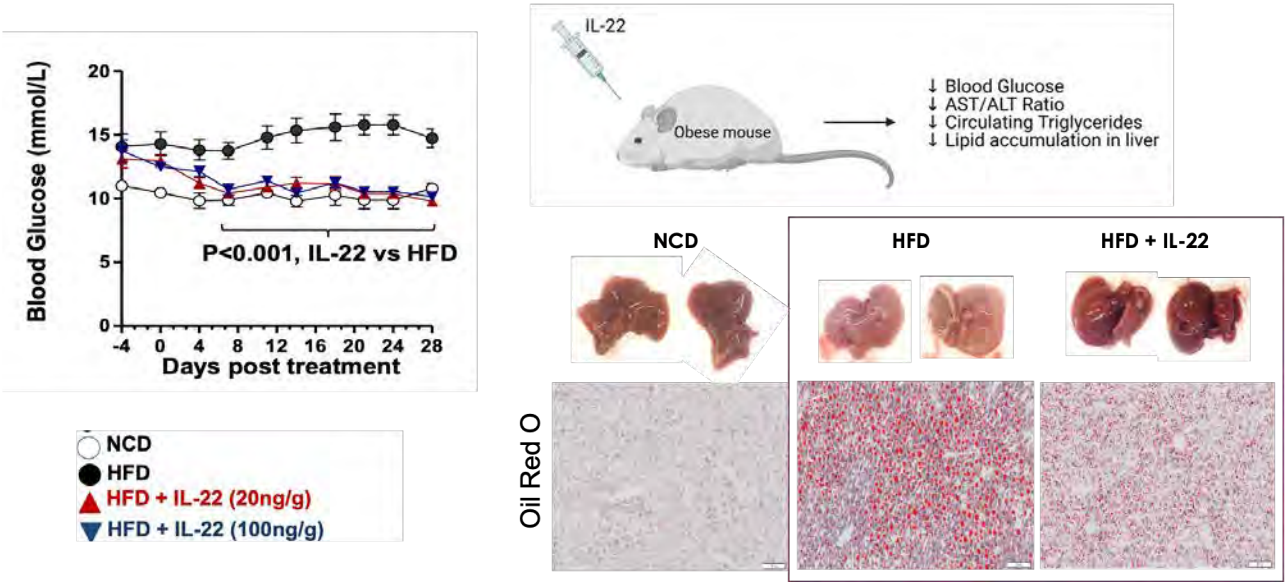
Background and aims: We discovered that the cytokine interleukin-22 (IL-22) is an efficient natural inhibitor of cellular stress. IL-22 is highly efficacious in metabolic disease with benefits including: complete restoration of glucose tolerance, suppression of fasting hyperinsulinaemia/hyperproinsulinaemia, and restoration of insulin sensitivity. Importantly, obese animals treated with IL-22, show significant improvements in circulating triglycerides, an improvement in liver function (AST:ALT ratio) and a reduction in lipid accumulation in the liver.

Method: To evaluate the role of endogenous IL-22 in metabolic tissue, we generated tissue specific IL-22 receptor (IL-22RA1) knockout mice lacking the receptor in pancreatic β-cells (IL-22RA1-/- Ins2Cre) and hepatocytes (IL-22RA1-/- AlbCre) and challenged these mice with a high fat diet (HFD). We designed and treated animals with fatty liver using prototype IL-22-based bispecific biologic drugs that are specifically targeted to metabolic tissue.

Results: Ablation of hepatic IL-22RA1 signalling induced insulin resistance, with marked upregulation of cellular stress (*Nos2, Grp78*), *Pdk4* and inflammatory cytokines (*Tnfa, Il1b, Il10, Ccl2*). Interestingly, increased hepatic inflammation, cellular stress was also observed in IL-22RA1-pancreatic β-cell knockouts. Moreover, β-cell-IL-22RA knockouts developed glucose intolerance with age which was exacerbated when challenged with HFD. Due to the multiple tissues expressing IL-22RA1 and its role in wound repair, prolonged administration of IL-22 in leads to off-target effects. This is apparent from the recent Phase I trial with dimerised IL-22, where the 100% of volunteers developed adverse events which were mainly skin emergent, including eczematous lesions. Therefore, we generated an IL-22-based bispecific biologic drug which is preferentially targeted towards the pancreas and liver and maintains efficacy in improving hyperglycemia. In-vivo, our pre-clinical experiments show that the IL-22-biologic reduced obesity-related liver steatosis by 4-fold, a marked reduction in hepatic expression of genes involved in cellular stress, insulin-stimulated glucose metabolism, inflammation, fatty acid and lipid production such as Sterol regulatory element-binding transcription factor 1c; and in cholesterol synthesis such as HMG-CoA reductase and fatty acid synthase (unpublished).

Conclusion: The critical point of the IL-22-bispecific technology is that it targets several different pathways active in non-alcoholic steatohepatitis and may influence fibrosis. We are currently de-risking our engineered IL-22 and providing important evidence for this to proceed to clinical trials.

Figure


Blood Glucose (mmol/L)
20
15
10
5
0
-4 0 4 8 12 16 20 24 28
Days post treatment
P<0.001, IL-22 vs HFD
NCD
HFD
HFD + IL-22 (20ng/g)
HFD + IL-22 (100ng/g)
IL-22
Obese mouse
↓ Blood Glucose
↓ AST/ALT Ratio
↓ Circulating Triglycerides
↓ Lipid accumulation in liver
NCD
HFD
HFD + IL-22
Oil Red O

PO-164
Selective modulation of metabotropic glutamate receptor 5 protects obese-high fat diet mice from oxidative stress and lipid accumulation

Clarissa Berardo[1], Marta Cagna[1], Laura Giuseppina Di Pasqua[1], Anna Cleta Croce[2], Ferdinando Nicoletti[3 4], Mariapia Vairetti[1], Andrea Ferrigno[1]

[1]University of Pavia, Internal medicine and therapeutics, PAVIA, Italy, *[2]Institute of molecular genetics, Italian National Research Council (CNR), Biology and biotechnology, Pavia, Italy*, *[3]Sapienza University of Rome, Physiology and pharmacology, Rome, Italy*, *[4]IRCCS Neuromed, Pozzilli, Italy*
Email: clarissa.berardo01@universitadipavia.it

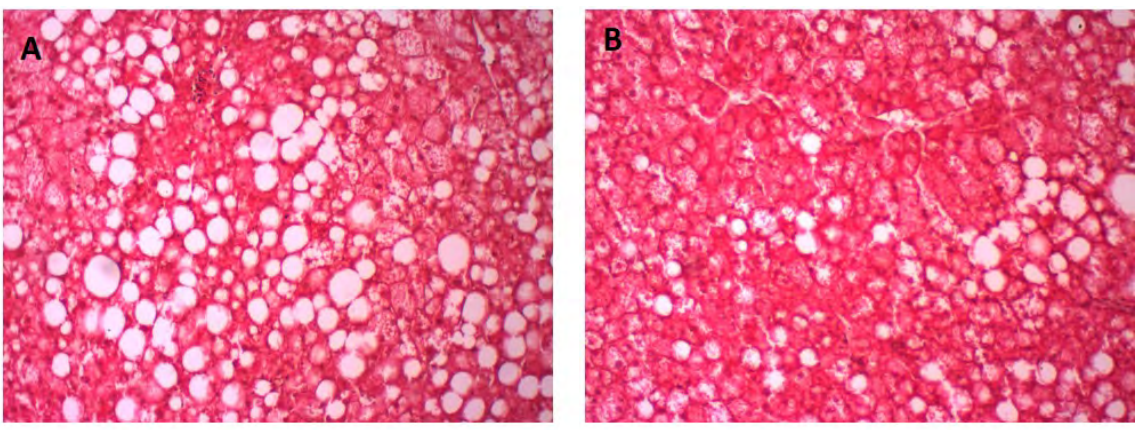
Background and aims: The metabotropic glutamate receptor 5 (mGluR5) is a main mediator of food intake in the central nervous system (Bradbury et al., 2005). Recently, its activity has been linked to alcoholic steatohepatitis (Choi et al., 2019). In addition, in an in vitro model of acute fat accumulation, we already proved that the administration of the mGluR5 agonist DHPG was responsible for the increase in lipid accumulation. In contrast, the use of the negative allosteric modulator MPEP was able to recover the injury induced by oleic and palmitic acids (Ferrigno et al., 2020). The present study was focused on the role of mGluR5 modulation in an in vivo model of hepatic steatosis.

Method: Male 606B6.V mice genetically modified for leptin gene (ob/ob mice) were fed for 7 weeks with high fat diet and concomitantly administered either with MPEP or vehicle (daily IP 20 mg/kg). At the sacrifice, liver biopsies were collected for the evaluation of lipid peroxidation and oxidative stress by TBARS and ROS assays, respectively. Protein analysis for mGluR5, mTOR, SREBP1, PPAR-alpha, were evaluated by Western blotting. The activation of NFkB was assessed by calculating the ratio between p65 phospho-NFκB and NFκB protein expression. Histological samples were fixed in formalin and stained with HandE. Lipid droplet areas were calculated by ImageJ software.

Results: No statistically differences are found in hepatic mGluR5 protein expression in the two groups considered. Hepatic TBARS and ROS levels significantly decrease after MPEP administration. MPEP-treated mice exhibited lower lipid accumulation with respect to the vehicle group, as showed by HandE staining. Moreover, lipid droplet areas are significantly reduced after MPEP treatment. In line with this finding, PPAR-alpha protein expression increases in MPEP group. NFκB is negatively regulated, probably via PPAR-alpha. Protein expression for mTOR and SREPB1 do not show any significant difference between the two groups.

Conclusion: Our data demonstrate that the selective blockade of mGluR5 improves steatosis in obese mice fed with high fat diet. In this chronic model of steatosis, oxidative stress parameters are decreased by MPEP treatment. Besides, intracellular lipid accumulation is prevented by PPAR-alpha in an mTOR-independent fashion. Even though the underlying mechanisms need to be further explored, this is the first in vivo study showing beneficial impact of MPEP in preventing fatty acid accumulation in the liver.

Figure:



H&E staining. **A.** ob/ob high fat diet + vehicle. **B.** ob/ob high fat diet + MPEP.

PO-167
Liver senescence is linked to the development of NAFLD in young mice independently of obesity

Ioannis I. Moustakas[1], Angeliki Katsarou[1], Aigli-Ioanna Legaki[1], Iryna Pyrina[2], Michael Koutsilieris[1], Antonios Chatzigeorgiou[1 2]

[1]National and Kapodistrian University of Athens, Department of Experimental Physiology, Athens, Greece, *[2]Institute for Clinical Chemistry and Laboratory Medicine, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany*
Email: johnmoustakas86@gmail.com

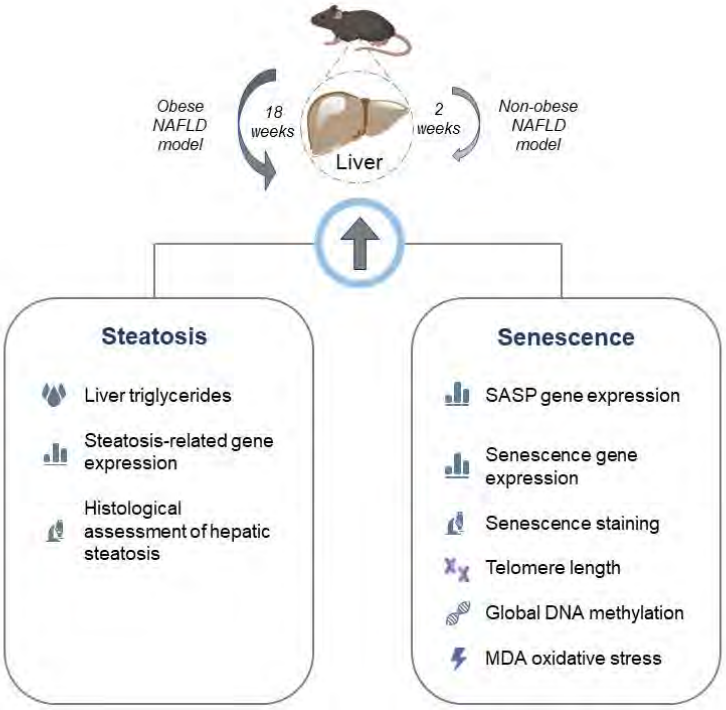
Background and aims: Senescence is considered to highly influence several inflammatory and metabolic disorders. Replicative senescence, characterized by telomere shortening as a result of aging, and stress-induced senescence provoked by extracellular or intracellular harmful stimuli are the two major senescence mechanisms. Accumulated data suggest that hepatocyte senescence is associated with the development of non-alcoholic fatty-liver disease (NAFLD); nevertheless, the main focus of preceding studies has been oriented towards age-dependent senescence during NAFLD. Herein, we sought to investigate whether the emergence of NAFLD is characterized by replicative- or stress-induced senescence, especially in non-aged organisms.

Method: Young mice were subjected into two different diet-induced NAFLD models, differing in the presence of obesity. Specifically, mice followed a high fat diet (HFD) for 18 weeks or a choline-deficient high fat diet (HFD-CD) for 2 weeks. At the end of the experiments, mice were sacrificed and the livers were excised. Immunohistochemistry (IHC) along with measurement of triglycerides in hepatic tissue and qPCR analysis of steatosis-related genes were used to evaluate establishment of liver steatosis. As indicators of senescence, a specific senescence staining along with the following parameters were determined: expression levels of senescence- and senescence-associated secretory phenotype (SASP)-related genes via qPCR, telomere length and levels of global DNA methylation, as well as measurement of lipid peroxidation in liver.

Results: Accumulation of liver fat, along with elevated hepatic mRNA expression of steatosis-related genes was observed in both models. The emergence of tissue senescence was also obvious in both HFD and HFD-CD-fed mice, as increased expression of senescence-associated genes and the presence of a robust hybrid histo-/immunochemical senescence-specific staining in the liver revealed. Interestingly, the length of telomeres and global DNA methylation did not show significant differences between NAFLD and healthy livers, while the oxidative-stress marker malondialdehyde was upregulated in steatotic livers of both models.

Conclusion: Such findings suggest that a strong link between senescence and NAFLD occurrence exists, even in non-aged organisms. At the same time, they underline the importance of stress-induced senescence in steatosis progression regardless of the presence of obesity.

Figure:


Obese NAFLD model
18 weeks
Liver
2 weeks
Non-obese NAFLD model
Steatosis
Liver triglycerides
Steatosis-related gene expression
Histological assessment of hepatic steatosis
Senescence
SASP gene expression
Senescence gene expression
Senescence staining
Telomere length
Global DNA methylation
MDA oxidative stress

PO-168
Fibroblast Growth Factor 21 (FGF21) is a hepatokine involved in NAFLD progression

Rocío Gallego-Durán[1 2], Javier Ampuero[1 2 3], HELENA PASTOR[1 2], Douglas Maya[1 2], Rocío Montero-Vallejo[1 2], Leticia Álvarez Amor[4 5], Jose Antonio Del Campo Castillo[6], María Jesús Pareja[7], M. Carmen Rico[1 2], Raquel Millan[1 2], Rocio Aller de la Fuente[8], Angela ROJAS ALVAREZ-OSSORIO[1 2], Rocio Munoz Hernandez[1 2], Antonio Gil-Gomez[1 2], Sheila Gato Zambrano[1 2], María del Rosario García-Lozano[1 2], Lucía Lopez-Bermudo[4 5], María Teresa Arias Loste[9], Javier Abad Guerra[10], José Luis Calleja Panero[10], Raul J. Andrade[11], Javier Crespo[9], Águeda Gónzalez-Rodriguez[12], Carmelo García-Monzón[12], Fausto Andreola[13], Rajiv Jalan[13], Franz Martin-Bermudo[4 5], Manuel Romero Gomez[1 2 3]

[1]Instituto de Biomedicina de Sevilla/CSIC/Universidad de Sevilla, SeLiver Group, Sevilla, Spain, *[2]Hepatic and Digestive Diseases Networking Biomedical Research Centre (CIBERehd)., Spain*, *[3]Hospital Universitario Virgen del Rocío, Digestive Diseases Unit, Sevilla, Spain*, *[4]Andalusian Center of Molecular Biology and Regenerative Medicine-CABIMER- University Pablo Olavide-University of Seville-CSIC, Sevilla, Spain*, *[5]Biomedical Research Network on Diabetes and Related Metabolic Diseases-CIBERDEM, Sevilla, Spain*, *[6]Hospital Virgen de Valme, Digestive Diseases Unit, Sevilla, Spain*, *[7]Hospital Virgen de Valme, Pathology Unit, Sevilla, Spain*, *[8]Hospital de Valladolid, Digestive Diseases Unit, Spain*, *[9]IDIVAL, Marqués de Valdecilla University Hospital, Digestive Diseases Unit, Spain*, *[10]Hospital Puerta de Hierro, Digestive Diseases Unit, Spain*, *[11]Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria, Universidad de Málaga, Digestive Diseases Unit, Málaga, Spain*, *[12]Hospital Universitario Santa Cristina, Instituto de Investigación Sanitaria Princesa, Liver Research Unit, Spain*, *[13]Institute for Liver and Digestive Health, Royal Free Hospital, Liver Failure Group, London, United Kingdom*
Email: mromerogomez@us.es

Background and aims: We aimed to assess the role of FGF21 in non-alcoholic fatty liver disease (NAFLD).

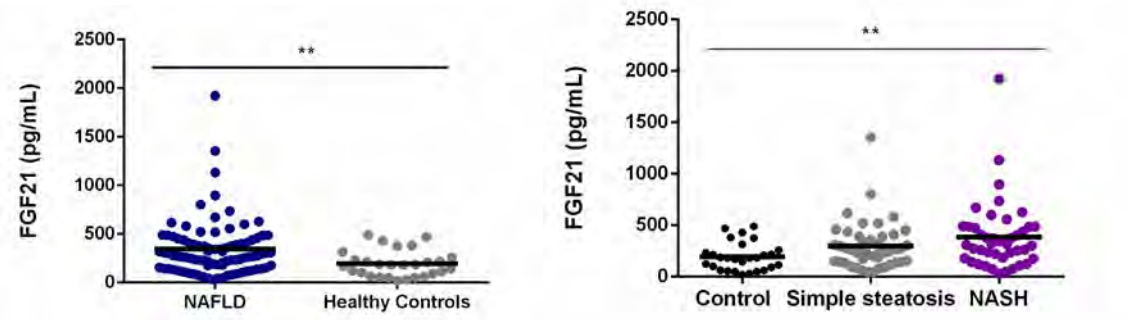
Method: We used human NAFLD pathology samples (n = 18) for FGF21 gene expression analyses (qPCR and RNAseq), serum (n = 117) to measure circulating FGF21 levels and DNA for genotyping (n = 387) FGF21 rs838133 variant. A hepatocyte-derived cell line (Huh7.5) was exposed to free fatty acids at different timepoints. Finally, C57BL/6 mice were fed with high-fat and choline-deficient diet supplemented with 0.1% methionine (CDA-HFD) for 16 weeks to assess liver FGF21 protein expression by immunohistochemistry and FGF21 circulating levels.

Results: A significant upregulation in FGF21 mRNA expression was observed in the liver analysed by both qPCR (fold change 5.32 ± 5.25 *vs*. 0.59 ± 0.66; p = 0.017) and RNA-Seq (3.5 fold; FDR: 0.006; p < 0.0001) in NAFLD patients *vs*. controls. Circulating levels of FGF21 were found to be increased in patients with steatohepatitis *vs*. bland steatosis (386.6 ± 328.9 *vs*. 297.9 ± 231.5 pg/ml; p = 0.009). Besides, sex, age, A-allele from FGF21, GG genotype from PNPLA3, ALT, type 2 diabetes mellitus and BMI were independently associated with NASH and significant fibrosis. *In vitro* exposure of Huh7.5 cells to high concentrations of free fatty acids resulted in an overexpression of FGF21 at 24, 48 and 72h (p < 0.001). Finally, Circulating FGF21 levels and hepatic FGF21 expression were found to be significantly increased (p < 0.001) in animals under CD-HFD.

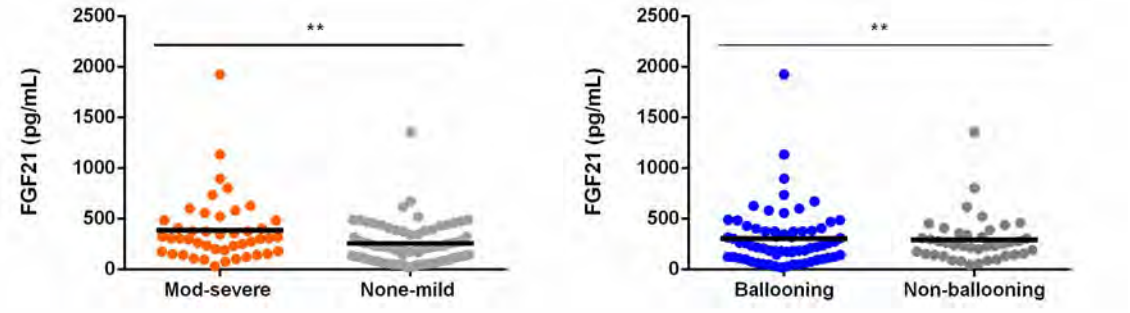
Conclusion: Both hepatic and circulating FGF21 expression was increased in NASH patients, in Huh7.5 cells under free fatty acids and in CDA-HFD animals. A-allele from rs838133 variant was also associated with an increased risk of steatohepatitis and significant fibrosis.

Figure: Serum FGF21 levels are raised in A) NAFLD patients vs healthy controls; B) NASH vs. NAFLD vs. healthy controls; C) according to steatosis degrees; D) according to hepatocyte ballooning presence ***p < 0.001; **p < 0.01; *p < 0.05.

B)



C) D)



PO-169
Quantification of EPCAM+ CD133+ microvesicles: a MAFLD biomarker

Sheila Gato Zambrano[1], Rocio Munoz Hernandez[1 2], Rocío Aller[3], Angela ROJAS ALVAREZ-OSSORIO[1 2], Rocío Montero-Vallejo[1 2], Rocío Gallego-Durán[1 2], Antonio Gil-Gomez[1 2], Douglas Maya[1 2], Vanessa García Fernández[1], M. Carmen Rico[1 4], Raquel Millan[1 4], javier ampuero[1 2 4], Manuel Romero Gomez[1 2 4]

[1]Instituto de Biomedicina de Sevilla, SeLiver Group, Spain, *[2]Hepatic and Digestive Diseases Networking Biomedical Research Centre (CIBERehd), Spain*, *[3]Valladolid University Hospital, Endocrinology and Nutrition, Spain*, *[4]Virgen del Rocío University Hospital, Digestive Diseases, Spain*
Email: mromerogomez@us.es

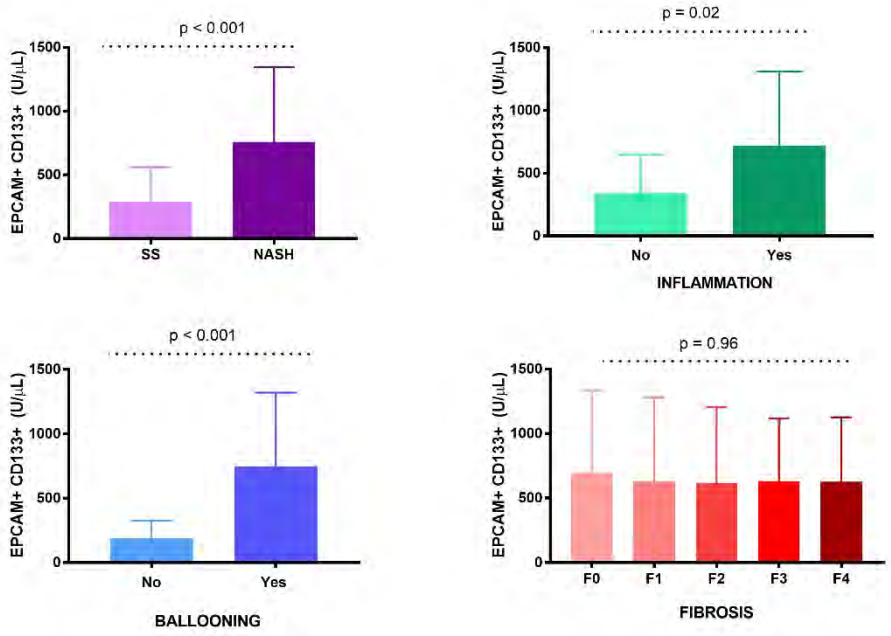
Background and aims: Microvesicles (MVs) are small membrane-derived vesicles that are shed from nearly all cells in the body in resting state, apoptosis or stimulation. MVs act as biological messengers to transfer information to other cells regulating biological processes like insulin resistance. Microvesicles from the liver could express Annexin V and EPCAM (Epithelial cell adhesion molecule) together with CD133. The aim of the study was to analyse the role of EPCAM+ CD133+ MVs in metabolic associated fatty liver disease (MAFLD) progression.

Method: One hundred twenty-five MAFLD proven liver biopsy patients [estimation cohort (n = 65) and validation cohort (n = 59)] were included. Patients were scored by SAF score; 15 patients were bland steatosis and 51 steatohepatitis in the estimation cohort, 27 patients were bland steatosis and 32 steatohepatitis in the validation cohort. MVs were determined in heparin plasma by Flow cytometry by size (0.2-1µm Megamix Plus SC protocol, BD) and phosphatidylserine expression measured by Annexin V. Cellular origin was determine using EPCAM and CD133. All samples were done in duplicate and the mean was taken.

Results: In the estimation cohort, patients with steatohepatitis showed significantly higher levels of microvesicles expressing AV+ EPCAM+ CD133+ than patients with bland steatosis (291.4 ± 268.7 vs. 758.6 ± 587.4; $p < 0.001$). Levels of MVs were higher in patients with inflammation vs. without inflammation (721.4 ± 588.3 vs. 341.9 ± 306.1; $p = 0.02$) and levels of MVs were higher in patients with ballooning compared to those vs. without (745.9 ± 573.7 vs. 188.9 ± 135.5; $p < 0.001$). AUROC = 0.89 (IC95%: 0.8-0.99); $p < 0.001$ was found in the detection of ballooning; sensitivity: 0.86 and specificity: 0.82. However, MV levels were not related with fibrosis stage ($p = 0.96$). These associations were confirmed in the validation cohort.

Conclusion: EPCAM+ CD133+ microvesicles were found increased in patients showing inflammation, ballooning and steatohepatitis irrespective of fibrosis stage. Identification and quantification of microvesicles could be a useful biomarker to detect the transition from simple steatosis to steatohepatitis in metabolic associated fatty liver disease.

Figure:


p < 0.001
EPCAM+ CD133+ (U/µL)
1500
1000
500
0
SS
NASH
p = 0.02
No
Yes
INFLAMMATION
p < 0.001
No
Yes
BALLOONING
p = 0.96
F0
F1
F2
F3
F4
FIBROSIS

PO-170
MiRNA-200b-3p overexpression is linked to NASH phenotype in both human and animal settings

Rocío Montero-Vallejo[1 2], Rocío Gallego-Durán[1 2], Leticia Álvarez Amor[3 4], Lucía Lopez-Bermudo[3 4], javier ampuero[1 2 5], HELENA PASTOR[1 2], M. Carmen Rico[1 2 5], Raquel Millan[1 2 5], Sheila Gato Zambrano[1 2], Douglas Maya[1 2], Rocio Munoz Hernandez[1 2], Angela ROJAS ALVAREZ-OSSORIO[1 2], Antonio Gil-Gomez[1 2], María Teresa Arias Loste[6], Javier Crespo[6], Franz Martin-Bermudo[3 4], Manuel Romero Gomez[1 2 5]

[1]Instituto de Biomedicina de Sevilla/CSIC/Universidad de Sevilla, Seville, Spain, *[2]Hepatic and Digestive Diseases Networking Biomedical Research Centre (CIBERehd), Spain*, *[3]Andalusian Center of Molecular Biology and Regenerative Medicine-CABIMER- University Pablo Olavide-University of Seville-CSIC, Seville, Spain*, *[4]Biomedical Research Network on Diabetes and Related Metabolic Diseases-CIBERDEM, Instituto de Salud Carlos III, Spain*, *[5]Digestive Diseases Unit, Hospital Universitario Virgen del Rocío, Seville, Spain*, *[6]Hospital Universitario Marqués de Valdecilla, Santander, Spain*
Email: mromerogomez@us.es

Background and aims: Liver biopsy remains as the gold standard method for NAFLD evaluation, despite its invasiveness. Therefore, the main aim of this study was to validate the diagnostic ability of miRNA-200b as a non-invasive biomarker in both patients and animal preclinical model of NAFLD.

Method: 59 biopsy-proven NAFLD patients were included in this study. Of them, 42/59 (71.2%) suffered from steatohepatitis (NASH) and 15/59 (25.4%) showed significant fibrosis (F2-F4). Both circulating and exosomal mir-200b-3p profiles were isolated from plasma, and their expression was analysed by qPCR. Further, rs738409 PNPLA3 SNP was genotyped in 42 patients by using Taqman probes. Finally, this microRNA was also evaluated in C57BL/6J mice fed with three different diets: control 16w (n = 3), choline-deficient, methionine supplemented high-fat diet for 16w (CDA-HFD, n = 5) and reversion group that underwent a nutritional intervention of 8w with CDA-HFD and further 8w with chow diet (n = 10). Histopathological findings were evaluated by Kleiner Score and SPSS v24.0 was used for the statistical analyses.

Results: In this cohort, 53% (31/59) were women, mean age 50 ± 12, and 48% (28/59) of patients suffered from type 2 diabetes mellitus. An increase in miRNA-200b was observed in NASH *vs.* simple steatosis patients, under both conditions, circulating (n = 42) (fold change 3.2 ± 5.6 *vs.* 0.51 ± 0.54; p = 0.011) and exosomal (n = 40) (fold change 7.7 ± 14.5 *vs*. 1.1 ± 0.8; p = 0.024). No association was found with fibrosis, diabetes or GG genotype of PNPLA3. After consuming the study diet, CDA-HFD mice exhibited liver fibrosis and NASH. An increase in hepatic expression of miRNA-200b-3p was observed compared to the control group (fold change 62.6 ± 46.4; p = 0.036). Finally, reversion mice showed a diminished liver miR-200b-3p expression when compared to the CDA-HFD mice (fold change 4.5 ± 11.7; p = 0.003), reaching the levels of the control group (p = ns) (Figure).

Conclusion: miRNA-200b-3p levels, both free and exosomes-encapsulated, were found to be increased in patients with NASH but not in those with liver fibrosis. Additionally, an animal model that recapitulated NAFLD features showed a hepatic overexpression of miRNA-200b. This microRNA could constitute a potential non-invasive tool for NASH diagnosis, avoiding the overlapping between NASH and fibrosis.

Figure:

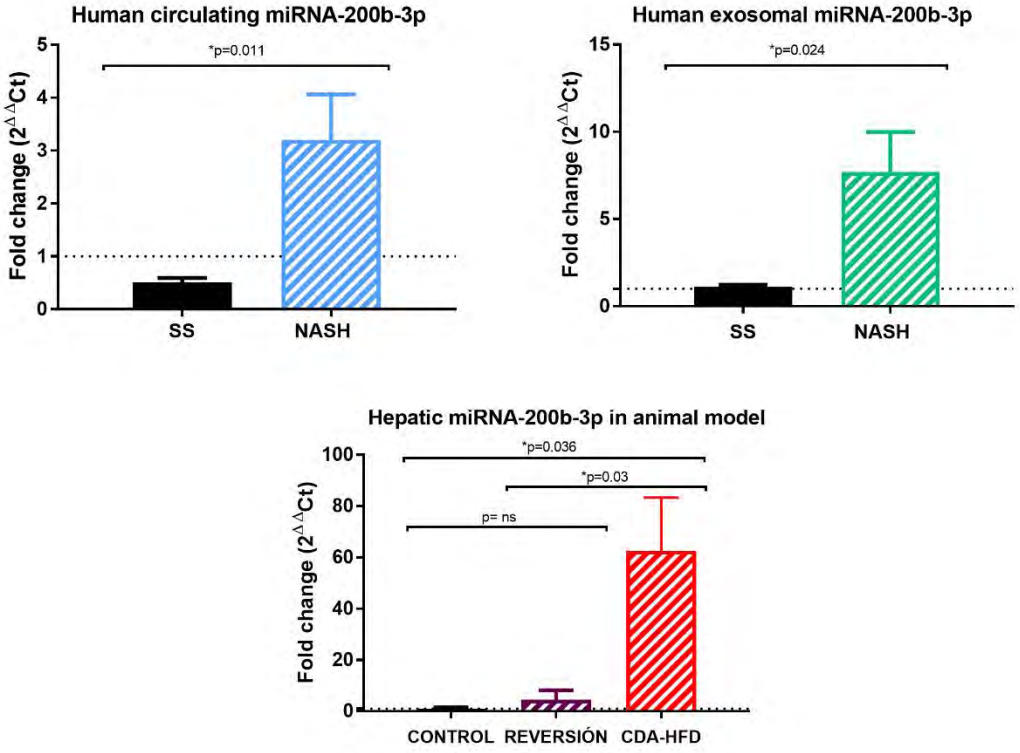


Figure. Fold change of circulating and exosomal miR-200b in patients and liver miR-200b in the animal model.

PO-172
Relationship between serum and hepatic levels of Zn and Fe versus fatty acid composition in a rat model of NASH

Laura Giuseppina Di Pasqua[1], Giuseppina Palladini[2], Clarissa Berardo[1], Marta Cagna[1], Barbara Mannucci[3], Anna Cleta Croce[4], Lucia Cucca[5], Andrea Ferrigno[1], Mariapia Vairetti[1]

[1]University of Pavia, Department of Internal Medicine and Therapeutics, Pavia, Italy, *[2]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*, *[3]Centro Grandi Strumenti-University of Pavia, Pavia, Italy*, *[4]Institute of Molecular Genetics-Italian National Research Council (CNR), Pavia, Italy*, *[5]University of Pavia, Department of Chemistry, Pavia, Italy*
Email: lauragiuseppin.dipasqua01@universitadipavia.it

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a heterogeneous disease including simple steatosis, non-alcoholic steatohepatitis (NASH) and cirrhosis. Alteration in Zn and Fe metabolism may be related to disease progression thus representing a potential therapeutic target. Aim of this study was to investigate the changes in serum and hepatic levels of Zn and Fe and their relationship with the hepatic fatty acid composition using rats fed with a methionine and choline deficient (MCD) diet as a model of NASH.

Method: Eight-week-old male Wistar rats (n = 24) fed for 2- and 8-week with an MCD diet and the corresponding control diet were used as nutritional model of NASH. Serum levels of hepatic enzymes (AST, ALT, ALPase) were quantified. Serum and hepatic levels of Fe and Zn were evaluated by ICP-OES, with external standard calibration. Liver fatty acid profiling and collagen deposition were performed by GC-MS analysis and Sirius red staining, respectively. Hepatic levels of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids have been quantified.

Results: Decreased serum and hepatic levels of Zn were found after 8-week MCD diet. Serum levels of Fe increased after 8-week MCD diet and, although not significant, the same trend occurred for hepatic Fe content. A time-dependent increase in serum enzymes as well as in hepatic collagen deposition was found in MCD rats. Liver PUFAs and UFAs grew in MCD rats after both 2- and 8-week MCD diet, while no changes were detectable for MUFA. A time-dependent lower content in SFA was detected in the MCD group. A significant inverse correlation was found when comparing serum and liver levels of Zn versus PUFAs and UFAs (serum: P = 0.02 and P = 0.009, respectively; liver: P = 0.03 and P = 0.02, respectively); conversely, a positive correlation was found with SFAs (p = 0.01 and P = 0.001, respectively). A positive correlation between serum and hepatic levels of Fe versus PUFA and UFA (serum: P = 0.01 and P = 0.02, respectively; liver: P = 0.04 and P = 0.005, respectively) and a negative correlation with SFA (p = 0.006 and P = 0.001, respectively) were also found.

Conclusion: MCD rats, that spontaneously progress to NASH, exhibit changes in serum and tissue levels of Zn and Fe associated with changes in hepatic PUFA, UFA and SFA. Our data support the evidences indicating the role of Zn and Fe in steatosis that progress to NASH and suggest their relationship with hepatic fatty acid composition.

PO-178
Neddylation inhibition reduces liver steatosis in MAFLD mice models by promoting hepatic fatty acid oxidation via DEPTOR-mTOR axis

Marina Serrano-Macia[1], Jorge Simón Espinosa[2], María J González Rellán[3], Mikel Azkargorta[4], Naroa Goikoetxea[2], Fernando Lopitz Otsoa[5], Diego Saenz de Urturi[6], Rubén Rodríguez Agudo[1], Sofia Lachiondo-Ortega[1], Maria Mercado-Gómez[1], Virginia Gutiérrez de Juan[5], Maider Bizkarguenaga[5], David Fernández Ramos[5], Guido Alessandro Baselli[7 8], Luca Valenti[7 8], Paula Iruzubieta[9 10], Javier Crespo[9 10], Erica Villa[11], Jesus Maria Banales[12], Matías A Avila[13], Jose Marin[14], James D Sutherland[15], Rosa Barrio[15], Ugo Mayor[16], Felix Elortza[4], Dimitris Xirodimas[17], Teresa Cardoso Delgado[2], María Luz Martínez-Chantar[2]

[1]Center for Cooperative Research in Biosciences (CIC bioGUNE), Liver Disease Lab, Spain, *[2]Center for Cooperative Research in Biosciences (CIC bioGUNE), Liver Disease Lab, DERIO, Spain*, *[3]Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Department of Physiology*, *[4]Center for Cooperative Research in Biosciences (CIC bioGUNE), Proteomics Platform, DERIO, [5]Center for Cooperative Research in Biosciences (CIC bioGUNE), Precision Medicine and Metabolism Lab, DERIO, Spain*, *[6]University of the Basque Country (UPV/EHU), Department of Physiology, Leioa, Spain*, *[7]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy*, *[8]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Transfusion Medicine and Hematology-Translational Medicine, Milan, Italy*, *[9]Marqués de Valdecilla University Hospital, Gastroenterology and Hepatology Department, Santander, Spain*, *[10]Research Institute Marqués de Valdecilla (IDIVAL), Clinical and Translational Digestive Research Group, Santander, Spain*, *[11]University of Modena and Reggion Emilia, Department of Gastroenterology, Modena, Italy*, *[12]Donostia University Hospital, Department of Liver and Gastrointestinal Diseases, San Sebastian, Spain*, *[13]Center for Applied Medical Research (CIMA), Hepatology Programme, Pamplona, Spain*, *[14]University of Salamanca, Experimental Hepatology and Drug Targeting (HEVEFARM), Salamanca, Spain*, *[15]Center for Cooperative Research in Biosciences (CIC bioGUNE), Ubiquitin-likes And Development Lab, DERIO, Spain*, *[16]University of the Basque Country (UPV/EHU), Department of Biochemistry and Molecular Biology, Leioa, Spain*, *[17]Univ. Montpellier, Centre de Recherche en Biologie cellulaire de Montpellier (CRBM-CNRS), Montpellier, France*
Email: mserrano@cicbiogune.es

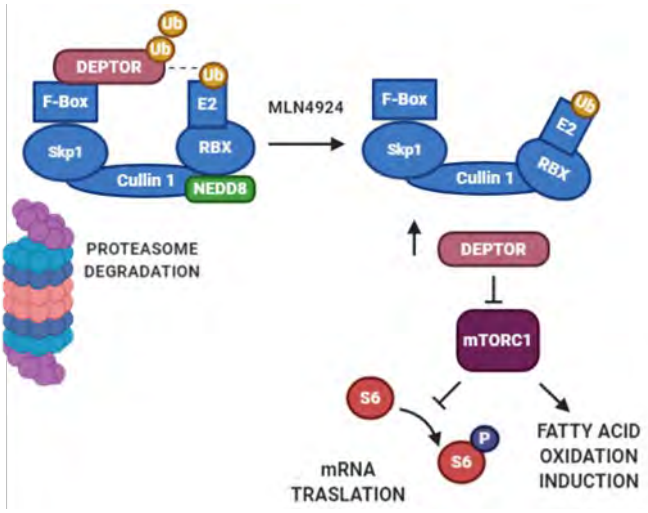
Background and aims: Metabolic-associated fatty liver disease (MAFLD) is a complex liver disease and comprehends a group of conditions being the massive accumulation of fat in the liver the main feature. Mechanistic target of rapamycin (mTOR) pathway plays an essential role in lipid metabolism and development of MAFLD. In recent years, the regulation of DEP domain-containing mTOR-interacting protein (DEPTOR), a negative regulator of mTOR pathway, has been involved in the alteration of lipid homeostasis. It is known that DEPTOR is degraded by SCF (Skp1-Cullin-F box proteins) E3 ubiquitin ligase, which needs to be neddylated to be active. Neddylation is a reversible ubiquitin-like post-translational modification upregulated in many diseases, including MAFLD. Therefore, we decided to evaluate the potential use of Pevonedistat (MLN4924), a neddylation inhibitor, in MAFLD therapy through regulation of mTOR signaling

Method: Neddylation inhibition was evaluated in mouse isolated hepatocytes. Moreover, male adult C57BL/6 mice (3-month old) fed either with 0.1% methionine and choline deficient diet (0.1%MCD diet) or with a choline-deficient high fat diet (CD-HFD) were used. After 2 weeks of 0.1%MCD diet or 3 weeks of CD-HFD, mice were treated during 2 or 3 more weeks, depending on the diet, with Pevonedistat (60mg/Kg) by oral gavage each 4 days. The effects of neddylation specific inhibition were also evaluated in male adult C57BL/6 AlfpCre mice infected with AAV-DIO-shNEED8 and maintained on CD-HFD for 6 weeks. Finally, the impact of hepatic neddylation in patients with MAFLD as well as the potential use of NEDD8 serum levels for MAFLD diagnostic purposes were evaluated.

Results: Neddylation inhibition using Pevonedistat, as well as silencing Nedd8 (neural precursor cell expressed, developmentally down-regulated 8) reduced lipid accumulation in oleic acid-stimulated mouse primary hepatocytes. Likewise, pharmacological neddylation inhibition and Nedd8 hepatic knockdown ameliorates liver steatosis preventing lipid peroxidation, hepatic oxidative stress and inflammation in mouse models of diet induced MAFLD. Increased Deptor levels and concomitant repression of mTOR signalling when neddylation is inhibited, is associated with augmented fatty acid oxidation and reduced lipid content. Deptor silencing in isolated mouse hepatocytes abolishes the anti-steatotic effects mediated by neddylation inhibition. Finally, serum NEDD8 levels correlate with hepatic neddylation both during disease progression in the clinical setting and during disease regression by therapeutic approaches in pre-clinical models.

Conclusion: Overall, upregulation of DEPTOR, driven by neddylation inhibition, is proposed as a novel effective target and therapeutic approach to tackle MAFLD. Besides, the results obtained enable to pose serum NEDD8 as a potential non-invasive biomarker in MAFLD.

Figure:



PO-182
Association of PNPLA3 with the development of arterial stiffness in young patients with non-alcoholic fatty liver disease

Anastasiia Radchenko[1], Olena Kolesnikova[1], Vilena Chupina[2], Laith Jnied[2]

[1]L.T. Mala Therapy National Institute of the National Academy of Medical Sciences of Ukraine, Department of Study of Aging Processes and Prevention of Metabolic-Associated Diseases, Kharkiv, Ukraine, *[2]Kharkiv National Medical University, Kharkiv, Ukraine*
Email: anastasha.radchenko@gmail.com

Background and aims: Genetic variants of the gene involved in lipid and glucose metabolism, rs738409 (C/G), which contains the Patatin-like phospholipase domain-containing protein 3 (PNPLA3), are associated with the risk of non-alcoholic fatty liver disease (NAFLD). The data on the relationship with the cardiovascular risk (CVR) remain controversial.
The aim of the study was to assess arterial stiffness (AS) in NAFLD patients with low, moderate and high CVR depending on different genotypes of the adiponutrin gene.

Method: We studied 273 asymptomatic young adults in the age range 25-44 years from 2018- 2020. We evaluated AS by measuring the pulse wave velocity (PWV), augmentation index (AI) and determined the following non-invasive indexes of liver steatosis: hepatic steatosis index (HSI), fibrosis-4 index (Fib-4). Serum level of resistin was also measured. A single variant in PNPLA3 (rs738409) was genotyped. We compared patients according to the SCORE scale. Multiple Linear Regression analysis was assessed for AS predictors.

Results: HSI (p = 0, 015), Fib-4 (p = 0, 056), and serum resistin (p = 0, 003) were significant predictors of AS in NAFLD patients with low CVR carrying PNPLA3 rs738409-G. Fib-4 and HSI became predictors of PWV in moderate CVR group (p = 0.001; p = 0.04). Fib-4 and AI were related to PWV (p = 0.015; p = 0.004) in NAFLD patients with high CVR carrying PNPLA3 rs738409-G.

Conclusion: Our research has shown the association between PNPLA3 rs738409-G and increased arterial stiffness in NAFLD patients, which may increase the CVR.

PO-184
Vitamin d regulated microRNA pathway and the molecular pathogenesis of metabolic-associated fatty liver disease

Zixuan Zhang[1], Rachel Moon[1], James Thorne[1], Bernadette Moore[1]

[1]*University of Leeds, School of Food Science and Nutrition, Leeds, United Kingdom*
Email: ml14z6z@leeds.ac.uk

Background and aims: MicroRNAs (miRNAs) play a critical role in both the progression of metabolic-associated fatty liver disease (MAFLD), and in mediating the cellular response to vitamin D and its post-transcriptional regulation of the vitamin D receptor (VDR). However, the potential roles for vitamin D regulated miRNAs in MAFLD pathogenesis has been relatively unexplored. This work aimed to critically review the literature evidence for a potential subset of miRNAs that are both modulated by vitamin D and dysregulated in MAFLD.

Method: Firstly, we comprehensively reviewed the data from profiling or mechanistic human studies investigating miRNA expression in liver tissues and/or serum in MAFLD pathogenesis, using the terms NAFLD, NASH, MAFLD, MASH and miRNA in PubMed. Secondly, we examined the evidence for the regulation of human serum miRNAs by vitamin D status, or in response to dietary intakes, or supplementation, along with the limited research that has specifically investigated the influence of vitamin D on liver-related miRNAs. Eventually, the evidence for miRNAs regulated by vitamin D and MAFLD was critically assessed after data integration.

Results: 73 human studies under comprehensive review identified 27 miRNAs that dysregulated in more than one MAFLD study. In contrast, only 13 studies, including a protocol for a trial in MAFLD, had examined miRNAs relative to vitamin D status, response to supplementation, or vitamin D in the context of the liver. Five miRNAs (miR-21, miR-30, miR-34, miR-122 and miR-146) were identified as dysregulated in multiple independent MAFLD studies, and their potential biological roles were summarized. While the modulation of miRNAs by vitamin D has been understudied, integrating the data suggests six vitamin D modulated miRNAs (miR-27, miR-155, miR-192, miR-223, miR-375 and miR-378) potentially relevant to MAFLD pathogenesis.

Conclusion: The summary tables within this review provide a significant resource to underpin future hypothesis-driven research, and we conclude that miRNAs modulated by vitamin D have not been studied sufficiently. Moreover, based on the evidence to date, a therapeutic benefit for vitamin D supplementation in MAFLD cannot be excluded.

Figure:

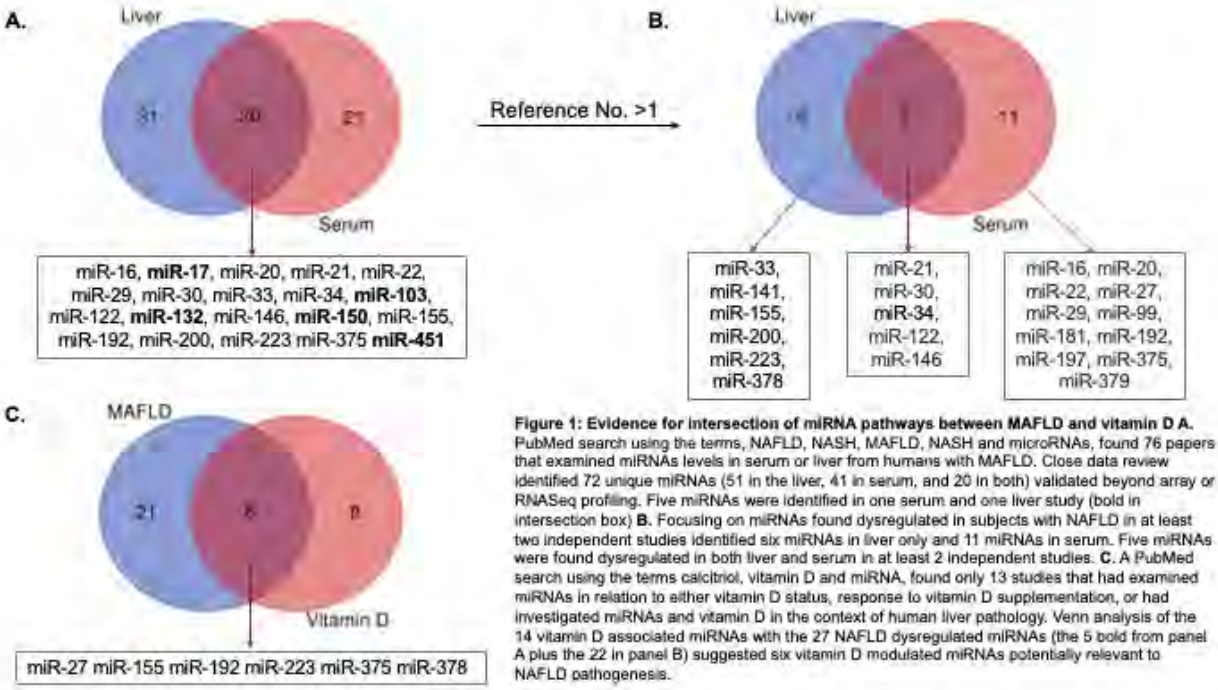


Figure 1: Evidence for intersection of miRNA pathways between MAFLD and vitamin D A. PubMed search using the terms, NAFLD, NASH, MAFLD, NASH and microRNAs, found 76 papers that examined miRNAs levels in serum or liver from humans with MAFLD. Close data review identified 72 unique miRNAs (51 in the liver, 41 in serum, and 20 in both) validated beyond array or RNASeq profiling. Five miRNAs were identified in one serum and one liver study (bold in intersection box) **B.** Focusing on miRNAs found dysregulated in subjects with NAFLD in at least two independent studies identified six miRNAs in liver only and 11 miRNAs in serum. Five miRNAs were found dysregulated in both liver and serum in at least 2 independent studies. **C.** A PubMed search using the terms calcitriol, vitamin D and miRNA, found only 13 studies that had examined miRNAs in relation to either vitamin D status, response to vitamin D supplementation, or had investigated miRNAs and vitamin D in the context of human liver pathology. Venn analysis of the 14 vitamin D associated miRNAs with the 27 NAFLD dysregulated miRNAs (the 5 bold from panel A plus the 22 in panel B) suggested six vitamin D modulated miRNAs potentially relevant to NAFLD pathogenesis.

PO-187
INT-747 ameliorates NASH pathogenesis by inhibiting hepatic diacylglycerol acyltransferase -2 mediated triglyceride synthesis in mice

Balasubramaniyan Vairappan[1], Anjana Chandrasekhar[1]

[1]*Jawaharlal Institute of Postgraduate Medical Education and Research, Biochemistry, Pondicherry, India*
Email: balamaniyan@gmail.com

Background and aims: We aimed to investigate whether superimposed inflammation increases DGAT (1and2) overexpression and associated hepatic TG synthesis in non-alcoholic steatohepatitis (NASH). Furthermore, the effect of FXR agonist, INT-747 on the regulation of hepatic DGAT-TG pathway in NASH was studied.

Method: 90 days after feeding HFD and chow, mice were orally administered INT-747 (Sigma, USA; 5mg/kg b.w. daily by gastric lavage) in the vehicle (corn oil) for 10 days or vehicle alone. In order to determine the effect of superimposed inflammation on the background of fatty liver, the treatment groups were given either lipopolysaccharide or vehicle (saline). Furthermore, HFD mice and that received LPS were treated with DGAT-1 inhibitor. Blood and hepatic tissue were collected for various analysis.

Results: When compared to naïve mice, NAFLD mice showed markedly elevated hepatic DGAT (1and2) protein expressions. LPS challenge to NAFLD mice showed a further significant increase of hepatic DGAT 2. INT-747 treatment to NAFLD mice and that received LPS showed significantly reduced both DGAT 1and2 and hepatic TG synthesis. Moreover, inhibition of DGAT-1 with its specific inhibitor reduced hepatic TG contents in NAFLD mice however, failed to reduce fat deposition or inflammation in NASH mice. NFκB, iNOS and 4-HNE protein expressions were significantly increased in NAFLD mice that received LPS. INT-747 treatment significantly ($p < 0.05$) lowered the above indices.

Conclusion: Our study is the first indication of evidence for an association between increased hepatic DGAT 2 and TG synthesis in NASH mice. INT-747 treatment attenuated hepatic TG accumulation by downregulating DGAT 2 overexpression and inflammation, thereby attenuating NASH pathogenesis.

PO-190
Single-cell analysis of NAFLD induced by high fat diet in rat

Fumihiko Takeuchi[1], Yi-Qiang Liang[1], Norihiro Kato[1]
[1]*National Center for Global Health and Medicine, Shinjuku City, Japan*
Email: fumihiko@takeuchi.name

Background and aims: Aiming to elucidate the molecular pathophysiology of non-alcoholic fatty liver disease (NAFLD) progression and the recovery by lifestyle-related improvement, we utilized an animal experimental model in which rats were loaded with a high-fat diet and then washed out with a normal diet.

Method: Male spontaneously hypertensive rats (SHR/Izm) were loaded with a high-fat diet. The ingredients of the high-fat diet consist of 68% of normal diet (SP feed), 25% of palm oil, 5% of cholesterol, and 2% of cholic acid. NAFL was caused by a high-fat diet load from 12 weeks for 4 weeks, and NASH was caused by a longer term loading up to 8 weeks. After 4 weeks of high-fat diet load, the fatty liver improved according to the duration of washout in the washout group who returned to the normal diet and were fed the normal diet until 20 or 32 weeks of age. Rats aged 16, 20, and 32 weeks on a normal diet were used as a control group. A total of 7 groups x 4 rats were used.
The liver consists of multiple cell types such as hepatocytes, stellate cells, vascular endothelial cells, and macrophages. Single-cell analysis was performed to observe in detail changes in the quantity and quality (gene expression) of each cell type due to changes in pathological conditions.
Using 0.1 to 0.4 g of frozen liver tissue, we performed ATAC-seq of bulk tissue on all samples. In addition, single-nucleus ATAC-seq (snATAC-seq) was performed on one sample each from the 5 groups of 16 to 20 weeks age.

Results: 13182 nuclei were observed by snATAC-seq. Based on the similarity of chromatin opening, the nuclei were classified into 14 clusters: hepatocytes (C1-C7), non-inflammatory macrophages (C8), inflammatory macrophages (C9), T cells and NK cells (C10), B cells (C11), stellate cells (C12), vascular endothelial cells (C13-C14). Using the snATAC-seq result, the cell type composition of bulk ATAC-seq samples was estimated. Inflammatory macrophages increased remarkably due to high-fat diet loading, stellate cells decreased after 4-week loading, and hepatocytes decreased after 8-week loading. For hepatocytes, a 4-week high-fat diet load inactivated lipid metabolism, and a longer-term (8-week) load activated the apoptotic pathway. The cell type composition and gene expression after washout recovered to almost the same level as in normal diet.

Conclusion: In this study, we were able to specifically observe changes in pathological conditions for each cell type.

PO-193

Anti-inflammatory and anti-fibrotic effect of icosabutate as mono- or combination therapy with a GLP-1 receptor agonist, a FXR agonist or an ACC inhibitor in a dietary mouse model of progressive fibrosis

David A. Fraser[1], Tore Skjaeret[1], Jacob Nøhr-Meldgaard[2], Sanne Veidal[2], Stephen Harrison[1], Cristina Alonso[3], Michael Feigh[2], Irantzu Pérez-Ruiz[3]

[1]Northsea Therapeutics, Amsterdam, Netherlands, *[2]Gubra Aps, Denmark*, *[3]OWL Metabolomics, Spain*
Email: david.fraser@northseatherapeutics.com

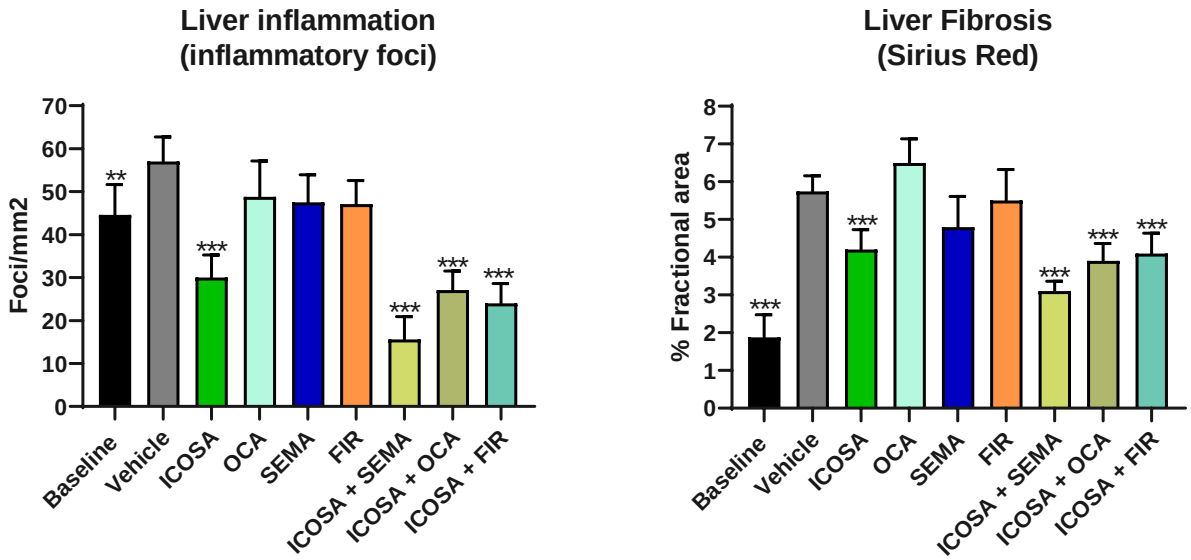
Background and aims: Icosabutate (ICOSA), a semi-synthetic fatty acid targeting FFAR4 and the arachidonic acid cascade, is currently in clinical development for the treatment of NASH (NCT04052516, results pending). To assess if additional anti-inflammatory and/or anti-fibrotic effects could be achieved via combination therapy, a comparison of ICOSA, firsocostat [FIR, a liver-targeted acetyl-coenzyme A carboxylase (ACC 1/2) inhibitor], semaglutide [SEMA, an injectable glucagon-like 1 (GLP-1) receptor agonist] or obeticholic acid [OCA, a farnesoid-X receptor agonist] as monotherapies was performed in a choline-deficient, L-amino acid defined high-fat diet fed (CDAA-HFD) mouse model. The effects of combining ICOSA with FIR, SEMA or OCA were simultaneously assessed.

Method: Male C57BL/6JRj mice were fed CDAA-HFD (A16092003, Research Diets) for 6 weeks before treatment start. A baseline group (n = 12) was terminated at study start. CDAA-HFD fed mice [n = 10-12 per group] received daily per oral (PO) treatment with vehicle (corn oil), ICOSA (112mg/kg), OCA (30mg/kg), SEMA (30nmol/kg SC), FIR (5mg/kg) as monotherapy or combinations of ICOSA + either SEMA, OCA or FIR (all dosing as for monotherapy) for 8 weeks. Inflammation and fibrosis was assessed in terminal liver biopsy by IHC, biochemical and morphometric assays.

Results: SEMA induced a 26% loss of bodyweight ($p < 0.001$) relative to baseline as both mono- and combination therapy. As monotherapy, icosabutate was the only anti-inflammatory compound, reducing inflammatory foci by 47% ($p < 0.001$ vs vehicle, see figure) with no significant effect observed for other compounds. A similar pattern was observed with galectin-3 (a macrophage marker), where only icosabutate achieved a significant reduction (-28%, $p < 0.001$ vs vehicle). All ICOSA combinations achieved significant reductions of both inflammatory foci and galectin-3, the most pronounced effect achieved by ICOSA + SEMA (-73% and -45% for inflammatory foci and galectin-3 respectively, $p < 0.001$ vs vehicle). Icosabutate was also the only compound that induced a significant anti-fibrotic effect as monotherapy as measured by sirius red (SR)-morphometry (see figure) whilst both ICOSA and SEMA significantly reduced hydroxyproline (HYP) content (by 33 and 22% respectively, both $p < 0.001$ vs vehicle). All combination therapies achieved significant reductions in fibrosis, the most pronounced effects observed for ICOSA + SEMA (-46% and -45% for SR-morphometry and HYP respectively, both $p < 0.001$ vs vehicle).

Conclusion: As monotherapy, icosabutate is a more potent anti-inflammatory/anti-fibrotic compound than SEMA, OCA or FIR in a delayed treatment CDAA-HFD NASH mouse model. Combination therapy provided additional benefits and, in particular, the ability of SEMA to reduce food intake is highly effective for enhancing the beneficial effects of icosabutate therapy.

Figure:



Values expressed as mean of n = 9-12 + SEM, Dunnett's test one-factor linear model
**: P < 0.01, ***: P < 0.001 compared to Vehicle

PO-196
Absence of key necroptotic genes protect macrosteatotic livers from ischaemia reperfusion injury

Raji Baidya[1 2], Jérémie Gautheron[3], Darrell Crawford[1 2], Kim Bridle[1 2]

[1]The University of Queensland, Faculty of Medicine, Brisbane, Australia, *[2]Gallipoli Medical Research Institute, Brisbane, Australia*, *[3]Sorbonne University, Inserm, Centre de Recherche Saint-Antoine (CRSA), Paris, France*
Email: r.baidya@uq.edu.au

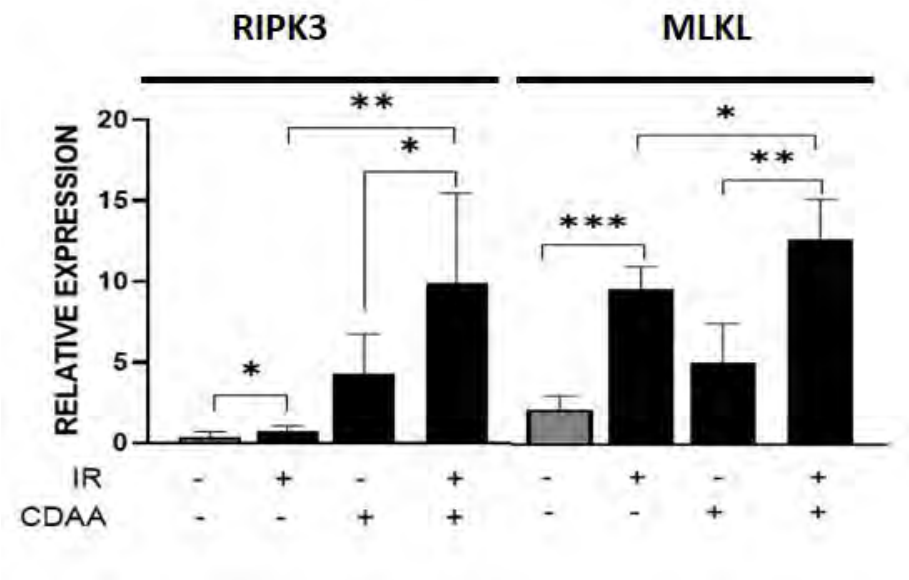
Background and aims: Steatotic donor livers are at a high risk of graft non-function due to susceptibility to ischaemia-reperfusion (I/R) during transplant. It is even debated whether donor livers with moderate macrosteatosis (30-60%) are suitable for transplantation. Necroptosis has been linked to I/R insult where receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL) are known to play fundamental roles. The aim of this study was to investigate the underlying cell death mechanisms during I/R injury using murine models of moderate hepatic macrosteatosis. Additionally, we investigated if targeting the execution step of necroptosis in a fatty liver I/R model, using RIPK3 and MLKL knockout (KO) mice, can improve liver function.

Method: Mice were fed a choline deficient, L-amino acid-defined (CDAA) diet for two weeks to induced macrosteatosis and subjected to 70% hepatic ischaemia reperfusion injury. Mice were euthanized after 24 hrs of reperfusion and blood and liver tissue samples were collected for further analysis.

Results: Our study showed that the expression of RIPK3 and MLKL in wild-type (WT) mice were increased after I/R in macrosteatotic livers. Histological analysis showed a significantly increased necrotic area after I/R in macrosteatotic livers compared to lean liver. Macrosteatosis exacerbated the liver injury sustained during I/R phase as demonstrated by a sharp increase in ALT level in WT mice ($p < 0.0001$) as well as the serum level of TNF alpha, IL6, and IL-1beta were significantly increased after I/R injury in steatotic liver compared to lean liver whereas their levels were reduced in both RIPK3-KO and MLKL-KO mice after I/R ($p < 0.01$). There was a significant increase in serum High mobility group box protein 1 (HMGB1) in I/R groups ($p < 0.001$) compared to control groups which was further elevated in steatotic livers compared to lean groups. The level of HMGB1 was markedly reduced in both the RIPK3-KO and MLKL-KO mice subjected to I/R injury. These results suggested that these KO mice were protected from liver I/R injury.

Conclusion: Our data suggest that absence of RIPK3 and MLKL protected the liver from I/R-injury. Further, HMGB1 could be a promising biomarker for graft outcome prediction. These findings suggest that attenuating fatty liver I/R injury using targeted interventions against RIPK3 and MLKL is a valid therapeutic strategy, with the ultimate aim to increase the availability of donor livers for transplant.

Figure:IR increased RIPK3 and MLKL protein expression after IR surgery in CDAA fed mice compared to control mice. *p < 0.05, **p < 0.001, ***p < 0.0001.



PO-203
4-methylpyrazole attenuates the development of diet-induced NAFLD

Katharina Burger[1], Finn Jung[1], Anja Baumann[1], Annette Brandt[1], Victor Sánchez[1], Ina Bergheim[1]

[1]*University of Vienna, Department of Nutritional Sciences, Wien, Austria*
Email: katharina.burger@univie.ac.at

Background and aims: Recent studies suggest that 4-methylpyrazole (4MP, fomepizole), known as a competitive inhibitor of alcohol dehydrogenase (ADH), may attenuate the development of acetaminophen-induced (APAP) liver injury. Results of these studies suggest that the protective effects of 4MP on APAP-induced liver damage were related to its effect on c-Jun N-terminal kinase (JNK) activation, known to be also involved in the development of non-alcoholic fatty liver disease (NAFLD). In the present study, using a pair-feeding dietary model of NAFLD, we assessed a concomitant intraperitoneal treatment of mice with 50mg 4MP/kg bodyweight protecting mice from the onset of NAFLD.

Method: Male C57BL/6J mice (6-8 weeks old, n = 8/group) were fed either a liquid control diet or a liquid fat-, fructose-, and cholesterol-rich diet (FFC) ± 50mg/kg bodyweight of 4MP or vehicle (0.9% NaCl) intraperitoneal three times per week for a duration of 8 weeks. Liver histology and markers of inflammation were determined at the end of the trial.

Results: While caloric intake was equal between all four groups, FFC-fed mice developed steatosis with beginning inflammation being significantly reduced in FFC-fed mice concomitantly treated with 4MP. Number of neutrophil granulocytes was significantly higher in livers of FFC-fed mice compared to controls whereas in livers of FFC+4MP-fed animals the number of neutrophils was almost at the level of controls. In line with these findings, both, expression of intercellular adhesion molecule 1 and monocyte chemoattractant protein-1 were also only significantly increased in livers of FFC-fed animals.

Conclusion: Our findings suggest that a treatment with the ADH inhibitor 4MP may attenuate the development of NAFLD in mice.

PO-205
Mitochondria-targeted antioxidant based on caffeic acid AntiOxCIN4 prevented hepatic lipid accumulation by avoided Western Diet-induced autophagic blockage

Ricardo Amorim[1 2 3], Inês C. M. Simões[4], José Teixeira[1], Pedro Soares[2], Yaiza Potes[4], Fernando Cagide[2], Sofia Benfeito[2], Adriana Carvalho[1], Ludgero Tavares[1 5], Susana P. Pereira[1], Rui F. Simões[1], Agnieszka Karkucinska-Wieckowska[6], Ivan Viegas[1 7], Michał Dąbrowski[4], Justyna Janikiewicz[4], Agnieszka Dobrzyń[4], John G. Jones[1], Fernanda Borges[2], Mariusz Więckowski[4], Paulo J. Oliveira[1]

[1]CNC-Center for Neuroscience and Cell Biology, CIBB-Centre for Innovative Biomedicine and Biotechnology, Coimbra, Portugal, *[2]Faculty of Sciences, University of Porto, CIQUP/Department of Chemistry and Biochemistry, Porto, Portugal*, *[3]Institute for Interdisciplinary Research (IIIUC), PhD Programme in Experimental Biology and Biomedicine (PDBEB), Coimbra, Portugal*, *[4]Nencki Institute of Experimental Biology, Laboratory of Mitochondrial Biology and Metabolism, Warsaw, Poland*, *[5]CIVG-Vasco da Gama Research Center, University School Vasco da Gama-EUVG, 3020-210 Coimbra, Portugal, Coimbra, Portugal*, *[6]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland*, *[7]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal.*
Email: ricardoamorim72@gmail.com

Background and aims: The incidence and prevalence of non-alcoholic fatty liver disease (NAFLD) are dangerously rising worldwide. Although mechanisms underlying disease pathophysiology are not fully clarified, mitochondrial dysfunction together with oxidative stress have been shown as potential key players. Driven by the lack of approved pharmacological therapies, we investigated the potential effect of a mitochondria-targeted hydroxycinnamic acid derivative (AntiOxCIN$_4$) in the prevention of NAFLD development.

Method: C57BL/6J mice daily supplemented with 2.5 mg AntiOxCIN$_4$ were then fed with standard diet (SD) or Western diet (WD) (30% high-fat, 30% high-sucrose) for 16 weeks.

Results: AntiOxCIN$_4$ supplementation decreased body (43%) and liver weight (39%) of WD-fed mice. Moreover, it also decreased the levels of plasmatic hepatocyte damage markers. The amelioration of hepatic-related parameters was associated with a reduction of fat accumulation (570%) and the remodelling of fatty acyl chain composition compared with WD-fed group. In accordance, we have also observed increased fatty acid oxidation related pathways in SD and WD-fed mice supplemented with AntiOxCIN$_4$. Additionally, AntiOxCIN$_4$ supplementation induced mitochondrial metabolism remodeling by improving mitochondrial respiration and increasing OXPHOS subunits levels in mice fed with WD + AntiOxCIN$_4$, triggered by the restoration of phospholipid profile and increased PGC-1α-SIRT3 axis. Finally, we uncovered that AntiOxCIN$_4$ supplementation could prevent WD-associated autophagic flux impairment, which can be prevented by an augment of lysosomal proteolytic capacity as observed *in vivo* model.

Conclusion: An amelioration of NAFL mice phenotype highlights the potential use of AntiOxCIN$_4$ in the prevention/treatment of NAFLD

PO-206
The activation of Liver X Receptor alpha in the gut protects the liver by the NASH-related damage

Gessica Lioci[1], Fabio Gurrado[1], Nadia Panera[2], Maria Rita Braghini[2], Cristiano De Stefanis[3], Monica Cucco[4], Anna Alisi[2], Gianluca Svegiati-Baroni[1 4]

[1]Marche Polytechnic University, Department of Gastroenterology, Ancona, Italy, *[2]Bambino Gesù Children's Hospital and IRCCS, Rome, Italy, Research Unit of Molecular Genetics of Complex Traits, Rome, Italy*, *[3]Bambino Gesù Children's Hospital and IRCCS, Rome, Italy, Core Facilities, Rome, Italy*, *[4]Marche Polytechnic University, Liver Injury and Transplant Unit, Ancona, Italy*
Email: anna.alisi@opbg.net

Background and aims: Non-alcoholic fatty liver disease (NAFLD) and its advanced form, non-alcoholic steatohepatitis (NASH), are becoming a worldwide health problem, but no approved medical treatment exists so far. In recent years the role of Liver X Receptor-alpha (LXR alpha), a nuclear hormone receptor involved in the regulation of hepatic lipid homeostasis and inflammation, has been studied in metabolic diseases, but its effects in the treatment of NASH are controversial. Here, we addressed the contribution of the intestinal activation of LXR alpha in hepatic lipid metabolism and fibrogenesis in NASH**.**

Method: We used FVBN (controls) and iVP16-LXR alpha (with constitutively activated LXR alpha in enterocytes) mice treated for 12 weeks with a control diet, or with (Carbon tetrachloride) CCl4 combined with a Western Diet (WD) as a model of NASH.

Results: After 12 weeks of treatment, WD/CCl4 iVP16-LXR alpha showed reduced liver weight, and hepatic content of triglycerides, total cholesterol, HDL and LDL, compared to WD/CCl4 FVBN.
WD/CCl4 iVP16-LXR alpha showed, compared to WD/CCl4 FVBN, decreased hepatic expression of genes involved in the uptake and storage of lipids (FABP4 and CD36) and de novo lipogenesis, such as fatty acid synthase.
In the liver of WD/CCl4 FVBN we observed an increased number of CD68+ macrophages compared to WD CCl4 iVP16-LXR alpha, suggesting a raised inflammatory pattern, while WD/CCl4 iVP16-LXR alpha exhibited preferentially anti-inflammatory M2 macrophages. Moreover, a reduced systemic and hepatic inflammation measured by inflammation antibody array and OpenArray gene expression technology was observed in WD/CCl4 iVP16-LXR alpha with respect to the WD/CCl4 FVBN. The reduced inflammatory activity in WD/CCl4 iVP16-LXR alpha mice resulted also in decreased hepatic collagen deposition, and in down-regulation of the expression of main fibrogenic genes (alpha-SMA, TGF-beta and type 1 and type 3 collagen). These results suggest that HDLs may exert an anti-inflammatory effect via the SRB1 receptor even though the mechanisms of action need to be further explored.

Conclusion: This work demonstrates that the specific activation of intestinal LXR alpha may exert beneficial effects overall on the process leading to NASH. Due to the lack of effective therapy in NASH, the selective activation of intestinal LXR alpha could represent a new specific treatment for the most common form of chronic liver disease.

PO-215
A 3D in vitro throughput-based discovery approach for selecting NASH drug candidates

Simon Ströbel[1], Katia Fiaschetti[1], Jana Rupp[1], Agnieszka Pajak[1], Katarzyna Sanchez[1], Manuela Bieri[1], Armin Wolf[1], Eva Thoma[1], Radina Kostadinova[1]

[1]*InSphero AG, Liver Discovery, Schlieren, Switzerland*
Email: simon.stroebel@insphero.com

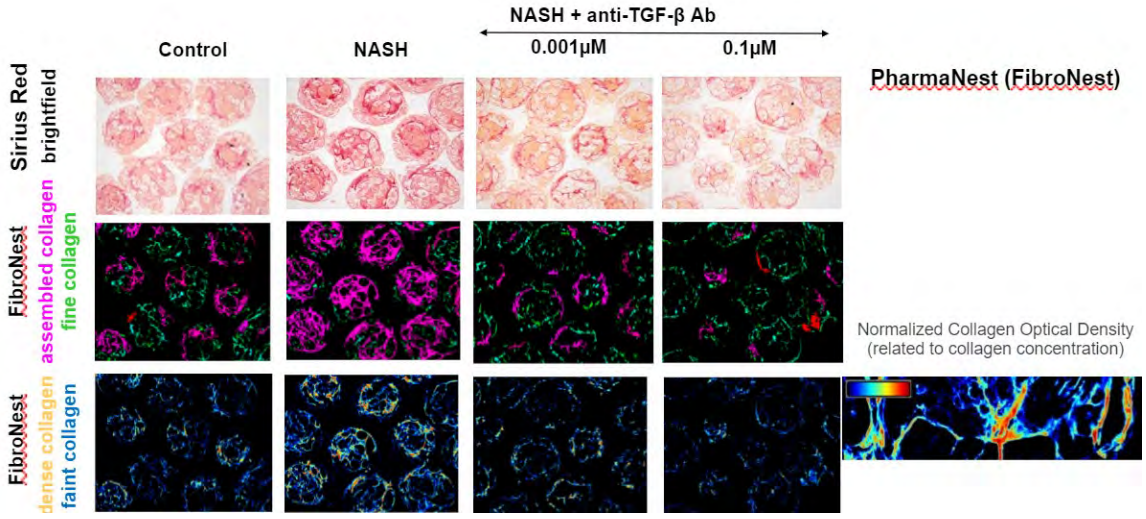
Background and aims: Non-alcoholic steatohepatitis is a severe progressive disease characterized by lipid accumulation, inflammation and fibrosis in the liver. To date, there are no approved drugs for NASH treatment and drug development has been impeded by the lack of predictive in vitro models reflecting the complex pathology of NASH. The aim of the study was to develop a human 3D *in vitro* NASH spheroid microtissue, based on a scaffold-free co-culture of primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells for high-throughput-compatible drug efficacy testing.

Method: Upon exposure to defined lipotoxic and inflammatory stimuli, such as free fatty acids and LPS in media containing high levels of sugar and insulin, our 3D NASH model displayed key disease pathophysiological features within 10 days of treatment. We established methods for assessing characteristic and quantifiable markers for NASH drug efficacy testing, including assays and end points for measuring triglyceride assays, secretion of pro-inflammatory cytokines/chemokines (Luminex), and secretion of pro-collagen type I/III (HTRF/ELISA).

Results: We observed increases in intracellular triglyceride content as indicator of lipid accumulation and the secretion of inflammatory markers such as IL-6, MIP-1alpha, TNF-alpha, IL-10, MCP-1 and IL-8 in our NASH-induced model as compared to the untreated control. Further, we detected increased fibril collagens deposition and secretion of procollagen type I and III peptides under NASH conditions. Whole transcriptome analysis of NASH-induced microtissue versus control revealed activation of pathways and differential regulation of genes associated with key lipid metabolism, inflammation, and fibrosis induction. Treatment with the anti-NASH TGF-beta antibody and ALK5i (TGFβRI inhibitor) concentration dependently decreased secretion of pro-collagen type I/III. Decreased deposition of fibrosis based on quantification of Sirius-Red-stained tissues (PharmaNest) was observed in the presence of anti-TGF-beta antibody and ALK5i. Importantly, biochemical readouts for 3D models treated with NASH drug candidates (Selonsertib and Firsocostat) were indicative of disease progression and the results generally reflected documented clinical observations.

Conclusion: In summary, using this rapid, high-throughput-compatible 3D NASH model for drug candidate efficacy testing in combination with multiplex end point assays, represents a promising approach for selection and decision making of the most effective drug candidates to move further in the development.

Figure:


NASH + anti-TGF-β Ab
Control
NASH
0.001µM
0.1µM
PharmaNest (FibroNest)
Sirius Red
brightfield
FibroNest
assembled collagen
fine collagen
Normalized Collagen Optical Density
(related to collagen concentration)
FibroNest
dense collagen
faint collagen

PO-221
TM6SF2/PNPLA3/MBOAT7 loss-of-function genetic variants impact on NAFLD development and progression both in patients and in in vitro models

Miriam Longo[1 2], Marica Meroni[1], Veronica Erconi[1], Fabrizia Carli[3], Chiara Macchi[4], Francesco Fortunato[5], Dario Ronchi[5], Silvia Sabatini[3 6], Erika Paolini[1 4], Emilia Rita De Caro[1], Anna Alisi[7], Luca Miele[8], Giorgio Soardo[9], Giacomo Comi[5 10], Luca Valenti[5 11], Massimiliano Ruscica[4], Anna Ludovica Fracanzani[1 5], Amalia Gastaldelli[3], Paola Dongiovanni[1]

[1]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, General Medicine and Metabolic Diseases, Milano, Italy, *[2]University of Milan, Department of Clinical Sciences and Community Health*, *[3]Institute of Clinical Physiology, 3National Research Council (CNR), Pisa, Italy*, *[4]University of Milan, Department of Pharmacological and Biomolecular Sciences, Milano, Italy*, *[5]University of Milan, Department of Pathophysiology and Transplantation, Milano, Italy*, *[6]University of Siena, Siena, Italy*, *[7]"Bambino Gesù" Children's Hospital IRCCS, Research Unit of Molecular Genetics of Complex Phenotypes, Roma, Italy*, *[8]Fondazione Policlinico Universitario A. Gemelli IRCCS, Area Medicina Interna, Gastroenterologia e Oncologia Medica, Roma, Italy*, *[9]University of Udine and Italian Liver Foundation, Department of Medical Area (DAME), Trieste, Italy*, *[10]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Neuromuscular and Rare Diseases Unit, Milano, Italy*, *[11]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Trasfusional Center-Translational Medicine*
Email: paola.dongiovanni@policlinico.mi.it

Background and aims: The I148M *PNPLA3* and E167K *TM6SF2* variants alongside the rs641738 polymorphism in *MBOAT7/TMC4* locus represent the main inherited factors for non-alcoholic fatty liver disease (NAFLD). We knocked-out *MBOAT7* in HepG2 cells, homozygous for the I148M PNPLA3 (MBOAT7$^{-/-}$), which developed giant lipid droplets (LDs). We aimed to:1) investigate the synergic impact of the 3 risk variants on liver disease severity and hepatocellular carcinoma (HCC) in NAFLD patients; 2) generate *in vitro* models of NAFLD by silencing *TM6SF2* in both HepG2 (TM6SF2$^{-/-}$) and MBOAT7$^{-/-}$ cells (MBOAT7$^{-/-}$TM6SF2$^{-/-}$).

Method: 1380 NAFLD patients including 121 HCC were stratified with a semi-quantitative score ranging from 0 to 3 according to the number of *PNPLA3*, *TM6SF2* and *MBOAT7* at-risk variants. TM6SF2 was silenced through CRISPR/Cas9. Organelles' morphology was assessed by transmission electron microscopy (TEM). Lipidomics was performed by LCMS-QTOF.

Results: At multivariate analysis adjusted for age, sex, BMI and diabetes, the co-presence of the 3 risk alleles correlated with the histological degree of steatosis ($p < 0.0001$), necroinflammation ($p < 0.0001$), ballooning ($p = 0.004$) and fibrosis ($p < 0.0001$). At nominal logistic regression analysis adjusted as above, patients carrying the 3 variants showed ~2-fold higher risk of fibrosis>2 ($p = 0.0003$), cirrhosis ($p = 0.0007$) and HCC ($p = 0.01$). *TM6SF2* silencing induced micro-vesicular LDs accumulation (median size: 0.87 μm^2) in TM6SF2$^{-/-}$ cells, whereas the MBOAT7$^{-/-}$TM6SF2$^{-/-}$ developed both micro/macro-LDs (median size: 4.60 μm^2). Lipidomic analysis revealed that the amount of saturated/monounsaturated triacylglycerols was increased in cells lacking *TM6SF2* gene, while the polyunsaturated ones were reduced. *TM6SF2* deletion strongly affected endoplasmic reticulum (ER) and mitochondrial architecture and, the compound knockout showed the highest levels of markers of ER/oxidative and DNA damage ($p < 0.05$). Both models increased proliferative rate, but it was still more exacerbated in the MBOAT7$^{-/-}$ TM6SF2$^{-/-}$ cells ($p < 0.05$), thus representing the first model generated *in vitro* which may fully reproduce features of NAFLD individuals bearing all the 3 at-risk variants.

Conclusion: The co-presence of the 3 at-risk mutations impacts on NAFLD spectrum, in humans and experimental models. *TM6SF2* silencing alone or combined with I148M PNPLA3 and MBOAT7 deletion affects LDs' distribution and contributes to hepatocellular damage and proliferation.

Figure:

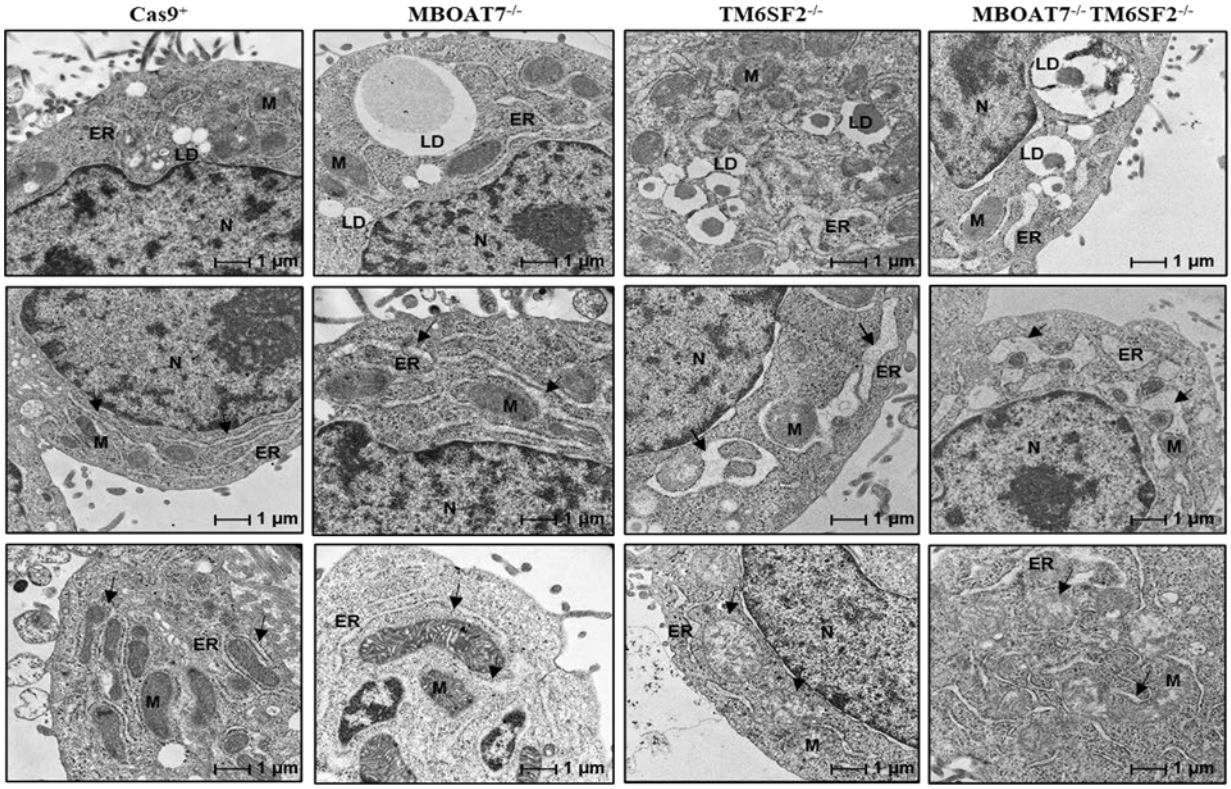


Figure: Representative TEM images of LDs, ER tubules, and degenerative mitochondria (black arrows) obtained by ultrathin 70 nm sections of hepatocytes (bar scale: 1 µm).

PO-229
Preclinical animal models for non-alcoholic steatohepatitis (NASH) and their pharmacological validation

Yishuang Wei[1], Jiliang Zhang[1], Mengnan Zhang[1], Miao Yuan[1], Chen Liu[1], Ping Chen[1], Yunhan Qiu[1], Luxia Wei[1], Mei Zhang[1], Feifei Zhu[1], Chaochen Yin[1], Lijuan Jiang[1], Xiaoyi Zhong[1], Huiru Zhao[1], Henry Lu[1], Deming Xu[1], Xiuxiu Shen[1], Kris Rutten[1]
[1]*WuXi AppTec*
Email: wei_yishuang@wuxiapptec.com

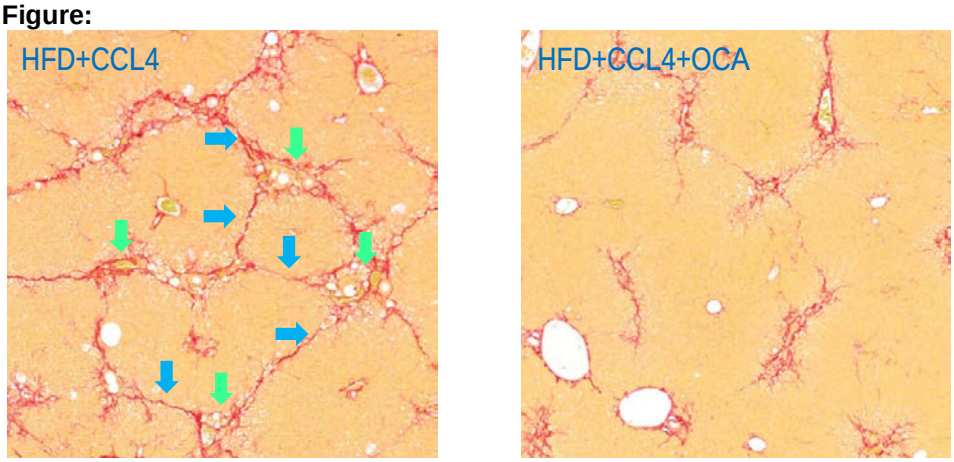
Background and aims: Preclinical animal models are essential to evaluating therapeutic agents for NASH (non-alcoholic steatohepatitis). Their clinical relevance underscores the validity of efficacy tests.

Method: We selected two animal models, MCD (induction with methionine and choline deficient diet) and HFD+CCL4 (sequential induction with high fat-diet followed by carbon tetrachloride) in mouse for histopathological analyses of hepatic steatosis, ballooning, inflammation and fibrosis, and efficacy tests with agents in clinical trials.

Results: Both models presented histopathological features that are consistent with the clinical definition of NAFLD activity score (NAS) and the hepatic fibrosis. However, periportal and perilobular bridging fibrosis (green and blue arrows in the figure below) was observed only in the HFD+CCL4 model (see Figure). The overall transcriptional changes (including inflammatory responses and lipid metabolism) in the livers of both HFD+CCL4 and MCD models displayed no correlation. The efficacy of obeticholic acid (OCA) was observed only in the HFD+CCL4 model, and that of selonsertib (SEL) only in the MCD model. The antifibrotic efficacy of OCA in the HFD+CCL4 model is exerted on perisinusoidal and portal tract fibrosis, and bridging fibrosis (figure below). In addition to OCA (targeting FXR) and SEL (ASK1), clinical agents targeting PPAR-alpha/delta, pan-PPAR, CCR2/CCR5, THR-beta, ACC1/2 and SSAO/VAP-1, and GLP1 were tested in the HFD+CCL4 model. Their efficacies were largely consistent with the respective clinical outcomes.

Conclusion: The HFD+CCL4 model is clinically relevant in histopathological findings. The results of pharmacological validation with clinical agents match the overall clinical outcomes.

Figure:



PO-230
Targeting the pro-inflammatory phenotype of adipose tissue macrophages using a dextran-nanocarrier ameliorates liver fibrosis and inflammation in a mouse model of NAFLD

Celia Martínez Sánchez[1 2], Mar Coll[3], Delia Blaya[3], Octavi Bassegoda[4], Hongping Deng[5], Pere Ginès[3 4], Kelly Swanson[5], Andrew Smith[6], Isabel Graupera[2 4]

[1]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Spain, *[2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Liver Unit, Barcelona, Spain*, *[3]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*, *[4]Hospital Clínic de Barcelona, Liver Unit, Barcelona, Spain*, *[5]University of Illinois at Urbana-Champaign, Champaign, United States*, *[6]University of Illinois at Urbana-Champaign, Materials Science and Engineering, Champaign, United States*
Email: mdcoll@clinic.cat

Background and aims: Non-alcoholic fatty liver disease (NAFLD) associated with obesity is characterized by a recruitment of macrophages in the liver and adipose tissue. Previous studies demonstrated that pro-inflammatory adipose tissue macrophages (ATMs) mediators induce hepatic inflammation in NAFLD although their role in fibrosis is still unknown. The aim of the present study is to modulate the pro-inflammatory phenotype of ATMs as a potential therapeutic strategy to reduce hepatic injury.

Method: Dextran (70kDa) nanocarriers conjugated with dexamethasone (dexa) were used to switch ATMs' phenotype of NAFLD and obese mice. NAFLD and obesity was mimicked by feeding mice for 6 months with high fat high cholesterol or high fat (HFD) diet, respectively. Both groups of mice were divided in those receiving dextran-conjugate or dextran-free drug *ip* for 5 weeks. To assess the efficiency of dextran internalization by ATMs, we administrated dextran-FITC to obese and NAFLD animals and analyzed it by flow cytometry. The modulation of hepatic and adipose macrophages' phenotype was addressed by flow cytometry and gene expression analysis. The anti-inflammatory effect of dexa-conjugates in liver and adipose tissue was evaluated by performing gene expression and immunostaining analysis of key inflammatory markers. Changes on hepatic fibrosis and NAS score were explored by histopathological analysis and by qPCR.

Results: NAFLD animals displayed an increased in liver and adipose tissue macrophage infiltration and exhibited an enhanced pro-inflammatory phenotype of macrophages in both territories. NAFLD mice showed an enhanced liver injury compared to obese animals as reflected by an increased serum transaminases, NAS score and fibrosis. Dextran-dexa conjugates were predominantly engulfed by ATMs compared to liver macrophages in NAFLD mice. Conjugated nanocarriers administration in NAFLD mice significantly switched the pro-inflammatory phenotype of ATMs but not liver macrophages. Dextran-dexa treatment produced a significant reduction of hepatic inflammation, NAS score, serum markers of liver injury and hepatic fibrosis in NAFLD mice

Conclusion: Dextran-dexamethasone conjugate is predominantly uptaken by adipose tissue macrophage and induce a switch of their phenotype which impacts on liver inflammation and fibrosis. There results demonstrate that modulating the pro-inflammatory phenotype of ATMs might be a good strategy to reduce hepatic fibrosis and inflammation in NAFLD.

PO-231
Cognitive dysfunction is associated with systemic inflammation and neuroinflammation in a rodent model of non-alcoholic steatohepatitis

Mohammed Eslam[1], Anne Catrine Daugaard Mikkelsen[2], Kristoffer Kjærgaard[2], Cecilie Bay-Richter[3], Annie Landau[3 4], Peter Lykke Eriksen[2], Rajiv Jalan[5], Stephen Hamilton-Dutoit[6], Fenghua Chen[3], Hendrik Vilstrup[2], Nils Erik Magnusson[7], Raj Mookerjee[2 5], Karen Louise Thomsen[2 5]

[1]Sydney University, *[2]Aarhus University Hospital, Department of hepatology and gastroenterology, Aarhus, Denmark*, *[3]Aarhus University, Translational neuropsychiatry unit, Aarhus, Denmark*, *[4]Aarhus University Hospital, Department of nuclear medicine and PET-centre, Aarhus, Denmark*, *[5]University College London, Institue for liver and digestive health, London, United Kingdom*, *[6]Aarhus University Hospital, Department of pathology, Aarhus, Denmark*, *[7]Aarhus University Hospital, Medical research laboratory, Aarhus, Denmark*
Email: ancami@clin.au.dk

Background and aims: Impaired cognition is well recognized in non-alcoholic fatty liver disease (NAFLD) and may affect up to 70% of NAFLD patients. The aim of this study was to investigate the hypothesis that cognitive dysfunction results from systemic and neuroinflammation in experimental NAFLD.

Method: Twenty male Sprague Dawley rats were fed a high-fat, high-cholesterol (HFHC) diet to induce NAFLD or a standard diet (n = 10 per group), for 16 weeks. The animals were studied for: behavioural changes-using validated neuropsychological tests; systemic inflammation-with a broad panel of plasma cytokines; neuroinflammation-using [^{3}H]PK11195 brain autoradiography.

Results: The HFHC diet induced non-alcoholic steatohepatitis (NASH) with extensive steatosis and lobular inflammation but no fibrosis. The HFHC rats demonstrated clear behavioural changes compared with standard diet: in Porsolt's Swim Test, they showed a depressive-like behaviour evidenced by increased immobility (p = 0.011) and reduced time swimming (p = 0.031); in the Novel Object Recognition test, they displayed impaired memory of previously encountered objects (p = 0.047). These changes were associated with elevated plasma pro-inflammatory cytokines (IFN-γ, GRO/KC, IL-1a, IL-2, IL-6, IL-10, IL-13, IL-17, MIP-1a, RANTES and MCP-1 (all p <0.05)) and, importantly, with increased microglia activation, demonstrated by increased specific-binding of [^{3}H]PK11195 in the prefrontal cortex (p <0.05).

Conclusion: Our data affirms that cognitive changes are present in preclinical NASH, even before fibrosis progression, and this is accompanied by systemic and neuroinflammation. The mechanistic pathways linking systemic and neuroinflammation require further elucidation, to highlight targets for therapy in patients.

PO-241
Melatonin mediated corrective changes in clock genes oscillations improves condition of experimentally induced Non-alcoholic fatty live in high fat high fructose fed C57BL/6J mice

Apeksha Joshi[1], Kapilkumar Upadhyay[2], Aliasgar Vohra[1], Kavita Shirsath[1], Ranjitsinh Devkar[1]

[1] Faculty of Science, The Maharaja Sayajirao University of Baroda, 1Division of Metabolic Physiology and Chronobiology, Department of Zoology, Vadodara, India, *[2]University of Michigan Medical School, Department of Internal medicine, Division of Gastroenterology and Hepatology, Michigan, United States*
Email: rv.devkar-zoo@msubaroda.ac.in

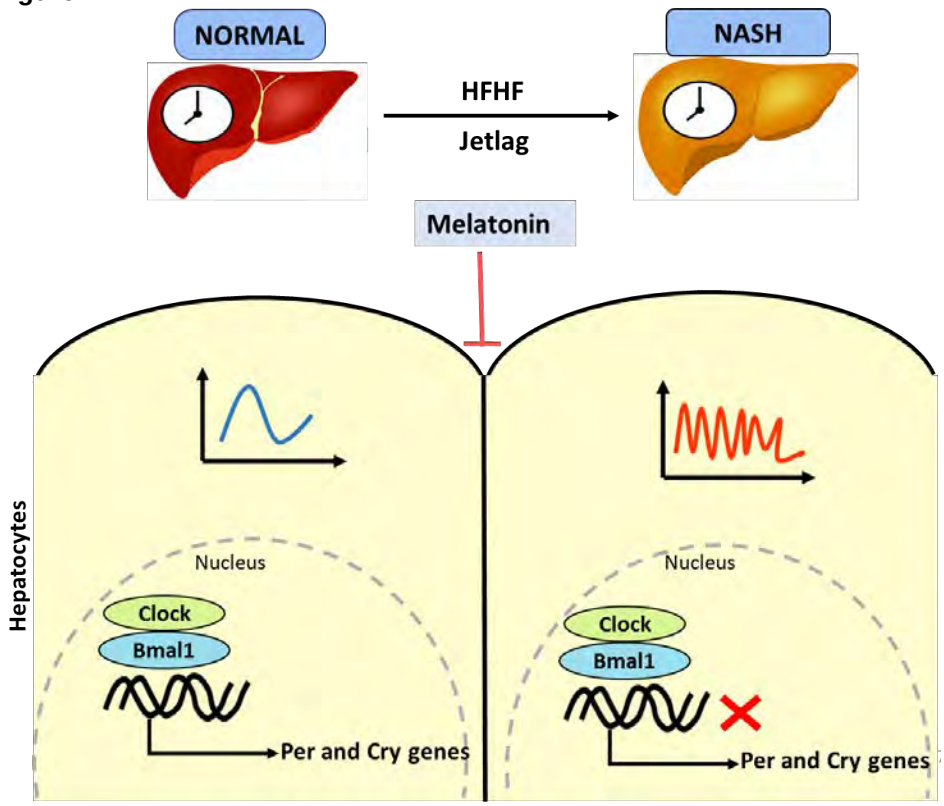
Background and aims: Epidemiological studies have shown that shift workers and transcontinental travellers exposed to photoperiodic changes have a high risk of metabolic disorders. The molecular clock network comprises of circadian locomotor output cycles kaput (Clock) and brain and muscle ARNT-like 1 (Bmal1) as activators, and period circadian protein homolog 1 (Per1), Per2, cryptochrome circadian regulator 1 (Cry1), and Cry2 as repressors, that function in transcriptional-translational feedback loop. Clock gene mutants display impaired glucose and lipid metabolism and are susceptible to diet-induced obesity and metabolic dysfunction. These evidence points towards a strong connection between the circadian clock and metabolic homeostasis. Non-alcoholic fatty liver disease (NAFLD) is associated with hepatic dysregulation of energy metabolism, lipid accumulation, oxidative stress and inflammation. This study investigates in detail, the shift in clock gene oscillations in high fat high fructose diet fed (HFHF) and/or Jet lag (JL) induced NAFLD, wherein merits of exogenous melatonin in making corrective changes has been contemplated.

Method: Male C57BL/6J mice were subjected to photoperiodic manipulations induced Jetlag (JL; 6h phase advance and phase delay) and/or high fat high fructose diet (HFHF) for 16 weeks, or melatonin (10 mg/kg) was injected intraperitoneally (after 8 weeks till the end of study). Serum was collected and liver function test (SGPT and SGOT) levels were studied in control and treated groups. Fresh frozen liver tissue was sectioned, and Oil red O staining was performed. Oscillation pattern of Clock genes (Bmal1, Clock, Cry2 and Per 1and2) was monitored in liver by qPCR followed by circwave analysis and immunoblots of Bmal1, Clock was performed at ZT = 0, 6, 12, 18, 24 h.

Results: C57BL/6J mice fed with HFHF and subjected to photoperiodic manipulations (jetlag) showed significantly elevated levels of AST and ALT as compared to control mice. Melatonin treatment recorded near normal levels of said parameter. HFHF and HFHF+JL groups recorded significant increment in fatty changes in hepatocytes as evidenced by ORO staining, but melatonin treatment showed beneficial effect in form of lowered lipid content in the liver. Further, hepatic mRNA and protein profiles of Bmal1 (at ZT6) and Clock (at ZT12) underwent corrective changes in oscillations, but moderate corrections were recorded in other components of clock genes (Per1, Per2 and Cry2). Exogenous melatonin partially restored the amplitude and peak time of said circadian oscillators.

Conclusion: Melatonin mediated corrective changes in hepatic core clock genes leads to an improvement in condition of HFHF and/or JL induced NAFLD implying towards its physiological role and possible use to alleviate lifestyle disorders.

Figure:


NORMAL
NASH
HFHF
Jetlag
Melatonin
Hepatocytes
Nucleus
Clock
Bmal1
Per and Cry genes
Nucleus
Clock
Bmal1
Per and Cry genes

PO-250
Human hepatocyte PNPLA3-148M exacerbates rapid non-alcoholic fatty liver disease development in chimeric mice

Mohammad Kabbani[1 2], Michailidis Eleftherios[1], Sandra Steensels[3], Clifton Fulmer[1 4], Joseph Luna[1], Jérémie Le Pen[1], Matteo Tardelli[3], Brandon Razooky[1], Inna Ricardo-Lax[1], Chenhui Zou[1 3], Briana Zeck[5], Ansgar Stenzel[1], Corrine Quirk[1], Lander Foquet[6], Alison Ashbrook[1], William M. Schneider[1], Serkan Belkaya[1 7], Gadi Lalazar[1 3], Yupu Liang[1], Meredith Pittman[3], Lindsey Devisscher[8], Hiroshi Suemizu[9], Neil Theise[5], Luis Chiriboga[5], David Cohen[3], Rob Copenhaver[6], Markus Grompe[6 10], Philip Meuleman[8], Baran Ersoy[3], Charles Rice[1], Ype de Jong[1 3]

[1]The Rockefeller University, New York, United States, *[2]Medizinische Hochschule Hannover, Hannover, Germany*, *[3]Weill Cornell Medicine, New York, United States*, *[4]Cleveland Clinic Main Campus, Cleveland, United States*, *[5]NYU Langone Health, New York, United States*, *[6]Yecuris Corporation, Tualatin, United States*, *[7]Bilkent University, Turkey*, *[8]Ghent University, Ghent, Belgium*, *[9]Central Institute for Experimental Animals, Kawasaki, Japan*, *[10]OHSU Hospital, Portland, United States*
Email: mokkabbani@gmail.com

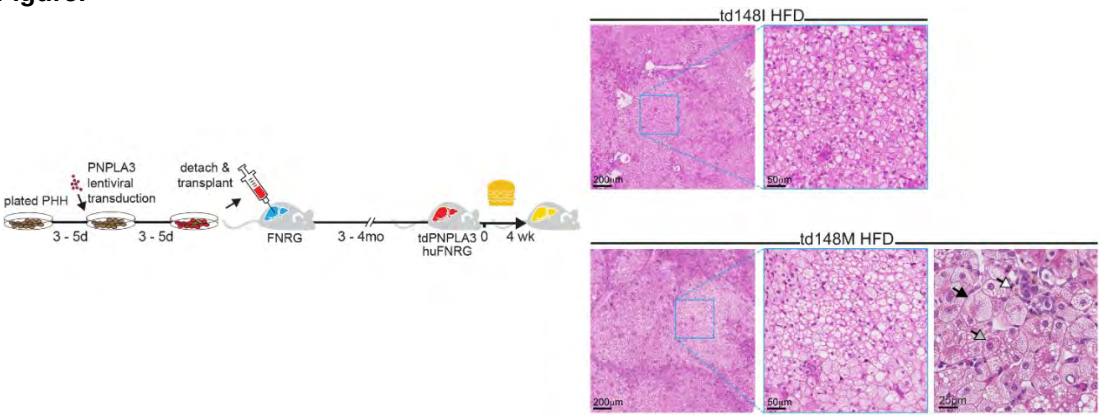
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a rapidly emerging global health problem associated with metabolic syndrome and predisposing genetic polymorphisms, most strikingly an isoleucine to methionine substitution in patatin-like phospholipase domain-containing protein 3 (PNPLA3-I148M). Despite mechanistic studies in rodent models and cell lines, the role of the 148M variant in human NAFLD progression remains to be further elucidated. Here we established a NAFLD model to study the impact of hypercaloric feeding in liver chimeric mice with PNPLA3-148I or -148M human grafts.

Method: Livers of immunodeficient *Fah-/-* mice (FNRG) were repopulated with primary human hepatocytes (PHH) from PNPLA3-148I or 148M donors. Once high human liver chimerism was achieved, mice were subjected to a Western-Style Diet (WD) consisting of sucrose in drinking water and a high fat (HFD). In addition, both PNPLA3 variants were overexpressed in PHH from a PNPLA3-148I homozygous donor prior to engraftment and chimeric mice were challenged with a WD or HFD.

Results: As early as 4 weeks on WD, humanized mice developed dyslipidemia, impaired glucose tolerance, and steatohepatitis selectively in the human graft, followed by pericellular fibrosis after 8 weeks of WD feeding. The PNPLA3-148M variant, either from a homozygous 148M PHH donor or overexpressed in a homozygous 148I PHH background, caused microvesicular steatosis, more active steatohepatitis, and Mallory Denk body formation in chimeric livers of mice on hypercaloric diets

Conclusion: PNPLA3-148I human hepatocytes develop steatosis and steatohepatitis with mild activity in chimeric mice on a hypercaloric diet. The PNPLA3-148M variant in human hepatocytes caused more active steatohepatitis. These models will facilitate mechanistic studies into the role of PNPLA3 in fatty liver disease progression. Human liver chimeric mice will also provide a platform to examine other human risk alleles associated with advanced fatty liver diseases.

Figure:


PNPLA3
lentiviral
transduction
plated PHH
detach &
transplant
3 - 5d
3 - 5d
FNRG
3 - 4mo
tdPNPLA3
huFNRG
0
4 wk
td148I HFD
200μm
50μm
td148M HFD
200μm
50μm
25μm

POSTER
ABSTRACT
PRESENTATION

CLINICAL SCIENCE

PO-10
Portohepatic hemodinamic disorders and increased blood ammonia at the steatohepatitis patients with initial liver fibrosis.

Tatiana Ermolova[1 1], Alexandr Petrov[2], MARIA BELOVICOVA[3]

[1]North-western state medical university named after I.I.Mecnnikov, Internal Medicine and Faculty, St.-Petersburg, Russian Federation, *[2]City Hospital 2, Internal medicine, St.-Petersburg, Russian Federation*, *[3]St.Elizabeth University of Health and Social Work, Health and Social Work, Bratislava, Slovakia*
Email: t.v.ermolova@mail.ru

Background and aims: there is data about decreased ureasynthesis at the non-alcoholic fatty liver disease with developing od hyperammoniemia. Ammonia becomes a new therapeutic target for treatment of fatty liver. Some experimental studies demonstrated effect of hypoammoniemic drugs for decrease of activity of hepatic stellate cells, liver microcirculation and prevention of liver fibrogenesis. Aims of our study are to estimate blood level of ammonia, intrahepatic microcirculation and efficacy of ornithine for correction of such disorders at the non-alcoholic steatohepatitis patients.

Method: We investigated 46 non-alcoholic steatohepatitis (NASH) and 35 HCV patients with initial fibrosis 0-2 stages. Level of ammonia was estimated by biochemical method (PocketChem BA, Arcray, Japan) in capillary blood at the patients and 29 healthy individuals (control).Intrahepatic hemodinamics are determined by polyhepatography (PHG)-modificated hepatic impedansometry, non-invasive method for integral estimation of intrahepatic blood flow by checking of tissue resistance to weak electric current. For correction of blood flow disorders we used hypoammoniemic drug ornithine (Hepa-Merz) in dosage 3 grams 2 times daily 4 weeks. Efficacy of LOLA we looked in 2 and 4 weeks via the control PHG and control of ammonia.

Results: Analysis of PHG demonstrated, that at all patients we revealed a liver microcirculation disorders- increased blood resistance, abnormal forms and amplitude of waves in sinusoidal level (out flow zone) at NASH patients and presinusoidal level (inflow zone) at viral patients. Level of ammonia in the NASH patients was 137.2 umol/L, in control group-39.2 umol/L ($p < 0.001$). Hyperammoniemia was higher at the NASH patients, compared with viral patients higher (102.3 umol) ($p < 0.01$). Analysis of efficacy of Hepa-Merz showed, that it was effective for correction of hepatic hemodinamic disorders at all patients, in 2 weeks of the treatment we observed normalization or improvement of the wave form, in 4 weeks-wave amplitude. Level of ammonia was decreased in 2 weeks.

Conclusion: NASH patients with initial stages of liver fibrosis are characterized by hyperammoniemia, which is more pronounced in comparison with viral hepatitis. NASH is accompanied by disorders of intrahepatic microcirculation disorders in out flow zone. LOLA improved liver microcirculation and decreases of blood ammonia level at the NASH and HCV patients.

PO-38
The sustainable development goals as a driver of multisectoral action on NAFLD: the NAFLD-SDG framework

Henry Mark[1], Massimo Colombo[2], Sandro Demaio[3], John Dillon[4], Jacob George[5], Hannes Hagström[6], Samantha Hocking[7], Nancy Lee[8], Mark Nieuwenhuijsen[9], Mary Rinella[10], Manuel Romero Gomez[11], Joan B. Soriano[12], Jörn Schattenberg[13], Frank Tacke[14], Emmanuel Tsochatzis[15], Luca Valenti[16], Shira Zelber-Sagi[17], Mae Dirac[18], Terry Huang[19], Jeffrey Lazarus[20]

[1]EASL International Liver Foundation, Genève, Switzerland, *[2]San Raffaele Hospital, Milano, Italy*, *[3]Victorian Health Promotion Foundation (VicHealth), West Melbourne, Australia*, *[4]School of Medicine-University of Dundee, Division of Molecular and Clinical Medicine, Dundee, United Kingdom*, *[5]The Westmead Institute for Medical Research, Westmead, Australia*, *[6]Karolinska University Hospital, Division of Hepatology, Sweden*, *[7]Royal Prince Alfred Hospital, Metabolism and Obesity Services, Camperdown, Australia*, *[8]Wilton Park, United Kingdom*, *[9]Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain*, *[10]Northwestern University Feinberg School of Medicine, Chicago, United States*, *[11]Virgen del Rocío University Hospital, UCM Digestive Diseases, ciberehd and IBIS, Sevilla, Spain*, *[12]Hospital de La Princesa, Respiratory Department, Madrid, Spain*, *[13]University Medical Center, Department of Medicine, Mainz, Germany*, *[14]Charité-Universitätsmedizin Berlin, Department of Hepatology and Gastroenterology, Berlin, Germany*, *[15]University College London, Institute for Liver and Digestive Health, United Kingdom*, *[16]University of Milan, Department of Pathophysiology and Transplantation, Milano, Italy*, *[17]University of Haifa, School of Public Health, Haifa, Israel*, *[18]University of Washington, Department of Family Medicine, Seattle, United States*, *[19]CUNY Graduate School of Public Health and Health Policy, New York, United States*, *[20]Barcelona Institute for Global Health (ISGlobal), Hospital Clínic, University of Barcelona, Barcelona, Spain*
Email: Jeffrey.Lazarus@isglobal.org

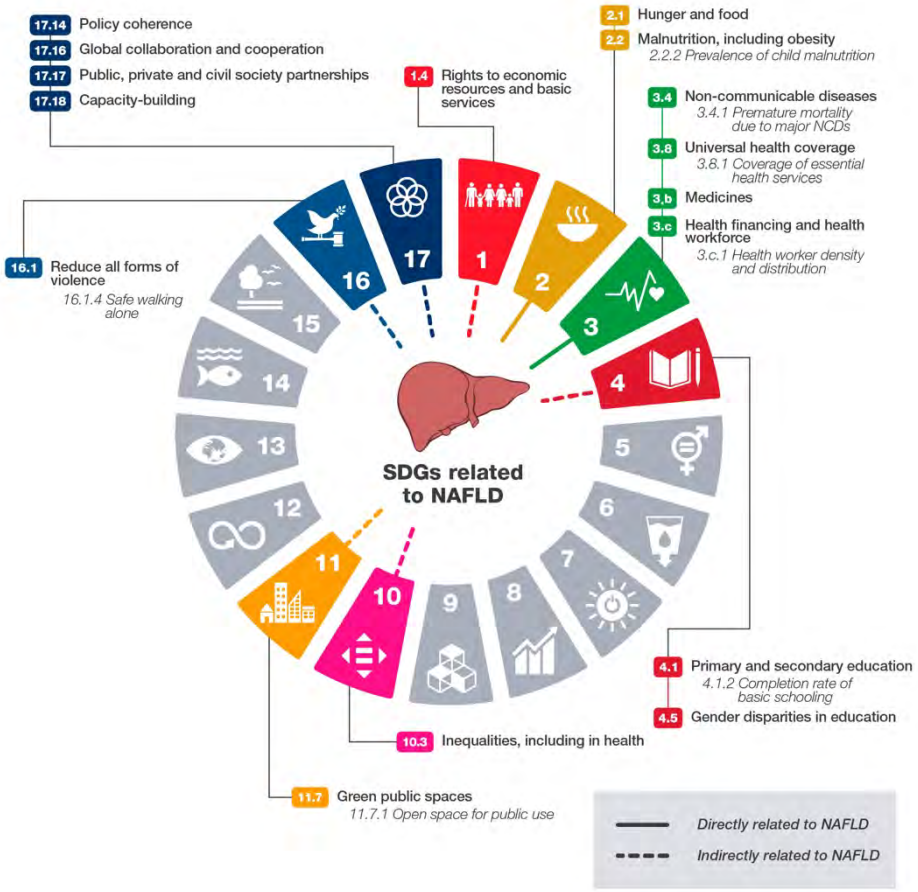
Background and aims: NAFLD is a highly prevalent condition that requires a comprehensive, cohesive, and coordinated response across sectors and disciplines. There is little awareness of the importance of multisectoral action on this issue or the measures required. The Sustainable Development Goals serve as the mainstay of the 2030 Agenda for Sustainable Development with clear priorities, from reducing social and economic inequalities to improving nutrition, health and education. The SDGs consist of 17 goals, followed by targets within each goal and indicators within each target. In the absence of a multisectoral framework for NAFLD, we developed a NAFLD-SDG framework to converge thinking about the design and delivery of public health responses.

Method: We developed the NAFLD-SDG framework following a two-stage process. Firstly, a core team of three researchers independently reviewed the 169 SDG targets and 231 unique indicators proposed by the Inter-Agency and Expert Group on SDG to develop a shortlist. Over two Delphi rounds, a multidisciplinary group of experts selected which of the shortlisted targets and indicators to include in the NAFLD-SDG framework. In the first Delphi round, respondents also provided written feedback on their selection. Following the second Delphi round, the targets and indicators with 75% or greater agreement were included in the final NAFLD-SDG framework.

Results: The final NAFLD-SDG framework comprises 16 targets and seven indicators across eight SDGs. This represents 9% of all SDG targets and 62% (16/26) of the shortlisted targets and 50% (7/14) of the shortlisted indicators and 3% of all SDG indicators. The selected targets and indicators cover a wide range of factors, from health, food and nutrition to education, the economy and the built environment (Figure 1).

Conclusion: Addressing the challenge of NAFLD will require re-envisioning the liver health landscape, with a greater focus on joined-up systems thinking and action across sectors and disciplines. The NAFLD-SDG framework can help guide this process by outlining the key stakeholders with whom the liver health community needs to engage and by providing a strategic advocacy tool to highlight the importance of cross-sectoral collaboration.

Figure:


17.14 Policy coherence
17.16 Global collaboration and cooperation
17.17 Public, private and civil society partnerships
17.18 Capacity-building
1.4 Rights to economic resources and basic services
2.1 Hunger and food
2.2 Malnutrition, including obesity
2.2.2 Prevalence of child malnutrition
3.4 Non-communicable diseases
3.4.1 Premature mortality due to major NCDs
3.8 Universal health coverage
3.8.1 Coverage of essential health services
3,b Medicines
3.c Health financing and health workforce
3.c.1 Health worker density and distribution
16.1 Reduce all forms of violence
16.1.4 Safe walking alone
SDGs related to NAFLD
4.1 Primary and secondary education
4.1.2 Completion rate of basic schooling
4.5 Gender disparities in education
10.3 Inequalities, including in health
11.7 Green public spaces
11.7.1 Open space for public use
Directly related to NAFLD
Indirectly related to NAFLD

PO-43
Effects of bariatric surgery on quality of life in patients with non-alcoholic fatty liver disease

Jesús Funuyet-Salas[1], maría ángeles pérez-san-gregorio[1], agustín martín-rodríguez[1], Manuel Romero Gomez[2]

[1]Faculty of Psychology. Department of Personality, Assessment, and Psychological Treatments. University of Seville, Spain., *[2]Digestive Diseases Unit. Virgen del Rocío University Hospital. SeLiver group at Institute of Biomedicine of Seville (IBIS). University of Seville, Spain.*
Email: jfunuyet1@us.es

Background and aims: Bariatric surgery shows a significant positive influence on quality of life (QoL), especially on physical functioning, in patients with obesity, although this is not clear in patients with non-alcoholic fatty liver disease (NAFLD). This study therefore compared QoL in NAFLD patients by whether or not they had undergone bariatric surgery.

Method: The sample of 243 biopsy-proven NAFLD patients was evaluated using the 12-Item Short-Form Health Survey (SF-12) and Chronic Liver Disease Questionnaire-Non-Alcoholic Fatty Liver Disease (CLDQ-NAFLD). The sample was divided into two groups, one with 81 patients (33 men and 48 women) who had undergone bariatric surgery (G_1), with a mean age of 50.02 years (*SD* = 10.59), and another group of 162 patients (66 men and 96 women) who had not undergone surgery, with a mean age of 50.74 years (*SD* = 10.48). This group was divided into two subgroups: 81 patients with obesity (G_2) and 81 without (G_3). Snedecor's *F* and Welch's *U* were computed to compare the QoL between groups. Tukey's *HSD* and Games-Howell tests were applied for post hoc multiple comparisons, and the Cohen´s *d* to measure effect size.

Results: Between-group differences (p = 0.000) were found in the total SF-12 scores in both the physical (PCS) and mental (MCS) component summary, and also in the total CLDQ-NAFLD. In the specific dimensions, the most outstanding differences (medium and large effect sizes) were found between the following groups: 1) G_1-G_2, with higher scores in G_1 in physical functioning (p = 0.000, d = 0.74), role-physical (p = 0.000, d = 0.79), bodily pain (p = 0.000, d = 0.62), general health (p = 0.000, d = 1.04), vitality (p = 0.000, d = 0.88), social functioning (p = 0.003, d = 0.53), role-emotional (p = 0.000, d = 0.71), mental health (p = 0.000, d = 0.72), PCS (p = 0.000, d = 0.77), MCS (p = 0.000, d = 0.68), abdominal symptoms (p = 0.000, d = 0.97), fatigue (p = 0.000, d = 0.88), systemic symptoms (p = 0.000, d = 0.92), activity (p = 0.000, d = 0.75), emotional (p = 0.000, d = 0.75), worry (p = 0.000, d = 0.80), and total CLDQ-NAFLD (p = 0.000, d = 1.06), and 2) G_2-G_3, with higher scores in G_3 in physical functioning (p = 0.000, d = -0.65), role-physical (p = 0.002, d = -0.54), general health (p = 0.001, d = -0.60), vitality (p = 0.000, d = -0.67), PCS (p = 0.001, d = -0.60), fatigue (p = 0.001, d = -0.56), systemic symptoms (p = 0.000, d = -0.76), activity (p = 0.000, d = -0.69), emotional (p = 0.002, d = -0.53), and total CLDQ-NAFLD (p = 0.000, d = -0.75).

Conclusion: NAFLD patients with obesity who had undergone bariatric surgery reported better physical and mental functioning than obese patients who had not. In addition, of those who had not undergone bariatric surgery, obese patients reported worse QoL, mainly physical, compared to non-obese patients. This study therefore confirmed the positive impact of bariatric surgery on QoL, and suggests the need to intervene in the QoL of NAFLD patients with obesity.

PO-47
Impact of coping, vitality and metabolic pathology on depressive symptoms of patients with non-alcoholic fatty liver disease

Jesús Funuyet-Salas[1], agustín martín-rodríguez[1], maría ángeles pérez-san-gregorio[1], Manuel Romero Gomez[2]

[1]Faculty of Psychology. Department of Personality, Assessment, and Psychological Treatments. University of Seville, Spain., *[2]Digestive Diseases Unit. Virgen del Rocío University Hospital. SeLiver group at Institute of Biomedicine of Seville (IBIS). University of Seville, Spain.*
Email: jfunuyet1@us.es

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is closely associated with more depressive symptoms than other chronic liver diseases. However, the influence of psychological variables such as coping or vitality on depressive symptoms, or the role of metabolic pathology in these relationships in NAFLD patients have not yet been sufficiently studied. Therefore, our objectives were to: 1) Find out whether vitality mediates the relationship between adaptive coping and depressive symptoms, and 2) Test whether type 2 diabetes mellitus (T2DM) and body mass index (BMI) exert a moderating effect on this relationship.

Method: We assessed 509 biopsy-proven NAFLD patients (300 men and 209 women, mean age 55.07 ± 11.85) using the Brief COPE (COPE-28), Beck Depression Inventory-II (BDI-II) and 12-Item Short-Form Health Survey (SF-12). The adaptive coping variable was constructed using patients' mean scores on the active coping, positive reframing and acceptance coping strategies. For the first objective, Model 4 mediation was performed using the SPSS PROCESS v3.5 macro, while for the second objective, Model 16 was used for moderated mediation. In both models, 5000 bootstrap samples were employed to test the indirect effects estimated, which were considered significant when the confidence interval (CI) at 95% did not include 0.

Results: Vitality mediated the association between adaptive coping and depressive symptoms (effect = -2.254, CI = -2.792 to -1.765). The direct effect of adaptive coping on depressive symptoms was significant after mediation analysis (effect = -3.665, p <0.001), showing partial mediation of vitality. T2DM (β = -0.043, p = 0.017) and BMI (β = -0.005, p = 0.009) moderated the negative effects of vitality on depressive symptoms. These indirect conditional effects were higher in diabetic patients than non-diabetic patients. In addition, the higher BMI was, the more these effects increased: low BMI (absence of T2DM, effect = -0.073, p <0.001; presence of T2DM, effect = -0.116, p <0.001); medium (absence of T2DM, effect = -0.097, p <0.001; presence of T2DM, effect = -0.140, p <0.001); and high (absence of T2DM, effect = -0.120, p <0.001; presence of T2DM, effect = -0.164, p <0.001).

Conclusion: These results showed that coping strategies and vitality are two psychological biomarkers influencing the mental health of NAFLD patients. In addition, the presence of T2DM and a higher BMI were found to be risk factors associated with increased depressive symptoms. In conclusion, low adaptive coping and reduced vitality could partly explain the stronger depressive symptoms of NAFLD patients usually observed. Considering the negative influence of poor mental health on therapeutic adherence, these factors should be considered in the design of future multidisciplinary NAFLD treatments, especially for patients with T2DM or obesity.

PO-48
Metabolic syndrome is associated with poor response to rifaximin in minimal hepatic encephalopathy

María Pilar Ballester[1 2], Juan José Gallego[2], Alessandra Fiorillo[2], Franc Casanova-Ferrer[2], Carla Gimenez-Garzo[3], Desamparados Escudero-García[1 4], Joan Tosca[1], María Pilar Rios[5], Cristina Montón[1], Lucía Durbán[5], Jose Ballester[1], Salvador Benlloch[5], Amparo Urios[2], Luis Aparicio[4], Miguel A Serra[1 4], Vicente Felipo[3], Carmina Montoliu[2 4]

[1]Hospital Clínic Universitari, Digestive Disease, València, Spain, *[2]INCLIVA Institut d'Investigació Sanitària, Valencia, Spain*, *[3]CIPF Centro de Investigación Príncipe Felipe, Laboratory of Neurobiology, Valencia, Spain*, *[4]University of Valencia, Medicine, València, Spain*, *[5]Hospital Arnau de Vilanova, Digestive Disease, València, Spain*
Email: mapibafe@gmail.com

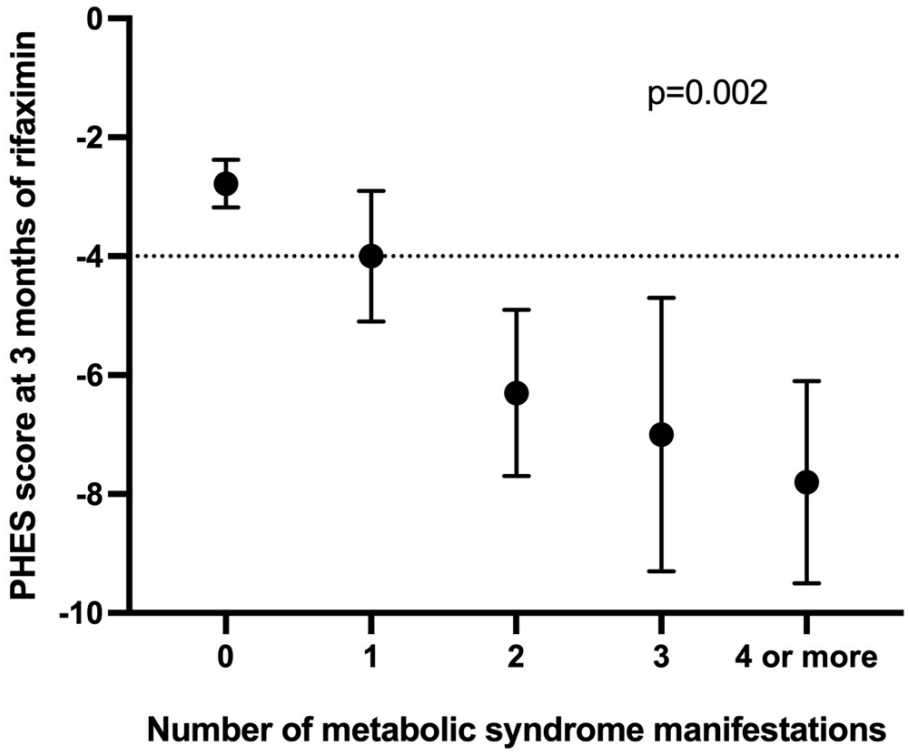
Background and aims: Patients with cirrhosis may show minimal hepatic encephalopathy (MHE), for which rifaximin is an effective and safe treatment. Several manifestations of metabolic syndrome may be associated with cognitive impairment. Our aims were to evaluate the influence of metabolic syndrome features on response to rifaximin for neurological and inflammatory alterations in MHE.

Method: A prospective cohort study was conducted in 63 cirrhotic patients from two tertiary centers recruited consecutively between 2015 and 2019. A group of 30 controls was included. Metabolic syndrome was defined according to the Adult Treatment Panel-III. Patients were classified into 31 without and 32 with MHE according to the Psychometric Hepatic Encephalopathy Score (PHES). All participants performed specific psychometric tests, and immunophenotype, cytokines, transcription factors and IgG levels were studied. Patients with MHE received rifaximin (400 mg/8h). Response was evaluated by PHES at 3 and 6 months. Risk analysis was used to compare response according to metabolic syndrome manifestations.

Results: The rifaximin response rate was 66%. Older age (p = 0.012) and all metabolic syndrome diseases (p < 0.05) were associated with non-response, plus an increase in risk as the number of manifestations rose (p < 0.001) (Figure 1). Patients with metabolic manifestations exhibited worse mental processing speed (p = 0.011), working memory (p = 0.005), visual coordination (p = 0.013) and lower proportion of activated T $CD4^+$ lymphocytes (p = 0.039) at baseline, as well as worse concentration (p = 0.030), bimanual coordination (p = 0.004) and higher levels of intermediate monocytes (p = 0.026), CX3CL1 (p < 0.05), IL-17 (p = 0.022), AHR (p = 0.010) and IgG (p < 0.05) at 3 and/or 6 months of rifaximin.

Conclusion: Patients with clinical signs of metabolic syndrome have poor response to rifaximin for MHE management, with a higher proportion of neurological alterations associated with a non-reversible pro-inflammatory environment.

Figure:


PHES score at 3 months of rifaximin
0
-2
-4
-6
-8
-10
p=0.002
0
1
2
3
4 or more
Number of metabolic syndrome manifestations

PO-73
Multifactorial effects of AXA1125 and AXA1957 observed on markers of metabolism, inflammation and fibrosis: a 16-week randomized placebo-controlled study in subjects with non-alcoholic fatty liver disease (NAFLD) with and without type 2 diabetes (T2D)

Stephen Harrison[1], Seth J. Baum[2], Nadege T. Gunn[3], Ziad H. Younes[4], Anita Kohli[5], Rashmee Patil[6], Manu Chakravarthy[7], Harinder Chera[8], Jeff Zhao[8], Margaret J. Koziel[8]

[1]Pinnacle Clinical Research, San Antonio, United States, *[2]Excel Medical Clinical Trials, LLC, Boca Raton, United States*, *[3]Pinnacle Clinical Research, Austin, United States*, *[4]Gastro One, Germantown, United States*, *[5]Arizona Liver Health, Chandler, United States*, *[6]Doctors Hospital at Renaissance Health, Edinburg, United States*, *[7]Carmot Therapeutics Inc, Berkeley, United States*, *[8]Axcella Health, Cambridge, United States*
Email: stephenharrison87@gmail.com

Background and aims: Effective and sustainable treatments for non-alcoholic steatohepatitis (NASH) will likely require simultaneously addressing metabolism, inflammation, and fibrogenesis. AXA1125 and AXA1957 are novel oral endogenous metabolic modulator (EMM) compositions of primarily amino acids. AXA1125-003 (NCT04073368) investigated the safety, tolerability, and physiologic effects of AXA1125 and AXA1957.

Method: This multicenter, randomized, placebo (PBO)-controlled study enrolled 102 adult NAFLD subjects ± T2D, with proton density fat fraction (PDFF) ≥10% and corrected T1 [cT1] ≥830 msec by multiparametric MRI. Subjects received twice-daily administration of either AXA1125 24 g, AXA1957 13.5 g or 20.3 g, or PBO for 16 weeks. Safety and tolerability were assessed by laboratory measures and adverse events (AEs). Physiologic assessments included change from baseline in key markers of metabolism (MRI-PDFF and homeostasis model assessment of insulin resistance [HOMA-IR]) and fibroinflammation (alanine aminotransferase [ALT], cT1, cytokeratin-18 [CK-18], N-terminal type III collagen propeptide [pro-C3]). Here, we report interim analysis (IA) data for subjects who completed ≥1 postbaseline MRI.

Results: Baseline characteristics were suggestive of NASH (average MRI-PDFF >20%, cT1 >900 msec, FibroScan >10 kPa, and pro-C3 >16 ng/ml). Among 62 subjects evaluable for this IA, those receiving AXA1125 and AXA1957 showed improved MRI-PDFF, ALT, cT1, and CK-18 relative to PBO as early as Week 8 and sustained at Week 16. Roughly 50% and 30% of subjects receiving AXA1125 and AXA1957, respectively, had ≥30% relative reduction from baseline MRI-PDFF. Fifty percent of subjects dosed with either composition had ≥40 msec absolute reduction in cT1. AXA1125 treatment led to marked absolute reductions in HOMA-IR. Subjects showed improvements in ALT, especially in those with a decrease >17 IU/L. Differential changes in CK-18 and pro-C3 were noted. Both compositions were safe and well tolerated, with stable lipid profiles. Overall, product-related AE rates were low, mostly mild, with minimal discontinuation and no product-related serious AEs. Top-line results from the complete data set will be shown.

Conclusion: AXA1125 and AXA1957 were safe, well tolerated, and led to clinically relevant multifactorial effects. These EMM compositions represent a novel mode of action with the potential to simultaneously address NASH and key comorbidities, including insulin sensitivity.

PO-78
A novel quantitative ultrasound technique for identifying non-alcoholic steatohepatitis

Feng Gao[1], Qiong He[2], Gang Li[1], Ou-Yang Huang[1], Liangjie Tang[1], Xiao-Dong Wang[1], Giovanni Targher[3], Chris Byrne[4], Jian-Wen Luo[2], Ming-Hua Zheng[1]

[1]the First Affiliated Hospital of Wenzhou Medical University, *[2]Department of Biomedical Engineering, School of Medicine, Tsinghua University*, *[3]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona*, *[4]Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton*
Email: gaofeng@wmu.edu.cn

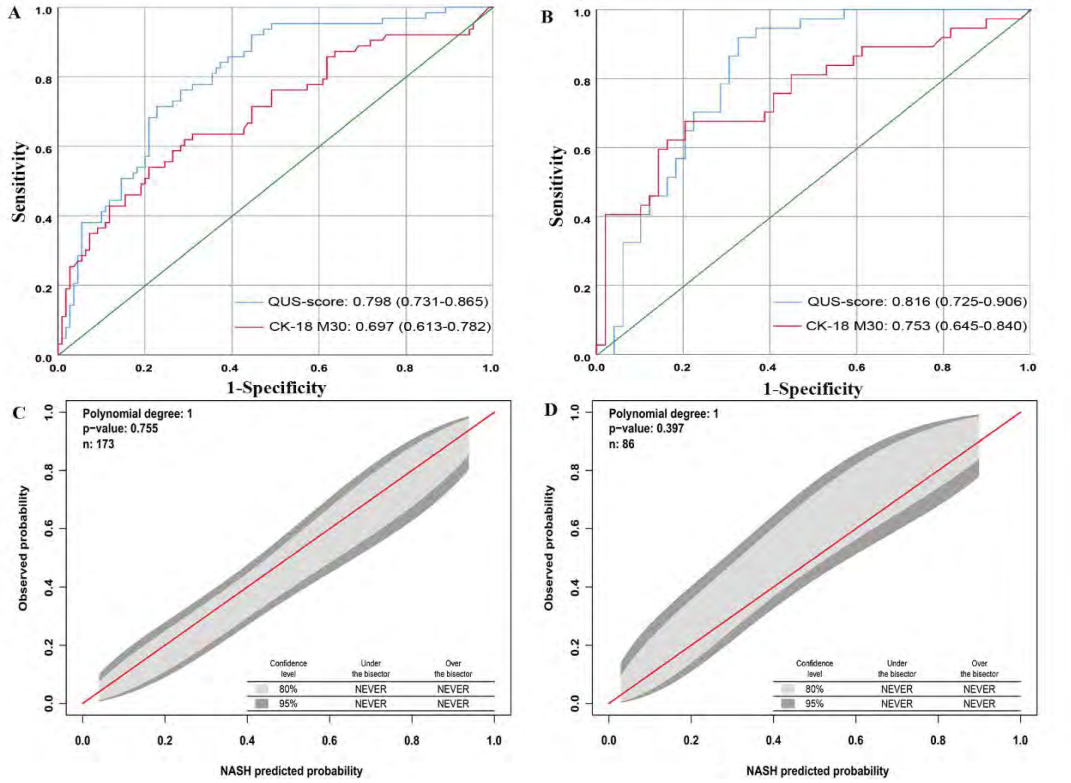
Background and aims: Non-alcoholic Steatohepatitis (NASH) has become the most rapidly increasing indication for liver transplantation in the United States. The correct identification of patients with NASH is important for prognosis and therapy decisions. The current expensive 'gold standard' for diagnosing and staging NASH, both in clinical practice and in investigational trials, is liver biopsy, which is an invasive procedure with associated risks, such as post-procedural bleeding. Therefore, there remains a need to develop a non-invasive, accurate, and easy-to-use tool to identify patients with NASH. Pre-clinical studies in animals have proved that quantitative ultrasound (QUS) parameters are closely related to the microstructure changes within liver tissue. We aimed to develop and validate a diagnostic tool, based on QUS analysis, for identifying NASH.

Method: We included 259 adult patients with biopsy-proven NAFLD, who underwent iLivTouch (Wuxi Hisky Medical Technologies Co., Ltd., China) examinations between December 2016 to September 2019 at the First Affiliated Hospital of Wenzhou Medical University (China). The histological spectrum of NAFLD was classified according to the NASH clinical research network scoring system. Radiofrequency (RF) data, raw data of iLivTouch was acquired for further QUS analysis. The toolbox of iLivTouch, LivQ-box, was utilized to obtain ultrasonographic features. The least absolute shrinkage and selection operator (LASSO) method was used to select the most useful predictive features. The study protocol was approved by the ethics committee and registered in the Chinese Clinical Trial Registry (ChiCTR-EOC-17013562).

Results: The mean age of participants was 42.8 years and 72.6% of them were male. NASH was histologically diagnosed in 100 subjects (38.6%). For the development of QUS-score, the study population was randomly assigned in a 2:1 ratio to training (n = 173) and validation (n = 86) sets. Eighteen candidate ultrasonographic features were reduced to two significant parameters by shrinking the regression coefficients with the LASSO method. We built a novel QUS-score based on these two features. The score formula as follows: QUS-score = 0.063*P7 + 0.019*P13. In the raw RF data, P7 is mainly related to intensity, and P13 is mainly related to scattering. The QUS-score yielded good discriminatory capacity and calibration for identifying NASH both in the training (area under the ROC curve [AUROC]: 0.798, 95% CI 0.731-0.865; Hosmer-Lemeshow test, p = 0.755) and validation sets (AUROC: 0.816, 95 %CI 0.725-0.906; Hosmer-Lemeshow test, p = 0.397) (Figure).

Conclusion: The results of our study shows that the newly developed and validated score, which was based on QUS technology, provides a non-invasive and practical way for accurately identifying the presence of NASH when lifestyle intervention is important.

Figure:


A
Sensitivity
1-Specificity
QUS-score: 0.798 (0.731-0.865)
CK-18 M30: 0.697 (0.613-0.782)
B
Sensitivity
1-Specificity
QUS-score: 0.816 (0.725-0.906)
CK-18 M30: 0.753 (0.645-0.840)
C
Polynomial degree: 1
p-value: 0.755
n: 173
Observed probability
NASH predicted probability
Confidence level
Under the bisector
Over the bisector
80%
NEVER
NEVER
95%
NEVER
NEVER
D
Polynomial degree: 1
p-value: 0.397
n: 86
Observed probability
NASH predicted probability
Confidence level
Under the bisector
Over the bisector
80%
NEVER
NEVER
95%
NEVER
NEVER

PO-80
Radiomics based on fluoro-deoxyglucose positron emission tomography predicts liver fibrosis in biopsy-proven NAFLD: a pilot study

Zhongwei Chen[1], Kun Tang[2], You-Fan Zhao[1], Yang-Zong Chen[2], Liangjie Tang[3], Gang Li[3], Ou-Yang Huang[3], Xiaodong Wang[4], Giovanni Targher[5], Chris Byrne[6], Xiang-Wu Zheng[1 2], Ming-Hua Zheng[3 4 7]

[1]Department of Radiology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, *[2]Department of Nuclear Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China*, *[3]NAFLD Research Center, Department of Hepatology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China*, *[4]Key Laboratory of Diagnosis and Treatment for the Development of Chronic Liver Disease in Zhejiang Province, Wenzhou, China*, *[5]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy*, *[6]Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Southampton, UK*, *[7]Institute of Hepatology, Wenzhou Medical University, Wenzhou, China*
Email: zhengmh@wmu.edu.cn

Background and aims: We aimed to evaluate the performance of radiomics based on ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) in predicting any liver fibrosis in individuals with biopsy-proven non-alcoholic fatty liver disease (NAFLD).

Method: A total of 22 individuals with biopsy-confirmed NAFLD, who underwent ^{18}F-FDG PET/CT, were enrolled in this study. Sixty-one radiomics features were extracted from whole liver region of interest in ^{18}F-FDG PET images. Subsequently, the minimum redundancy maximum relevance (mRMR) method was performed and a subset of two features mostly related to the output classes and low redundancy between them were selected according to an event per variable of 5. Logistic regression analysis was used to build predictive regression models based on selected features. The predictive performance was assessed by the receiver operator characteristic (ROC) curve analysis. The model calibration was assessed by the Hosmer-Lemeshow goodness of fit test.

Results: The mean (SD) age of the included subjects was 38.5 (10.4) years and 17 subjects were men. 12 subjects had histological evidence of any liver fibrosis. The coarseness of neighborhood grey-level difference matrix (NGLDM) and long-run emphasis (LRE) of grey-level run length matrix (GLRLM) were selected to predict fibrosis. The AUROC was 0.817 [95% confidence interval (CI), 0.595-0.947] for prediction of liver fibrosis (Table 1). The result of the Hosmer-Lemeshow goodness of fit showed a good calibration for the predicting model.

Conclusion: These preliminary data suggest that ^{18}F-FDG PET radiomics may have clinical utility in assessing early liver fibrosis in NAFLD.

Figure:

Table 1. Operating characteristics of radiomics based on ^{18}F-FDG PET for discriminating any fibrosis in biopsy-proven NAFLD.

	Fibrosis
AUROC (95%CI)	0.817 (0.595-0.947)
Sensitivity, % (n/N)	83.33 (10/12)
Specificity, % (n/N)	80.00 (8/10)
Accuracy, % (n/N)	81.82 (18/22)
PPV, % (n/N)	83.33 (10/12)
NPV, % (n/N)	80.00 (8/10)
LR+	4.17
LR-	0.21
Diagnostic odds ratio	19.9

Abbreviations: ^{18}F-FDG, fluorine-18-fluorodeoxyglucose; PET, positron emission tomography; NAFLD, non-alcoholic fatty liver disease; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predict value; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

PO-82
Sex influence the association between appendicular skeletal muscle mass to visceral fat area ratio and non-alcoholic steatohepatitis in patients with biopsy-proven NAFLD

Gang Li[1], Rafael Rios[2], Xin-Xin Wang[3], Yue Yu[4], Kenneth I. Zheng[1], Ou-Yang Huang[1], Liangjie Tang[1], Hong-Lei Ma[1], Giovanni Targher[5], Chris Byrne[6], Xiao-Yan Pan[7], Ming-Hua Zheng[1 8 9]

[1]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, NAFLD Research Center, Department of Hepatology, wenzhou, China, *[2]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, NAFLD Research Center, Department of Hepatology, China*, *[3]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Department of Gastroenterology, wenzhou, China*, *[4]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Department of Gastroenterology, China*, *[5]University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy;, Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, wenzhou, Italy*, *[6] University Hospital Southampton, Southampton General Hospital, Southampton, UK;, Southampton National Institute for Health Research Biomedical Research Centre, United Kingdom*, *[7]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Department of Endocrinology, wenzhou, China*, *[8]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Institute of Hepatology, Wenzhou Medical University, Wenzhou, China;, wenzhou, China*, *[9]the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China, Key Laboratory of Diagnosis and Treatment for The Development of Chronic Liver Disease in Zhejiang Province, Wenzhou, China, wenzhou, China*
Email: zhengmh@wmu.edu.cn

Background and aims: Sarcopenic obesity is regarded as a risk factor for the progression and development of non-alcoholic fatty liver disease (NAFLD). Since male sex is a risk factor for NAFLD and skeletal muscle mass markedly varies between the sexes, we examined whether sex influences the association between appendicular skeletal muscle mass to visceral fat area ratio (SVR), i.e., an index of skeletal muscle mass combined with abdominal obesity, and the histological severity of NAFLD.

Method: SVR was measured by bioelectrical impedance in a cohort of 613 (M/F = 443/170) Chinese middle-aged individuals with biopsy-proven NAFLD. Multivariable logistic regression as well as subgroup analyses were used to test the association between SVR and the severity of NAFLD (i.e., non-alcoholic steatohepatitis (NASH) or NASH with presence of any stage of liver fibrosis). NASH was identified by a NAFLD activity score≥5, with a minimum score of 1 for each of its categories. Presence of fibrosis was classified as having a histological stage ≥1.

Results: SVR was inversely associated with NASH in men (adjusted-odds ratio 0.62; 95%CI 0.42-0.92, P = 0.017 for NASH, adjusted-odds ratio 0.65; 95%CI 0.43-0.99, P = 0.043 for NASH with presence of fibrosis); but not in women 1.47 (0.76, 2.83), P = 0.25 for NASH, and 1.45 (0.74, 2.83), P = 0.28 for NASH with presence of fibrosis. There was a significant interaction for sex and SVR ($P_{interaction}$ = 0.017 for NASH and $P_{interaction}$ = 0.033 for NASH with presence of fibrosis).

Conclusion: Our findings show that lower skeletal muscle mass combined with abdominal obesity is strongly associated with the presence of NASH only in men.

PO-83
Biomaging, biochemical and genetic markers to stratify patients with metabolic associated fatty liver disease (MAFLD) in clinical practice: a single center cohort study

Antonio Salvati[1], Laura De Rosa[2], Francesco Faita[3], Daniela Cavallone[1], Gabriele Ricco[1], Piero Colombatto[1], Barbara Coco[1], Veronica Romagnoli[1], Filippo Oliveri[1], Ferruccio Bonino[4], Maurizia Brunetto[2 5]

[1]Pisan Hospital Cisanello, Hepatology Unit, Pisa, Italy, *[2]Pisan University Hospital Cisanello, Hepatology Unit, Pisa, Italy*, *[3]CNR PISA, Institute of Clinical Physiology, Pisa, Italy*, *[4]National Research Council, Institute of Biostructure and Bioimages, Napoli, Italy*, *[5]University of Pisa, Department of Clinical and Experimental Medicine, Pisa, Italy*
Email: maurizia.brunetto@unipi.it

Background and aims: MAFLD is a multifaceted syndrome affecting millions of patients (pts) with different clinical features. Only a small part of MAFLD pts progress to cirrhosis and eventually HCC, so the early identification of clinic-pathologic features predictive of higher risk of liver disease progression is mandatory. The aims of our single center cohort study were to analyze: (a) the prevalence of different subgroups of MAFLD among pts with hepatic steatosis at presentation; (b) the relationship between their clinic-pathologic characteristics, non-invasive biochemical markers of liver damage and inflammation and bioimaging markers of hepatic stiffness and intrahepatic fat to identify factors which correlate with progressive liver disease; (c) the prevalence across different MAFLD subgroups of the single-nucleotide polymorphisms (SNPs) of PNPLA3 and TM6SF2 genes which are involved in the transmembrane lipid transport at the hepatocyte level.

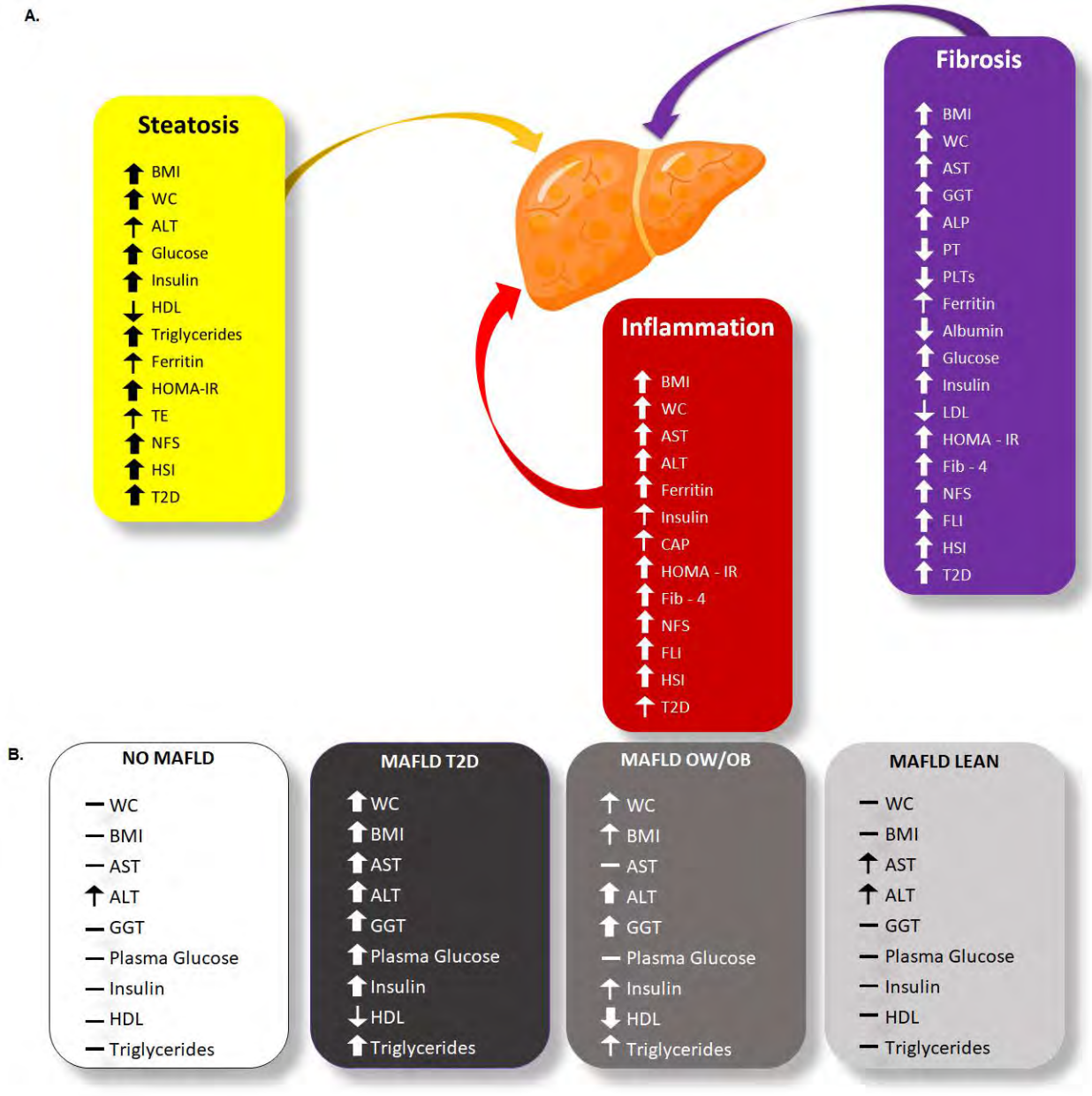
Method: We studied the distribution of bioimaging, biochemical and genetic markers in 419 pts with ultrasound steatosis who were classified according to MAFLD criteria: 52 pts (12.4%) without MAFLD, 367 (87.6%) with MAFLD of whom 72 with T2D, 268 overweight/obese and 27 BMI<25 kg/m^2.

Results: Overall MAFLD pts were older and with higher WC, BMI, AST/ALT and plasma glucose, insulin, triglycerides and lower HDL than pts without MAFLD. Liver stiffness (TE) correlated ($p < 0.001$) with WC, FIB-4 and Nafld Fibrosis Score (NFS), platelet-counts; with inflammatory markers (AST, $p = 0.002$; ferritin, $p = 0.005$) only in pts with TE <9.7 kPa. T2D MAFLD pts had higher TE ($p < 0.001$) and higher prevalence of TE ≥9.7kPa, $p = 0.033$. CAP correlated ($p < 0.001$) with BMI, WC, insulin, HOMA-IR, FLI and HSI, but not with TE. PNPLA3 rs738409 C >G homozygosity was found in 84 of 400 (21%) pts: it positively correlated with ALT ($p = 0.003$) and negatively with platelet-count ($p < 0.001$). TM6SF2 rs58542926 C >T homozygosity was rare (5 of 400 pts, 1.25%) and associated with lower cholesterol/triglycerides ($p = 0.002$, $p < 0.001$).

Conclusion: Our findings show that a multiparametric approach to identify differential profiles of steatosis, inflammation and fibrosis across subgroups is mandatory to warrant a personalized management of MAFLD patients.

Figure: Significant variations of bioimaging, biochemical and genetic markers associated (A) with Steatosis (yellow), Inflammation (red) and Fibrosis (purple) and (B) MAFLD groups.

The arrow thickness indicates the different degree of significance of the biomarker variation (⬆ = p <0.001; ↑ = p <0.05) directions of the arrow:-normal, ↑ increased and ↓ decreased values, respectively. BMI = Body mass index, WC = waist circumference, HOMA-IR = homeostasis model assessment-estimated insulin resistance index, T2D = type 2 diabetes, TM6SF2 = transmembrane 6 superfamily



member 2, PNPLA3 = Patatin Like Phospholipase Domain Containing 3.

PO-91
Diagnostic accuracy of liver steatosis measurements using artificial intelligence

Michael House[1], Miriam Vos[2], Eduardo Castillo-Leon[2], Jack Knight-Scott[3], Alton B. "Brad" Farris[4], Susanna Katay[5], Tim St Pierre[6]

[1]Resonance Health, Burswood, Australia, *[2]Emory University, Pediatrics, United States*, *[3]Children's Healthcare of Atlanta, Radiology, United States*, *[4]Emory University, Pathology and Laboratory Medicine, United States*, *[5]Resonance Health, United Kingdom*, *[6]The University of Western Australia, Physics, Australia*
Email: michaelh@resonancehealth.com

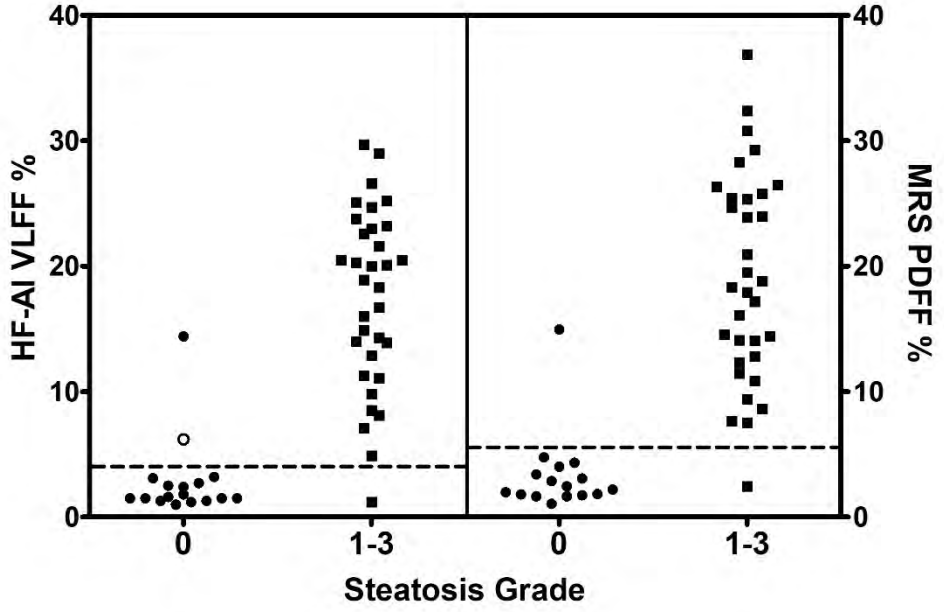
Background and aims: Liver steatosis is an important indicator of liver health that can be accurately measured using magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) methods. Artificial neural networks (ANN) now offer the ability to deliver quantitative assessments of steatosis in near real-time without the need for special expertise or training in image data analysis. The aim of this study is to compare the diagnostic ability of an ANN for predicting liver steatosis grades in biopsy against MRS measurements.

Method: Fifty patients (36 with NAFLD) aged 7 to 19 years undergoing percutaneous liver biopsy at Children's Healthcare of Atlanta for any reason were recruited. MRI scans (1.5T Siemens Aera) were performed within 3 days of the liver biopsy. Liver spectra were acquired using the HISTO sequence and volumetric liver fat fraction (VLFF) data were acquired via the HepaFat-AI® protocol. MRI data were automatically processed using a FDA-cleared ANN (HepaFat-AI®) for simultaneously measuring VLFF, proton density fat fraction (PDFF) and steatosis grade. Liver PDFFs were obtained from automatically generated HISTO reports. The area under the receiver operating characteristic curve (AUROC) was used to assess the diagnostic accuracy of each method against histology.

Results: ANN VLFF (HepaFat-AI®) returned AUROCs of 0.95 (0.88-1.00), 0.96 (0.92-1.00), and 0.97 (0.94-1.00) for the detection of liver steatosis at grade 0 vs 1-3, grade 0-1 vs 2-3, and grade 0-2 vs 3, respectively. MRS-PDFF showed AUROCs of 0.97 (0.92-1.00), 0.97 (0.94-1.00), and 0.98 (0.94-1.00). There were no statistically significant differences between the ANN-VLFF and MRS-PDFF AUROCs at any threshold. Excluding one case that had an ANN result, but no MRS result, the two techniques classified exactly the same cases at the grade 0 vs 1-3 boundary (Figure).

Conclusion: The accuracy of the ANN for diagnosing different grades of liver steatosis is equivalent to a conventional MRS technique. Using the ANN eliminates the need for image analysis or other technical expertise and speeds up reporting to an extent that point-of-care screening could be performed and costs associated with analysis greatly reduced.

Figure:



PO-92
Metabolic association and liver fibrosis relationship in patients with non-alcoholic fatty liver disease and gallstone disease

Tatyana Krolevets[1], Maria Livzan[1], Daria Popello[1]
[1]*Omsk State Medical University, therapy and gastroenterology, Omsk, Russian Federation*
Email: mts-8-90@mail.ru

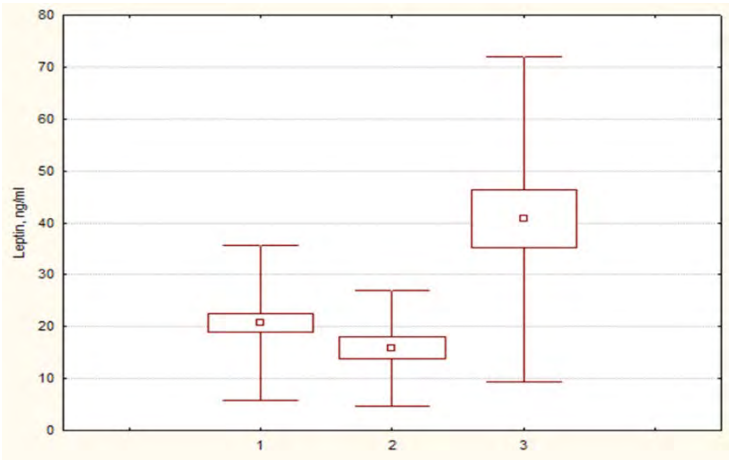
Background and aims: Nowadays, liver fibrosis is considered as a universal prognostic criterion for the development of non-alcoholic fatty liver disease (NAFLD) and patient survival. We want to assess the progression of liver fibrosis in a comorbid course of NAFLD and gallstone disease (GD) detecting the clinical and laboratory markers associated with the advanced fibrosis.

Method: 183 patients with NAFLD (127 men (69.4%) and 56 women (30.6%), average age 49 years) were included into the open comparative study. A standard laboratory and instrumental examination, including elastography were carried out. Furthermore, serum concentrations of insulin, leptin, its soluble receptor, and adiponectin were determined. The diagnostic value of the parameters obtained by the comparative and correlation methods and multiple regression analysis were studied in the groups of patients with NAFLD and GD (n = 88) with different stage of liver fibrosis in comparison with the patients with NAFLD without GD (n = 95).

Results: A high prevalence of ischemic heart disease was detected in patients with GD who underwent cholecystectomy (CE) (X^2 = 7.8269, p <0.01, r_s = 0.207, p≤0.05). The positive associations between the liver fibrosis and indications of CE and type 2 diabetes in patients with NAFLD (r_s = 0.234, p≤0.05 and r_s = 0.226, p≤0.05) were detected.
The phenomenon of insulin and leptin resistance were typical for patients with NAFLD and GD.The level of leptin is statistically significantly higher in patients with NAFLD and GD especially in patients who underwent CE (H = 16.63344, p <0.01, r_s = 0.336, p≤0.05). Comorbid patients with advanced stages of fibrosis had a high leptin level and a low level of adiponectin (U = (- 2.21648), p <0.01 and U = 2.04448, p <0.01). Hyperleptinemia positively correlated with the progressive stage of fibrosis in patients with GD and NAFLD (r_s = 0.363, p≤0.05). According to the results of multiple regression analysis, the highest significance in relation to liver fibrosis in patients with NAFLD were CE and type 2 diabetes in anamnesis.

Conclusion: The progression of liver fibrosis in patients with NAFLD and GD is associated with clinical and biochemical activity of the disease (NASH), dyslipidemia, type 2 diabetes and hyperleptinemia with a leptin resistance; CE can be considered as an unfavourable factor in the development of advanced stages of fibrosis in patients with NAFLD.

Figure: Figure 1. Level of leptin in comparative analysis of study groups. Group 1: Comparison group; Group: Non-alcoholic fatty liver disease (NAFLD) + gallbladder disease (GD); Group 3: NAFLD + GD + cholecystectomy (CE).



PO-115
Lower serum zinc levels are associated with severe hepatic necro-inflammation in patients with metabolic dysfunction-associated fatty liver disease (MAFLD)

Sui-Dan Chen[1] [1], Huai Zhang[2], Rafael Rios[3], Yang-Yang Li[1], Pei-Wu Zhu[4], Yi Jin[1], Hong-Lei Ma[3], Liangjie Tang[3], Gang Li[3], Ou-Yang Huang[3], Kenneth I. Zheng[3], Chris Byrne[5], Giovanni Targher[6], Ming-Hua Zheng[7]

[1]the First Affiliated Hospital of Wenzhou Medical University, Department of Pathology, Wenzhou, China, *[2]the First Affiliated Hospital of Wenzhou Medical University, Department of Biostatistics and Medical Record, Wenzhou, China*, *[3]the First Affiliated Hospital of Wenzhou Medical University, Department of Hepatology, Wenzhou, China*, *[4]the First Affiliated Hospital of Wenzhou Medical University, Department of Clinical Laboratory, Wenzhou, China*, *[5]University Hospital Southampton, Health Research Biomedical Research Centre, Southampton, United Kingdom*, *[6]University and Azienda Ospedaliera Universitaria Integrata of Verona, Section of Endocrinology, Diabetes, and Metabolism, Verona, Italy*, *[7]the First Affiliated Hospital of Wenzhou Medical University, Key Laboratory of Diagnosis and Treatment for The Development of Chronic Liver Disease, Wenzhou, China*
Email: zhengmh@wmu.edu.cn

Background and aims: zinc is an essential trace element that plays an important role in maintaining health, affecting gene expression, signal transduction, and regulation of apoptosis. It is currently uncertain whether serum zinc concentrations are altered in patients with metabolic dysfunction-associated fatty liver disease (MAFLD). We aimed to investigate the association between serum zinc levels and severity of hepatic necro-inflammation in patients with MAFLD.

Method: liver disease severity was evaluated histologically using NAS criteria. We undertook univariable and multivariable analyses of the association between serum zinc concentrations and severe hepatic necro-inflammation, and then further explored this association with a smoothing function regression model and threshold level analysis.

Results: 561 adult patients with biopsy-proven MAFLD were enrolled. Serum zinc concentrations were inversely and independently associated with severe hepatic necro-inflammation (adjusted-odds ratio: 0.91, 95% CI: 0.87-0.96), even after adjusting for known risk factors and potential confounders. Threshold saturation effect analysis suggested a threshold effect at high zinc concentrations, with an inverse association below the threshold and a trend toward a positive association above this threshold.

Conclusion: lower levels of serum zinc are significantly associated with greater severity of hepatic necro-inflammation in patients with MAFLD. There was a strong trend toward a positive association between zinc concentration and severe hepatic necro-inflammation at high zinc concentrations above the threshold effect concentration.

Figure: association between serum zinc level and risk of severe hepatic necro-inflammation in the whole population.

PO-139
The incremental Intrahepatic and extrahepatic cholangiocarcinoma risks in NAFL, NASH, and MAFLD patients: systematic review and meta-analysis

Boby Pratama Putra[1], Felix Nugraha Putra[2]

[1]Medical doctor, Blitar, Indonesia, *[2]Universitas Airlangga, Faculty of Medicine, Indonesia*
Email: boby_pratama_putra@yahoo.co.id

Background and aims: A new term, metabolic-associated fatty liver disease (MAFLD), is defined as the incidence of non-alcoholic steatohepatitis (NASH) with metabolic dysfunction. The non-alcoholic fatty liver disease (NAFLD) spectrum has been suggested as intrahepatic (ICCA) and extrahepatic (ECCA) cholangiocarcinoma risk factors, although the results were still inconsistent. This study aims to evaluate the association between NAFL, NASH, and MAFLD with the ICCA and ECCA incidence.

Method: Comprehensive searching using predefined keywords was conducted in online databases to include all relevant literature from 2000 until 2021. This study followed the PRISMA guideline. All NAFLD observational studies that access the association with the ICCA or ECCA incidence were included for analysis. Bias risk was accessed by using the Newcastle-Ottawa Scale. Analysis was performed to calculate the pooled odds ratio (OR) with 95% confidence interval (CI) using random-effect heterogeneity test.

Results: One cohort and 10 case-control studies with total of 467, 699 participants met our inclusion criteria. The overall NAFLD spectrum is significantly associated with ICCA (pooled OR = 2.31, 95% CI 1.64 to 3.24, $p < 0.00001$, $I^2 = 77\%$) and ECCA (pooled OR = 1.66, 95% CI 1.09 to 2.52, $p = 0.02$, $I^2 = 87\%$). The NASH subgroup has greater significant incremental ICCA risk (pooled OR = 3.37, 95% CI 1.86 to 6.10, $p < 0.00001$, $I^2 = 0\%$). The NAFL patients whose metabolic dysfunction, which consists of type-2 diabetes mellitus, hypertension, and dyslipidemia, has increased ICCA risk although not statistically significant (OR = 2.37, 95% CI 0.11 to 52.8). Otherwise, the NASH whose metabolic dysfunction or MAFLD patients have the greatest significant incremental ICCA risk (OR = 6.29, 95% CI 1.18 to 33.3).

Conclusion: The NAFL, NASH, and MAFLD patients are associated with increased ICCA and ECCA risks. However, further studies are warranted to establish the association and causality, particularly the MAFLD.

PO-144
NAFLD vs MAFLD: South Asian NAFLD patients don't favour name change

Prajna Anirvan[1], Amna Subhan[2], Ananta Shrestha[3], Anuradha Dassanayake[4], Bashir Ahmed Shaikh[5], Mamun Al-Mahtab[6], Shivaram Prasad Singh[1]

[1]SCB Medical College and Hospital, Cuttack, Gastroenterology, Cuttack, India, *[2]Aga Khan University Hospital, Medicine, Karachi, Pakistan*, *[3]Alka Hospital, Hepatology, Kathmandu, Nepal*, *[4]Colombo North Centre for Liver Disease, University of Kelaniya, Faculty of Medicine, Ragama, Colombo, Sri Lanka*, *[5]Shaheed Mohtarma Benazir Bhutto Medical University, Medicine, Larkana, Pakistan*, *[6]Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, Hepatology, Dhaka, Bangladesh*
Email: spsingh.cuttack@gmail.com

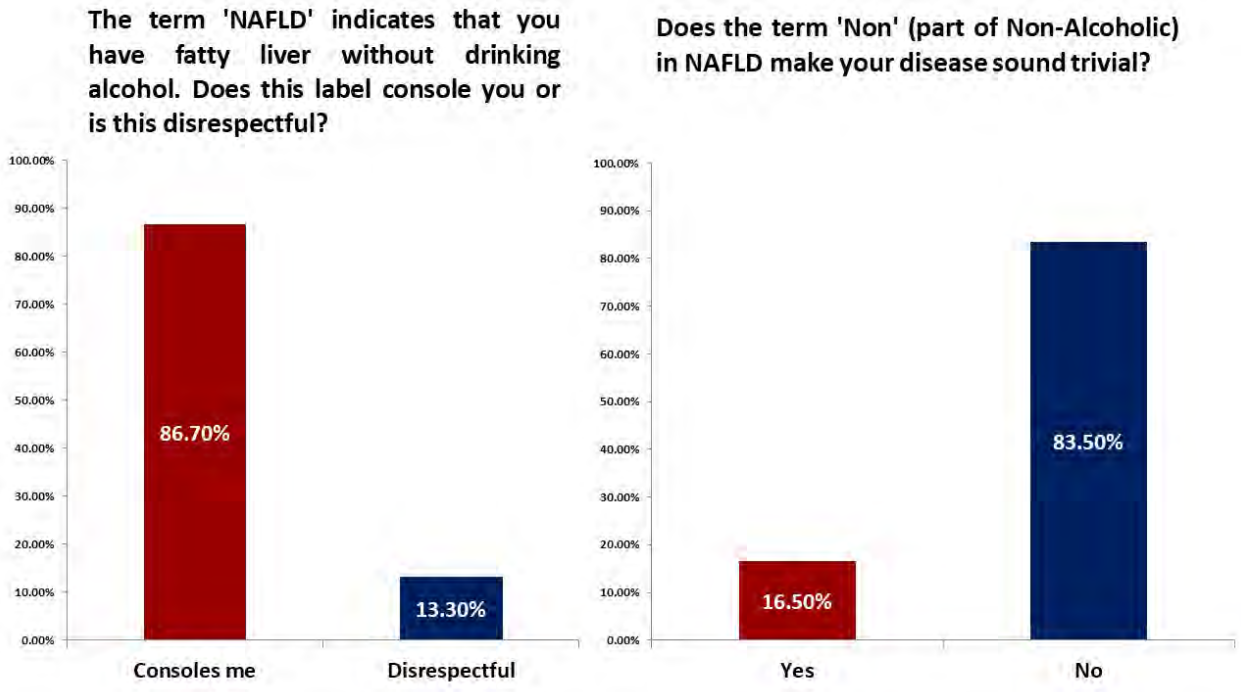
Background and aims: There has been an aborted attempt to change the nomenclature of Non-alcoholic Fatty Liver Disease (NAFLD) to MAFLD (Metabolic Associated Fatty Liver Disease). It has been claimed that patient groups have been insisting on the change; however, this is not based on any credible evidence. Hence, we decided to conduct a study among South Asian NAFLD patients to obtain their perspective, especially regarding change in name from NAFLD to MAFLD. The aim of the study was to assess awareness of the disease among NAFLD patients and their views regarding the proposed name change.

Method: The study was conducted at multiple centres across South Asia from January 2021 to June, 2021. An 8-question survey questionnaire was developed and responses were categorized by multiple-choice format. Survey responses were collated and analysed.

Results: Of 219 patients surveyed, 80.3% of the patients were not aware of the entity ‘NAFLD’ prior to diagnosis. 74.3% patients admitted to being questioned about alcohol intake at first diagnosis. Of the patients who were not questioned, 75.9% were females. After being labelled NAFLD, 92.1% patients were never grilled regarding alcohol intake at subsequent visits. While 86.7% patients found the term ‘NAFLD’ consoling, 83.5% patients didn’t think that ‘Non’ in NAFLD trivialised their problem. Only 7.2% patients were scared of developing cardiovascular disease.

Conclusion: Awareness of NAFLD among South Asian NAFLD patients is poor. The term ‘NAFLD’ destigmatises them of the taboo associated with alcohol use. Repeated queries about alcohol intake are humiliating to patients. Most patients found the term ‘NAFLD’ consoling and wanted NAFLD to stay since this label protected them from further persistent questioning about alcohol intake. Besides, the majority did not feel that it trivialises their problem. The proponents of MAFLD need to understand the cultural diversity across populations and recognise the sensitivities of South Asians before attempting to change the name.

Figure:


The term 'NAFLD' indicates that you have fatty liver without drinking alcohol. Does this label console you or is this disrespectful?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
86.70%
13.30%
Consoles me
Disrespectful
Does the term 'Non' (part of Non-Alcoholic) in NAFLD make your disease sound trivial?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
16.50%
83.50%
Yes
No

PO-149
One in ten cases of severe liver disease in the general population is attributable to type 2 diabetes

Ville Tapio Männistö[1], Miika Vuorinen[1], Veikko Salomaa[2], Annie Britton[3], Antti Jula[2], Satu Männistö[2], Iris Erlund[2], Jouko Sundvall[2], Annamari Lundqvist[2], Markus Perola[2], Fredrik Åberg[4]

[1]Kuopio University Hospital, Department of Medicine, *[2]Finnish Institute for Health and Welfare*, *[3]Institute of Epidemiology and Health Care, University College London*, *[4]Transplantation and Liver Surgery Clinic, Helsinki University Hospital*
Email: ville.mannisto@kuh.fi

Background and aims: The incidence of liver cirrhosis and hepatocellular carcinoma (HCC) is increasing, and alcohol-related liver disease and non-alcoholic fatty liver disease (NAFLD) are the leading etiologies in Western countries. Type 2 diabetes strongly associates with NAFLD and advanced liver disease. Actually, even a family history of diabetes has been linked to and liver fibrosis in NAFLD. Type 2 diabetes predicts mortality regardless of the primary etiology of liver disease, and diabetes combined with even mild alcohol use is associated with severe liver-related outcomes in the general population. However, the fraction of liver-related outcomes in the general population that are attributable to diabetes remains unclear.

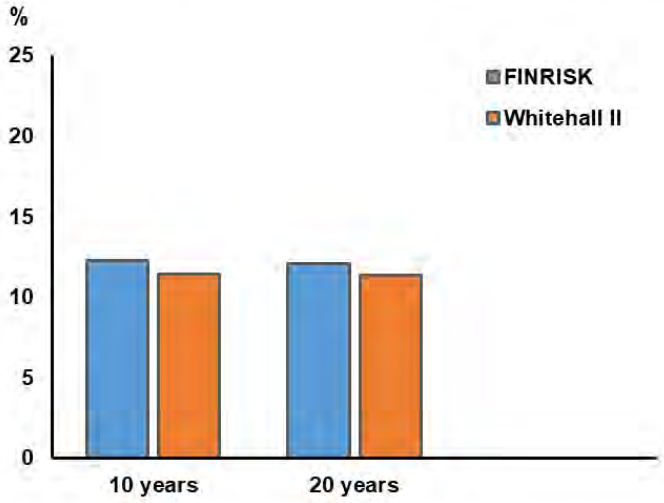
Method: The population attributable fraction (PAF) for liver disease of diabetes as a time-dependent exposure was estimated in the FINRISK 1997-2012 studies (n = 28787) and British Whitehall II study (n = 10063). We also assessed the predictive ability of a family history of diabetes for liver-related outcomes. Incident diabetes data were from drug purchase/reimbursement registries (FINRISK) or follow-up examinations (Whitehall II). Incident severe liver outcomes were identified through linkage with national registries for hospitalization, cancer, or death.

Results: Type 2 diabetes was associated with a 2-fold risk of liver-related outcomes in both the FINRISK (HR 1.92; 95%CI 1.36-2.67, P < 0.001) and Whitehall II (HR 2.92; 95%CI 1.36-2.67, P < 0.001) cohorts, and this remained significant after adjusting for age, sex, BMI, weekly alcohol use and smoking (HR 1.50, P = 0.024 and HR 1.91, P = 0.005 in FINRISK and Whitehall II, respectively). PAF-analyses demonstrated that diabetes explained 11-12% of the increased risk for severe liver-related outcomes after 10 and 20 years of follow-up (Figure), and this estimate was robust and significant also when adjusting for covariates described above, and in subgroups by alcohol use. Maternal diabetes increased the risk of liver-related outcomes (HR 1.43 (1.01-2.03), P = 0.044) in subjects without baseline diabetes.

Conclusion: Approximately 10% of severe liver-related outcomes are attributable to diabetes at the population level. The association between maternal, but not paternal, diabetes history and liver disease might suggest a mitochondrial genetic mechanism.

Figure:

Population attributable fraction (PAF) of diabetes for liver events in FINRISK and Whitehall II cohorts. Adjusted for age and sex.



PO-151
Use of non-invasive tests to assess NAFLD patients in routine clinical practice

Jeffrey Lazarus[1 2], Henry Mark[2], Laurent Castera[3], Sven Francque[4], Emmanuel Tsochatzis[5 6]

[1]ISGlobal, Barcelona, Spain, *[2]EASL International Liver Foundation, Genève, Switzerland*, *[3]Paris Diderot University, Paris, France*, *[4]University of Antwerp, Antwerpen, Belgium*, *[5]University College London, Institute for Liver and Digestive Health, United Kingdom*, *[6]Royal Free Hospital, Sheila Sherlock Liver Centre, United Kingdom*
Email: Jeffrey.Lazarus@isglobal.org

Background and aims: An enduring challenge for addressing NAFLD is ensuring that patients with significant or advanced fibrosis are identified and linked to appropriate care. Non-invasive tests (NITs) are proposed as a practical first step assessment of fibrosis risk. By applying a single cut-off or a low (rule-out) and high (rule-in) cut-off to NIT results, the risk of a patient having a pre-defined level of fibrosis can be predicted. While NITs are becoming more widely utilised, little data is available on their application modalities in clinical practice.

Method: We developed a survey to capture information about the NITs being used in clinical practice and the corresponding cut-offs being employed. The survey was circulated to a convenience sample of more than 200 NAFLD clinicians and researchers. Data collection started in March 2021 and is ongoing.

Results: As of 1 July 2021, surveys had been submitted from 24 different secondary and tertiary healthcare settings in 22 countries. Respondents from 10 countries (45%), reported a written national risk stratification pathway that outlines the NIT strategy and/or cut-offs; four respondents (17%) reported a written sub-national risk stratification pathway. The median number of different NITs used at each clinic was 3.5 (IQR 3-4, range 1-8). FIB-4 was the most used NIT, employed in 21/24 clinics (87.5%), followed by Fibroscan® (20/24 clinics; 83%) and NAFLD Fibrosis Score (15/24 clinics; 63%). The FIB-4 cut-offs varied among clinics. The most used lower cut-off was <1.3, nine clinics used this in all adults. Seven clinics used age-specific cut-offs (<1.3 if under 65 and <2.0 if 65 or over). Three clinics used a low cut-off of <1.45. Eleven clinics used an upper cut-off of >2.67 for all ages, while four used an upper cut-off of >3.25. For NAFLD Fibrosis Score 12/15 clinics employed lower and upper cuts-offs of <-1.455 and >0.676 respectively. Upper cut-off for liver stiffness by Fibroscan® (M probe) varied widely, from >8kPa to >15kPa; 9.6kPa was the most common higher cut-off, used by four different clinics.

Conclusion: NITs are considered a valuable tool to identify patients with NAFLD who require specialist care. The current interim findings show that the cut-offs being used in routine practice vary widely between healthcare settings. As lower and upper cut-offs have important implications for sensitivity and specificity of the test, these findings can inform ongoing discussions around the benefits of standardised setting and population specific cut-offs.

PO-153
Oral supplementation of phosphatidylcholine diminishes the onset of a diet-induced non-alcoholic fatty liver disease

Victor Sánchez[1], Annette Brandt[1], Raphaela Staltner[1], Sophie Haselsteiner[1], Anja Baumann[1], Ina Bergheim[1]

[1]*University of Vienna, Department of Nutritional Sciences, Vienna, Austria*
Email: ina.bergheim@univie.ac.at

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is by now one of the most common liver diseases worldwide affecting ~25% of the global general population with still increasing prevalence. General overnutrition, and a diet rich in fat and sugar are considered to be key risk factors of the disease development. Among many other changes, decreases in phosphatidylcholine (PC) levels in liver tissue and blood have been associated with the development of NAFLD in humans. However, data on the effects of an oral supplementation of PC on the development of NAFLD are limited. Here, we assessed the effects of an oral supplementation of egg yolk PC (12.5 mg/g diet) in a pair-feeding model of diet-induced NAFLD in C57BL/6J mice.

Method: Female C57BL/6J mice were either pair-fed a liquid control diet (C) ± 12.5 mg PC/g diet, or a fat-, fructose- and cholesterol rich diet (FFC) ± 12.5 mg PC/g diet for 8 weeks. A glucose tolerance test was performed and indicators of liver damage and inflammation were assessed.

Results: While only having slightly altered indices of glucose metabolism, FFC-fed mice developed macrovesicular steatosis and early signs of inflammation. Despite similar caloric intake and body weight gain, development of steatosis and inflammation was significantly attenuated in FFC+PC-fed mice. Furthermore, number of neutrophil granulocytes and myeloperoxidase activity in liver tissue were significantly lower in livers of FFC+PC-fed mice than in FFC-fed animals ($p < 0.05$), with both parameters being almost at the level of controls in livers of FFC+PC-fed animals. PC treatment of FFC-fed mice also attenuated the increased formation of nitric oxide (NOx) found in livers of FFC-fed mice.

Conclusion: Our results indicate that an oral supplementation of phosphatidylcholine may at least in part attenuate the onset of a diet-induced NAFLD in mice and that this may be related to alteration of NOx formation in the liver.

PO-157
AGILE3+ development and validation: novel FibroScan based score to diagnose advanced fibrosis in non-alcoholic fatty liver disease patients

Zobair Younossi[1], Stephen Harrison[2], Philip N Newsome[3], Wah-Kheong Chan[4], Yusuf Yılmaz[5], Victor de Lédinghen[6], Charlotte Costentin[7], Ming-Hua Zheng[8], Vincent Wai-Sun Wong[9], Magdy Elkhashab[10], Ryan Huss[11], Robert Myers[11], Julie Foucquier[12], labourdette aymeric[12], Marie Destro[12], Céline Fournier[12], Véronique Miette[12], Laurent Sandrin[12], Jerome Boursier[13], Arun Sanyal[14]

[1]Inova Fairfax Hospital, Falls Church, United States, *[2]University of Oxford, United Kingdom*, *[3]National Institute for Health Research, Birmingham Biomedical Research Centre, Birmingham, United Kingdom*, *[4]Universiti Malaya KL Gate, Kuala Lumpur, Malaysia*, *[5]Marmara University, Turkey*, *[6]Haut-Lévêque University Hospital, Pessac, France*, *[7]Grenoble-Alpes University Hospital, Grenoble, France*, *[8]Wenzhou Medical University, Wenzhou, China*, *[9]The Chinese University of Hong Kong, Hong Kong, China*, *[10]Toronto Liver Centre, Toronto, Canada*, *[11]Gilead Sciences, Inc., Foster City, United States*, *[12]Echosens, Paris, France*, *[13]Angers University Hospital Center, Angers, France*, *[14]VCU School of Medicine, Richmond, United States*
Email: jeboursier@chu-angers.fr

Background and aims: Available non-invasive tests, including FIB-4 and liver stiffness measurement (LSM) by Vibration Controlled Transient Elastography (VCTE) are highly effective in excluding advanced fibrosis (AF; F ≥3) yet their ability to rule it in is moderate. Our objective was to develop and validate a new score (Agile 3+), combining LSM with routine clinical parameters to identify AF in non-alcoholic fatty liver disease (NAFLD) patients, with optimized positive predictive value (PPV) and reduced cases with indeterminate results.

Method: This multi-national, retrospective study included 7 cohorts of NAFLD adults with liver biopsy, LSM by VCTE, and blood sampling in routine clinical practice or during clinical trials screening. The population was randomly divided into a training set (TS; n = 1434), on which the best fitting logistic regression model was built, and an internal validation set (VS; n = 700), on which performance and goodness of fit of the model were assessed. Furthermore, Agile 3+ was externally validated in the NASH CRN cohort (8 US centers, n = 585) and the French NAFLD cohort (3 centers, n = 1042). Cut-offs (≥85% sensitivity/≥90% specificity) in the TS were derived to rule-out and rule-in AF, respectively for FIB-4, LSM and Agile 3+ and tested in the VS.

Results: Agile 3+ combined LSM, AST/ALT ratio, platelets, gender, age and presence of diabetes mellitus. Calibration plots of Agile 3+ indicated excellent goodness of fit. The area under the receiver operating characteristic curves (AUROC) of Agile 3+ ranged from 0.86 to 0.90. In all datasets, Agile 3+ outperformed LSM and FIB-4 in terms of AUROC, percentage of patients with indeterminate results and that of patients with AF with a score above the high cut-off. Moreover, in the TS and internal VS, patient rate with F <3 with a score below the low cut-off and PPV for patients above the high cut-off were higher with Agile 3+ compared to FIB-4 and LSM (Figure).

Conclusion: A novel non-invasive score including LSM by VCTE and routine clinical parameters improves the identification of AF among NAFLD patients and may reduce the necessity of liver biopsy. Moreover, external validation on primary and secondary care centers could assess its potential as a new tool to refer patients to liver specialists.

Figure:

	Training set			Internal VS			NASH CRN cohort			French NAFLD cohort**		
N	1434			700			585			1042		
Prevalence F≥3	54%			54%			37%			38%		
	FIB-4	LSM	Agile 3+	FIB-4	LSM	Agile 3+	FIB-4	LSM	Agile 3+	FIB-4	LSM	Agile 3+
AUROC [95% CI]	0.82 [0.80;0.84]	0.86 [0.84;0.88]	0.90 [0.88;0.91]	0.84 [0.81;0.86]	0.85 [0.82;0.88]	0.90 [0.88;0.92]	0.78 [0.74;0.82]	0.83 [0.80;0.87]	0.86 [0.84;0.89]	0.78 [0.76;0.81]	0.84 [0.81;0.86]	0.87 [0.85;0.89]
Delong test *p* (vs Agile 3+)	< 0.0001	< 0.0001	NA	< 0.0001	< 0.0001	NA	< 0.0001	0.0042	NA	<0.0001	0.0011	NA
% correctly classified patients	58%	65%	74%	59%	63%	72%	54%	66%	70%	52%	65%	70%
Rule out cut-off (≥85% Se)	*<1.12*	*<9.2*	*<0.451*	*<1.12*	*<9.2*	*<0.451*	*<1.12*	*<9.2*	*<0.451*	*<1.12*	*<9.2*	*<0.451*
% patients	37%	40%	44%	36%	41%	42%	41%	55%	54%	35%	57%	53%
Se/Sp	0.85/0.62	0.85/0.69	0.85/0.78	0.84/0.61	0.83/0.69	0.87/0.76	0.86/0.56	0.76/0.73	0.82/0.75	0.88/0.49	0.75/0.77	0.83/0.75
NPV	0.87*	0.89*	0.90*	0.87*	0.88*	0.91*	0.88	0.84	0.88	0.87	0.83	0.87
Indeterminate zone [85%Se ; 90%Sp[												
% patients	30%	23%	13%	28%	24%	17%	31%	20%	16%	32%	20%	18%
Rule in cut-off (≥90% Sp)	*≥1.81*	*≥13.6*	*≥0.679*	*≥1.81*	*≥13.6*	*≥0.679*	*≥1.81*	*≥13.6*	*≥0.679*	*≥1.81*	*≥13.6*	*≥0.679*
% patients	33%	37%	43%	36%	36%	42%	28%	25%	30%	33%	23%	29%
Se/Sp	0.53/0.90	0.61/0.90	0.71/0.90	0.57/0.90	0.57/0.90	0.69/0.91	0.50/0.84	0.53/0.91	0.61/0.87	0.56/0.82	0.48/0.92	0.61/0.90
PPV	0.76*	0.78*	0.81*	0.77*	0.77*	0.81*	0.64	0.78	0.73	0.65	0.79	0.79

* PPV and NPV were adjusted on a prevalence of F≥3: 37% (prevalence of external VS)

** Analysis performed by Pr Boursier and his team.

PO-160
Prognostic value of AGILE scores in patients with non-alcoholic fatty liver disease

Jerome Boursier[1], Clémence M Canivet[1], Marine Roux[2], Lannes Adrien[1], Isabelle Fouchard[1], Frédéric Oberti[1]

[1]Angers University Hospital Center, Angers, France, *[2]University of Angers, Angers, France*
Email: jeboursier@chu-angers.fr

Background and aims: Recently, Agile 4 and Agile 3+, two scores combining liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) with routine clinical parameters were proposed to diagnose cirrhosis and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD) patients, respectively[1]. The study objective was to assess the prognostic accuracy of Agile 4 and Agile 3+ for the prediction of liver-related events (LRE) and to compare them to LSM alone.

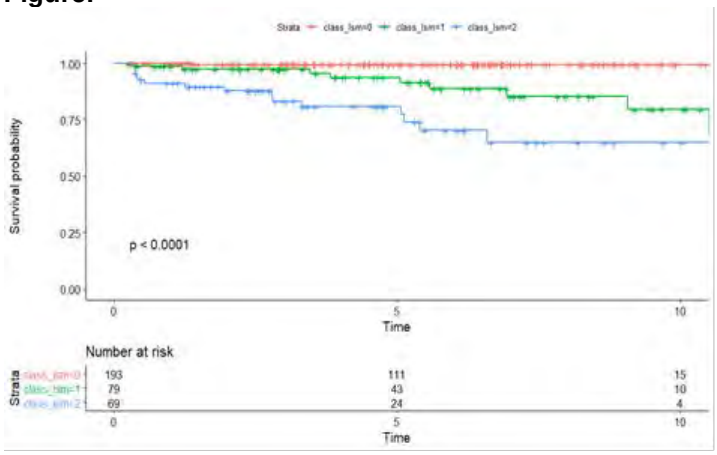
Method: This retrospective study included NAFLD adults from a French tertiary care center who underwent LSM and blood sampling as part of routine clinical practice. The main study outcome was LRE, a composite end point combining cirrhosis complication or hepatocellular carcinoma. LRE were ascertained by chart review. Cut-off values of Agile 4 and Agile 3+ previously determined[1] and Baveno cut-off values for LSM (10kPa-15kPa) were used to define the rule-out, indeterminate and rule-in zones at baseline. Kaplan-Meier curves were compared using the Log-rank test.

Results: 341 NAFLD patients were included in the study (median age: 58 years, male sex: 65%, diabetes: 36%). LRE occurred in 27 (7.9%) patients after a median follow-up of 5.2 years (1st and 3rd quartiles: 2.9-7.2). The patient rate included in the rule-out/indeterminate/rule-in zones of the Agile 3+ and Agile 4 were respectively 56%/15%/29% and 83%/9%/8%. Kaplan-Meier curves (**Figure**) for Agile 4 and Agile 3+ showed significant differences between the rule-out and the rule-in zones ($p < 0.001$ for both) and between indeterminate and rule-in zones ($p \leq 0.002$ for both), while the difference between indeterminate and rule-out zones was not significant. By comparison, the patient rate included in the rule-out/indeterminate/rule-in zones with LSM were 57%/23%/20%. Using LSM, patients experiencing a LRE were initially either in the indeterminate or the rule-in zones and consequently, a significant difference ($p < 0.001$) between the rule-out and the indeterminate zone was observed while the difference between the indeterminate and rule-in was less significant ($p = 0.03$).

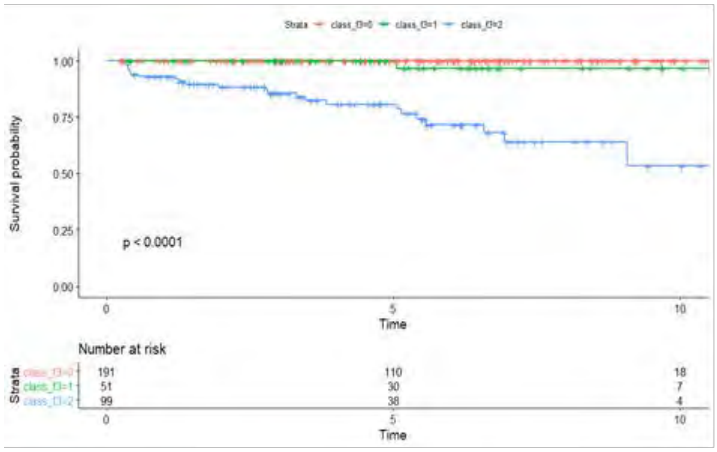
Conclusion: Agile 4 and Agile 3+ well predict the occurrence of LRE in NAFLD patients. Particularly, rule-in cut-offs of both scores better identify at-risk patients than LSM alone. These results demonstrate the interest of those scores in the identification of patients requiring hepatocellular carcinoma and esophageal varices screening.

1.Younossi, ZM et al. AASLD 2020 LP12.

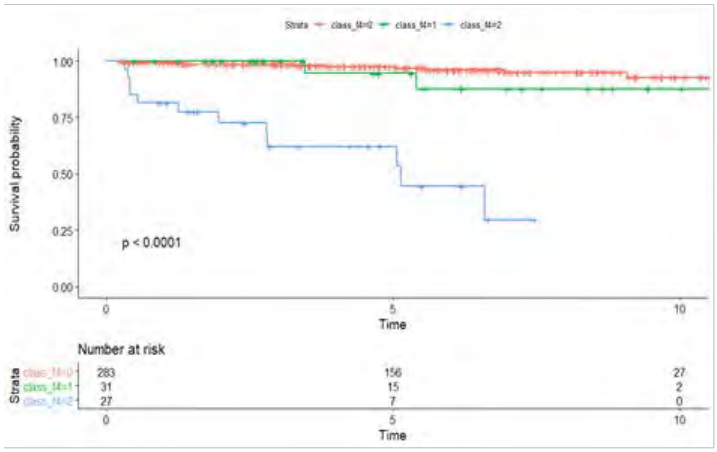
Figure:



a. Kaplan-Meier curves for LSM



b. Kaplan-Meier curves for Agile 3+



c. Kaplan-Meier curves for Agile 4

PO-162
Cross-validation of non-invasive diagnostics for NAFLD in individuals with type 1 diabetes

jonathan mertens[1 2 3], Eveline Dirinck[1 2], Floris Vanhevel[4], Maarten Spinhoven[4], Christophe De Block[1 2], Sven Francque[1 3]

[1]University of Antwerp, Laboratory of Experimental Medicine and Paediatrics, Wilrijk, Belgium, *[2]Antwerp University Hospital, Endocrinology, Diabetology and Metabolism, Edegem, Belgium*, *[3]Antwerp University Hospital, Gastroenterology and Hepatology, Edegem, Belgium*, *[4]Antwerp University Hospital, Radiology, Edegem, Belgium*
Email: jonathan.mertens@student.uantwerpen.be

Background and aims: Liver steatosis defines non-alcoholic fatty liver disease (NAFLD), which is increasingly prevalent. The exact prevalence of NAFLD in individuals with type 1 diabetes (T1D) is uncertain, partly due to the lack of cross-validation of diagnostic methods for steatosis. This study aims to determine the accuracy of non-invasive diagnostics in a T1D cohort.

Method: Patients underwent ultrasound (ultrasound steatosis score [USS] 0-3), elastography (Fibroscan®) with controlled attenuation parameter (CAP), both with M (M-CAP) and XL (XL-CAP) probe (when technically valid), and magnetic resonance spectroscopy (MRS). A liver fat content ≥5.6 % was considered diagnostic for steatosis. The fatty liver index (FLI) was also calculated.

Results: 94 adults were included. Prevalence of steatosis was 11.8 % (MRS). According to the other methods, prevalence of steatosis was 27.2 % (FLI ≥60), 29.7 % (USS ≥1 criterion) and 78.3 % (based on a CAP ≥248 dB/m on either probe). The area under the receiver-operator curve (AUROC) was 0.85 (0.75- 0.96) for the M-CAP and 0.67 (0.49-0.84) for the XL-CAP, while the FLI yielded an AUROC of 0.56 (0.38-0.75). The AUROC for the USS was 0.88 (0.76-1.00). The optimal M-CAP cut-offs were 255 and 271 dB/m, and 244 and 307 dB/m for the XL-CAP. The USS ≥1 criterion yielded a good sensitivity of 0.91, with a specificity of 0.79. The optimal FLI cut-offs were similar to the conventional ones, namely <0 and ≥60. Correlation was strong between the three continuous indices: FLI vs. M-CAP r = 0.60, FLI vs. XL-CAP r = 0.63 and M- vs. XL-CAP r = 0.67 (p <0.001 for all). To evaluate agreement between the two probes, we constructed a Bland-Altman plot from 47 subjects that had both valid M- and XL-CAP values. Linear regression of the differences between the probes, compared to the mean ruled out proportional bias. When compared qualitatively, there is fair agreement (k = 0.41, p = 0.003) between the two probes.

Conclusion: Ultrasound and CAP are accurate diagnostic, while FLI seems unreliable in T1D. Although the precision of M- compared to XL-CAP is adequate, both probes have distinctively different cut-offs. Non-invasive imaging needs to be studied further in T1D.

Figure: Accuracy analysis of non-invasive diagnostic tools.

Modality	Cut-off	Sensitivity	Specificity	LR +	LR -
USS	≥1 criterion	0.91	0.79	4.33	0.11
M-CAP	255 dB/m	1.00	0.63	2.70	0.00
M-CAP	271 dB/m	0.88	0.74	3.38	0.16
XL-CAP	244 dB/m	0.89	0.41	1.51	0.27
XL-CAP	307 dB/m	0.56	0.74	2.15	0.59
FLI	<30	0.64	0.61	1.64	0.59
FLI	≥60	0.36	0.74	1.38	0.01

PO-165
Increased gut permeability may be associated with bacterial protease activity, not intestinal inflammation in patients with significant liver disease due to NAFLD

Roberta Forlano[1], Benjamin H. Mullish[1], Tong Liu[1], Evangelos Triantafyllou[1], Charlotte Skinner[1], Mark Thursz[1], Julian Marchesi[1], Pinelopi Manousou[1]

[1]*Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, London, United Kingdom*
Email: r.forlano@imperial.ac.uk

Background and aims: The disruption of the gut-liver axis represents a crucial step towards the development of metabolic disorders, such as Non-alcoholic fatty liver disease (NAFLD). Here, we investigated the presence and the determinants for gut permeability in diabetic patients with and without NAFLD and different disease severity.

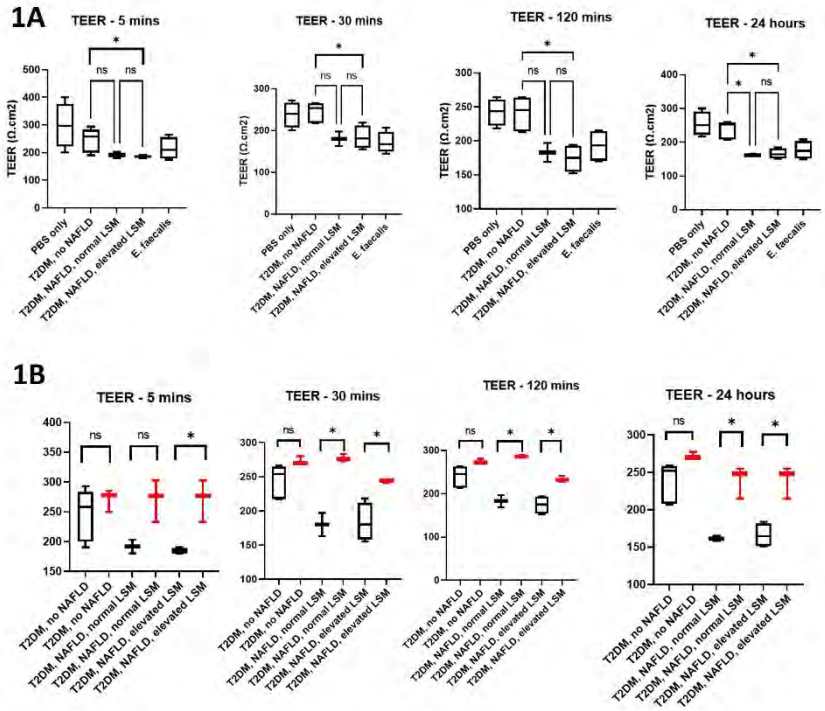
Method: We developed an *in-vitro* model of gut barrier using monolayers of Madin-Darby canine kidney cells (MDCK), cultured on Transwell inserts for 48-72 hours at 37.5°C-5% humidity. Monolayer's permeability was measured as Transepithelial electrical resistance (TEER) using a voltammeter. Samples of fecal water (FW) were obtained from stools of diabetic patients who were screened for NAFLD using blood tests, ultrasound and liver stiffness measurement (LSM). Aliquots of FW were normalized for protein content (300ug protein/250ul solution FW+ phosphate-buffered saline (PBS)) and added to the apical compartment of the barrier model, while TEER was measured at different time points after incubation. Experiments were replicated adding a commercial bacterial protease inhibitor cocktail to the FW. *E. faecalis* spent medium and PBS) were used as positive and negative control respectively. The level of faecal cytokines was also measured using pro-inflammatory plate with Meso scale discovery (MSD).

Results: Experiments were carried out using FW from 12 diabetic patients. Specifically, 5 patients had no liver disease, 3 patients had NAFLD with normal LSM and 4 patients NAFLD with significant liver disease (elevated LSM, LSM >8.1 kPa). Overall, TEER was significantly lower where monolayers were incubated with FW from patients with elevated LSM vs those without liver disease. The difference was significant after 5 min (185 vs 258Ωcm^2, *Kruskal Wallis with post-hoc correction,* $p = 0.04$), 30 mins (132 vs 247Ωcm^2, $p = 0.032$), 60 mins (180 vs 254Ωcm^2, $p = 0.043$) and 24 hours (164 vs 252Ωcm^2, $p = 0.002$) (Figure 1A). When the experiments were replicated adding bacterial protease inhibitors, TEER in the monolayers incubated with FW from significant liver disease, was significantly higher compared to TEER without inhibitor (Figure 1B), suggesting a component of bacterial protease activity. Finally, on MSD, there was no difference in terms of fecal cytokines across the groups (*Kruskal Wallis with post-hoc correction)*.

Conclusion: Our model *in-vitro* suggests that diabetic patients with liver disease due to NAFLD may have increased gut permeability compared to diabetics with normal liver. In this model, gut permeability may be associated with bacterial protease activity rather than with intraluminal inflammation.

Figure:

Figure 1: Figure 1A) TEER measurement across MDCK monolayers measured at different time points after incubating the monolayers with fecal water, PBS only (negative controls) or *E.faecalis* only (positive controls). Figure 1B) Differences between TEER measurement between monolayers incubated with fecal water with (red) and without (black) bacterial protease inhibitors.



PO-166
The BAST score performs predicts the presence of liver disease better than FIB-4 and NAFLD fibrosis score in a cohort of patients with type-2 diabetes mellitus in primary care

Roberta Forlano[1], Benjamin H. Mullish[1], Michael Yee[2], Mark Thursz[1], Pinelopi Manousou[1]

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Imperial college London, London, United Kingdom, *[2]Department of Endocrinology, Imperial college london, United Kingdom*
Email: r.forlano@imperial.ac.uk

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with Type-2 diabetes mellitus (T2DM), with diabetics being at higher risk for adverse outcomes. The aim of this study was to establish prevalence and develop a screening pathway for NAFLD referral/management.

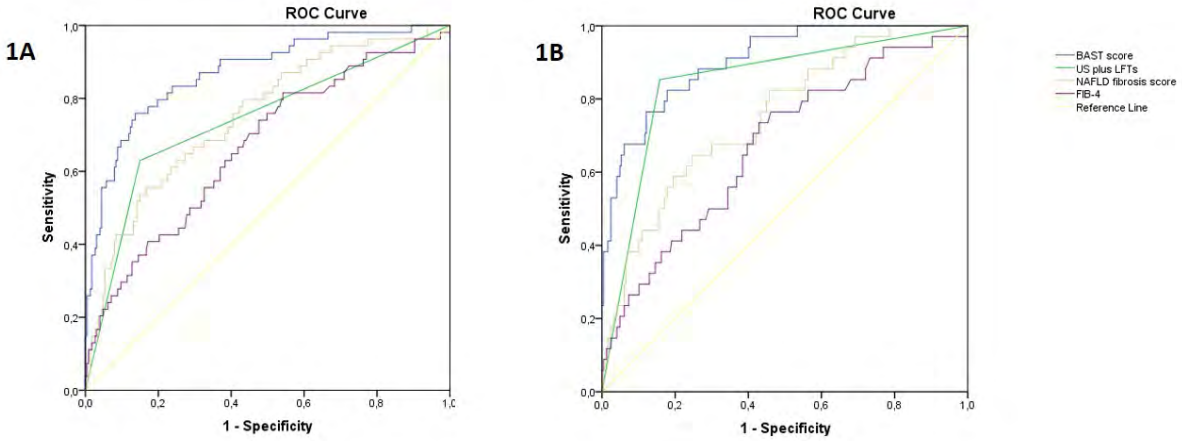
Method: Consecutive patients with T2DM were enrolled from North-West London Community Care. Patients were screened for liver disease by bloods (including NAFLD fibrosis score and FIB-4), ultrasound (US) and fasting liver stiffness measurement (LSM) and CAP score.

Results: Of 300 patients enrolled, 287 were included;13 withdrew. Overall, 184 (73%) had NAFLD, 28 (10%) other causes of liver disease (BAFLD and HBV) and 75 (26%) no liver disease. Those with NAFLD had larger waist (p = 0.0001) and hip (p = 0.0001) circumferences and higher BMI (p = 0.0001) compared to those without NAFLD. They also had higher ALT (p = 0.0001), AST (p = 0.0001), GGT (p = 0.0001) and HbA1c (p = 0.0001). Among those with NAFLD, 50/183 (28%) and 50/287 (17%) of total had increased LSM (>8.1 kPa). Those with increased LSM had larger waist (p = 0.0001) and hip (p = 0.0001) circumferences and higher BMI (p = 0.0001) compared to those with normal LSM (n = 134). Moreover, those with increased LSM also presented higher ALT (p = 0.001), AST (p = 0.0001), GGT (p = 0.0001) and HbA1c (p = 0.0001).
Overall, patients were randomly split into 2:1 to the derivation (n = 192) and validation (n = 95) cohort.
In the derivation cohort, waist circumference (OR 1.089, p = 0.006), BMI (OR 1.24, p = 0.005) and AST (OR 1.092, p = 0.0001) were independent predictors of increased LSM. The BAST score was derived by logistic regression: 0.086* (Waist circumference, cm) + 0, 08* (BMI, kg/m2) + 0, 025* (AST, IU/L)-14, 607. With a cut-off or 0.063 (optimised by Youden-index), the BAST score predicted the presence of significant liver disease (LSM>8.1 kPa) with AUROC 0.847 (95%CI 0.779-0.914, p > 0.0001), sensitivity 94%, specificity 43%, prevalence-adjusted PPV 32%, NPV 99% and DA 65%. Hosmer-Lemeshow test for the BAST score was 0.88, confirming that the model fitted the population well. In the validation cohort. the AUROC of BAST score for predicting LSM>8.1 kPa was 0.86 (95%CI: 0.81-0.98, p < 0.0001) (Figure 1A).
In the whole population, the BAST score performed better than conventional screening tool for NAFLD, including FIB-4 and NAFLD fibrosis score (pairwise comparison of AUROC curves, De Long test). Similarly, the BAST score performed better when predicting the presence of advanced fibrosis (LSM>12 kPa) in the same population (Figure 1B).

Conclusion: We have developed and validated internally a simple score (the BAST score) based on BMI and AST. The BAST score may predict the presence of liver disease due to NAFLD better than FIB-4 and NAFLD fibrosis score in diabetic patients within primary care. This model may be used to shape a referral management pathway in this population.

Figure:

Figure 1. BAST score vs FIB-4 and NAFLD fibrosis score for predicting LSM and fibrosis stage in the whole study population.



PO-171
Statin use is associated with significantly lower risk of high-risk NAFLD among patients with type 2 diabetes. A propensity-matched analysis from NHANES 2017-2018

Eduardo Vilar Gomez[1], Raj Vuppalanchi[1], Samer Gawrieh[1], Niharika Samala[1], Naga Chalasani[1]

[1]*Indiana University School of Medicine, Gastroenterology and Hepatology, Indianapolis, United States*
Email: evilar@iu.edu

Background and aims: Limited data exist regarding the beneficial effects of statin on risk of NAFLD progression among patients with type 2 diabetes mellitus (T2DM). Our study examined the association between statin use and high-risk NAFLD in patients with T2DM.

Method: Our study included data from the NHANES, a nationwide probability sample survey designed to collect information on the health and nutritional status of the U.S. population. NHANES 2017-2018 includes 5494 persons with reliable vibration controlled transient elastography (Fibroscan ®) measurements. After excluding other causes of liver disease, steatogenic medications and excessive alcohol intake, 850 individuals with T2DM were at risk for NAFLD. Of them, 438 with statin consumption were propensity matched (1:1) to 412 who did not receive statin while adjusting for gender, race/ethnicity, and BMI. FibroScan-AST (FAST) score was used to non-invasively identify participants with NASH and clinically significant fibrosis. A score ≥0.67 was indicative of high-risk NAFLD. Data are shown in weighted mean and percentage with their SEs. Logistic regression models were used to examine the effect of statin use on high-risk NAFLD.

Results: Our propensity matched cohort comprised 372 statin users vs 372 non-users. Most of the baseline features were well balanced between statin exposed and non-exposed participants, except for age and LDL levels (Table). Atorvastatin (51%) and simvastatin (24%) were the most prescribed statin. The average duration of statin therapy was 6.64 ± 0.47 years. Fatty liver (defined as CAP ≥285 dB/m) was present in 66% and 71% of statin users and nonusers. The risk of fatty liver was not significantly different between statin exposed and non-exposed after controlling for relevant confounders (Adj. OR: 0.79, 95% CI: 0.54-1.15) (p = 0.21). The mean FAST score was significantly lower in statin users (0.14 ± 0.01) vs non-users (0.22 ± 0.02) (p <0.01). The prevalence of high-risk NAFLD was 1.3% ± 0.6 in statin exposed vs 7.1% ± 2.2 in non-exposed (p = 0.01). After adjusting for relevant confounders, the exposure to statin was associated with a 87% lower presence of high-risk NAFLD (Adj. OR: 0.13; 95% CI: 0.05-0.37).

Conclusion: In a nationally representative sample of the U.S. population with T2DM, the statin use was not associated with lower risk of fatty liver but highly significant lower prevalence of NASH with clinically significant fibrosis. This suggests statins may lower the risk of progression from fatty liver to NASH with fibrosis.

Figure:

	Weighted mean or percentage ± SEs		
	Statin users N = 372	**Non-users** N = 372	P value
T2DM duration, y	10 ± 0.6	8 ± 1.2	0.09
Age	64 ± 1.2	57 ± 1.03	<0.01
Race/ethnicity			0.88
NH Whites	58 ± 0.04	59 ± 0.05	
NH Blacks	16 ± 0.03	15 ± 0.04	
NH Asians	8 ± 0.02	7 ± 0.02	
Hispanics	18 ± 0.03	19 ± 0.02	
Male	45 ± 0.04	47 ± 0.04	0.68
BMI	33.77 ± 0.42	34.49 ± 0.71	0.29
HbA1c >7%	46 ± 0.04	38 ± 0.03	0.13
LDL-C (mg/dL)	77.1 ± 3.57	113.8 ± 4.05	<0.01

PO-174
Waist and hip circumference are independently associated with risk of liver disease in population-based studies

Oscar Danielsson[1], Markku Nissinen[1], Fredrik Åberg[2], Antti Jula[3], Veikko Salomaa[3], Satu Männistö[3], Annamari Lundqvist[3], Markus Perola[3]

[1]Clinic of Gastroenterology, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland, *[2]Transplantation and Liver Surgery, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland*, *[3]Finnish Institute for Health and Welfare, Finland, Finland*
Email: oscar.danielsson@hus.fi

Background and aims: While several anthropometric measures predict liver disease, the waist-hip ratio (WHR) has shown superiority in previous studies. We analyzed independent and joint associations of waist circumference (WC) and hip circumference (HC) with liver disease and liver-related risk factors.

Method: A cross-sectional study (*n* = 6619) and a longitudinal cohort (*n* = 40, 923) comprised individuals from the Health 2000 and FINRISK 1992-2012 studies. Prevalent and viral liver diseases were excluded. The longitudinal cohort was linked with national healthcare registers for severe incident liver disease. Linear regression and Cox proportional hazards models were used to analyze anthropometric, lifestyle, metabolic, and bioimpedance-related parameters; liver enzymes; and 59 liver-related genetic risk variants.

Results: WC and HC showed independent and opposite associations with both liver enzymes and incident liver disease among men (HR for liver disease: WC, 1.07, 95% CI 1.03-1.11; HC, 0.96, 95% CI 0.92-0.99; *P* range 0.04 to <0.001) and women (HR for liver diseases: WC, 1.06, 95% CI 1.02-1.10; HC, 0.93, 95% CI 0.89-0.98; *P* range 0.005 to 0.004). HC modified the associations between WC and liver enzymes, and between WC and incident liver disease, particularly among men. Liver enzymes and risk of liver disease increased with increasing WC, more so among individuals with high WHR compared to with low WHR. WC and HC jointly reflected both body fat distribution and muscle mass, which was largely mirrored by the WHR, with some sex-specific differences.

Conclusion: WC and HC exhibit independent and joint associations with liver disease, which are largely reflected by the WHR. Both body fat distribution (subcutaneous vs. visceral fat) and muscle mass contribute to these anthropometric measures.

PO-175
The relationship between the frequency of drinking and incidence of fatty liver in Japanese cohort undergoing health checkups during the period 2008-2019

Aya Sugiyama[1], Akemi Kurisu[1], Serge Ouoba[1], Bunthen E[1], Ko Ko[1], Anvarjon Rakhimov[1], Md Razeen Ashraf Hussain[1], Tomoyuki Akita[1], Takayuki Harakawa[2], Toru Sako[2], Makoto Koshiyama[3], Junko Tanaka[1]

[1]Graduate School of Biomedical and Health Sciences, Hiroshima University, Department of Epidemiology, Infectious Disease Control and Prevention, Hiroshima, Japan, *[2]General Affairs, Foundation for Community Health and Medicine Promotion in Hiroshima Prefecture, Hiroshima, Japan*, *[3]Iwate Prefectural Preventive Medicine Association, Iwate, Japan*
Email: aya-sugiyama@hiroshima-u.ac.jp

Background and aims: The relationship between the frequency of drinking and the incidence of fatty liver is still poorly understood. This study analyzed data from a large cohort who underwent health checkups in Japan to investigate the incidence as well as prevalence of fatty liver and risk factors for fatty liver by alcohol consumption.

Method: The study population consisted of residents who underwent ultrasonography at health checkups in Japan between 2008 and 2019 (N = 75, 670). The prevalence of fatty liver diagnosed with ultrasonography was calculated by alcohol consumption. The incidence of fatty liver in 31, 062 residents who underwent ultrasonography at least twice during 2008-2019 without fatty liver at the first time was calculated using the person-year method. Multivariate logistic analysis was performed to investigate risk factors associated with the prevalence and incidence of fatty liver.

Results: The prevalence of fatty liver was 27.6% (95% confidence interval [CI], 27.2-27.9) in non-drinkers, 28.5% (27.5-29.5) in moderate-drinkers, and 28.0% (26.0-29.9) in heavy-drinkers. The incidence of fatty liver was 3, 084/100, 000 person-years (2, 997-3, 172/100, 000) in non-drinkers, 3, 754/100, 000 person-years (3, 481-4, 042/100, 000) in moderate-drinkers, and 3, 861/100, 000 person-years (3, 295-4, 497/100, 000) in heavy-drinkers. The prevalence and incidence of fatty liver were not associated with drinking status. Obesity was the most important independent risk factor (prevalence: adjusted odds ratio [AOR], 6.3; 95% CI, 6.0-6.5; incidence: AOR, 2.4; 95% CI, 2.3-2.6).

Conclusion: Based on this large data set analysis, drinking status does not affect the prevalence or incidence of fatty liver in residents undergoing health checkups in Japan. From a public health perspective, measures for obesity must be prioritized to reduce the burden of disease of fatty liver in Japan.

Figure:

Age and sex-specific incidence of fatty liver stratified by alcohol consumption among residents who underwent ultrasonography at health checkups in Japan

PO-176
The natural history of pediatric Non-alcoholic Fatty Liver Disease: a long term follow-up study

Laura Draijer[1], Maaike Voorhoeve[1], Marian Troelstra[2], Meeike Kusters[3], Eric de Groot[4], Aart Nederveen[2], Marc Benninga[1], Bart Koot[1]

[1]Amsterdam UMC, Pediatric Gastroenterology, Hepatology and Nutrition, Amsterdam, Netherlands, *[2]Amsterdam UMC, Radiology and Nuclear Medicine, Amsterdam, Netherlands*, *[3]Amsterdam UMC, Pediatrics, Amsterdam, Netherlands*, *[4]Imagelabonline and Cardiovascular, Erichem, Netherlands*
Email: l.g.draijer@amsterdamumc.nl

Background and aims: The long term hepatic outcome of pediatric Non-alcoholic Fatty Liver Disease (NAFLD) remains unclear due to the lack of robust longitudinal data on the natural history of pediatric onset NAFLD into adulthood. Between 2008-2012, an unselected cohort of 133 children with severe obesity was screened for NAFLD, using Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) and the Enhanced Liver Fibrosis Test (ELF test). The aim of this follow-up study is to determine the 10-year natural history of pediatric onset NAFLD.

Method: All participants of the original study were invited to participate in this prospective follow-up study. All subjects underwent ^{1}H-MRS to assess change in steatosis and ELF test to assess change in fibrosis. Steatosis was defined as ^{1}H-MRS >1.8%, which was validated to correspond with >5% steatosis at histology. Risk factors for disease progression were determined.

Results: In total, 51 of the 133 subjects from the original cohort were included in the present study (38%).
The mean follow-up time was 10.3 years (range 7-13 years). At follow-up, sixty-five percent was female, mean BMI was 40.06 kg/m2, 92% had obesity and median ALT was 21 IU/L. The median ^{1}H-MRS and proportion of subjects with steatosis did not change significantly: 1.72% (IQR 0.83-4.51) vs 1.67% (IQR 0.96-4.34) and 24/50 (46%) vs 24/50 (46%) at baseline and follow-up, respectively (Table 1). In those with NAFLD, nine subjects had a change in ^{1}H-MRS of >5%, of which six subjects showed a decrease and three showed an increase. The ELF test did not significantly change: 8.81 ± 0.66 at baseline versus 8.52 ± 0.72 at follow-up (p = 0.118). Five subjects had a change in ELF test of >1.0, of which four showed a decrease and one showed an increase in ELF test. At univariate linear regression, change in steatosis was significantly associated with change in BMI z-score, triglycerides and ALT within those with NAFLD at baseline. There were no significant associations between change in ELF test and change in risk factors.

Conclusion: This 10-year follow-up study suggests that progression of steatosis and fibrosis is uncommon in adolescents and young adults with childhood onset obesity and NAFLD. The established metabolic risk factors for steatosis were also longitudinally associated with change in steatosis. These data support that it is justified to screen for NAFLD and monitor for progression of fibrosis at a low frequency, unless metabolic risk factors worsen.

Figure: Table 1. Comparison of clinical outcomes

	NAFLD at baseline (n=24)			**No NAFLD at baseline (n=27)**		
	Baseline	**Follow-up**	**p-value**	**Baseline**	**Follow-up**	**p-value**
Female, n (%)	12 (50)	-	-	21 (78)	-	-
BMI z-score	4.16 (4.01-4.48)	4.05 (2.90-4.59)	0.414	4.13 (3.58-4.45)	3.79 (3.62-4.16)	0.195
BMI (kg/m2)	39.95 ± 4.81	40.48 ± 8.28	0.806	37.31 ± 5.68	40.01 ± 6.66	0.869
BMI > 30 kg/m2, n (%)	-	23 (96)	-	-	24 (89)	-
Waist circumference (cm)	108.7 ± 8.7	120.7 ± 20.0	**0.014**	100.8 ± 9.8	112.9 ± 16.0	0.453
Bariatric surgery, n (%)	0	9 (38)	-	0	7 (26)	-
Biochemical						
ALT, IU/L	36 (22-49)	26 (15-64)	0.401	21 (15-24)	27 (16-36)	**0.036**
AST, IU/L	-	18 (22-32)	-	-	20 (18-25)	-
yGT, IU/L	-	23 (13-65)	-	-	18 (14-26)	-
Triglycerides, mmol/L	0.89 (0.74-1.35)	0.98 (0.77-1.59)	0.506	0.61 (0.47-1.04)	0.76 (0.50-1.07)	0.415
HDL, mmol/L	-	1.17 (1.04-1.44)	-	-	1.24 (1.14-1.44)	-
HOMA-IR	4.60 (2.58-6.68)	2.28 (1.30-3.70)	**0.007**	1.90 (1.10-3.10)	2.30 (1.38-3.50)	0.181
Steatosis						
1H-MRS (%)	4.68 (2.76-9.48)	3.47 (1.52-5.84)	0.241	0.83 (0.52-1.25)	1.40 (0.94-2.12)	**0.005**
Δ 1H-MRS >5%, n (%)	-	9 (37.5)	-	-	0 (0)	-
Steatosis, n (%)	24 (100)	13 (54)	-	0 (0)	6 (22)	-
Fibrosis						
ELF test	8.81 ± 0.66	8.52 ± 0.72	0.118	-	-	-
Δ ELF > 1.0 (%)	-	5 (10)		-	-	-

PO-181
Discriminatory changes in circulating metabolites as a predictor of hepatocellular cancer in patients with MAFLD

rohini sharma[1], Haonan Lu[1], Jacob George[2], Mohammed Eslam[2], Augusto Villanueva[3], Luigi Bolondi[4], Helen L. Reeves[5], Misti McCain[5], Caroline Sands[1], Matthew Lewis[1]

[1]imperial college london, *[2]Storr Liver Centre*, *[3]Icahn School of Medicine at Mount Sinai*, *[4]University of Bologna*, *[5]Newcastle University Translational Clinical Research Institute*
Email: r.sharma@imperial.ac.uk

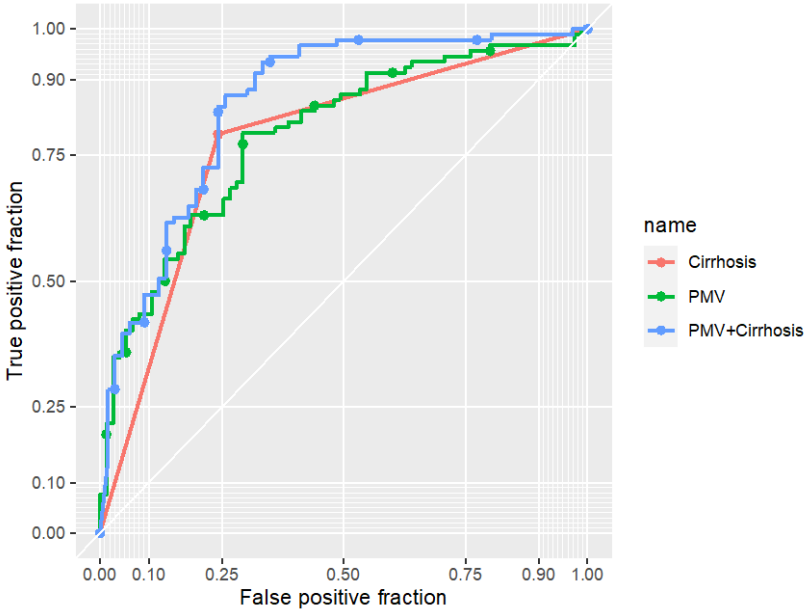
Background and aims: The burden of metabolic (dysfunction) associated fatty liver disease (MAFLD) is rising mirrored by an increase in hepatocellular cancer (HCC). MAFLD and its sequelae are characterised by perturbations in lipid handling, inflammation and mitochondrial damage. The profile of circulating lipid and small molecule metabolites with the development of HCC is poorly characterized in MAFLD and could be used in future studies as a biomarker for HCC

Method: We assessed the profile of 273 lipid and small molecule metabolites by ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-MS) in serum from patients with MAFLD (n = 113) and MAFLD-associated HCC (n = 144) from six different centres. Regression models were used to identify a predictive model of HCC using minimal circulating features

Results: Twenty lipid species and one metabolite, reflecting changes in mitochondrial function and sphingolipid metabolism, successfully predicted the presence of cancer on a background of MAFLD with high accuracy (AUC 0.789), which was enhanced with the addition of cirrhosis to the model (AUC 0.855). In particular, the presence of these metabolites was associated with cirrhosis in the MAFLD subgroup ($p < 0.001$) (fig). When considering the HCC cohort alone, the metabolic signature was an independent predictor of overall survival (HR 1.42, 95%CI: 1.09-1.83, $p < 0.01$)

Conclusion: These exploratory findings reveal a metabolic signature in serum which is capable of accurately detecting the presence of HCC on a background of MAFLD. This unique serum signature will be taken forward for further investigation of diagnostic performance as biomarker of early stage HCC in patients with MAFLD in the future

Figure:


1.00
0.90
0.75
0.50
0.25
0.10
0.00
True positive fraction
0.00
0.10
0.25
0.50
0.75
0.90
1.00
False positive fraction
name
Cirrhosis
PMV
PMV+Cirrhosis

PO-186
Short-term gluten-free diet is effective in reducing controlled attenuated parameter and body mass index in patients with Non-alcoholic steatohepatitis

Angelo Armandi[1 2], Helena Bespaljko[2], Alexander Mang[2], Yvonne Huber[2], Christian Labenz[2], Peter Galle[2], Detlef Schuppan[3], Jörn Schattenberg[2]

[1]Division of Gastroenterology and Hepatology, Department of Medical Sciences, University of Turin, Turin, Italy, *[2]Metabolic Liver Disease Research Program, I. Department of Medicine, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany*, *[3]Institute of Translational Immunology and Research Center for Immunology, University Medical Center, Johannes-Gutenberg-University, Mainz, Germany*
Email: angelo.armandi@unito.it

Background and aims: Amylase Trypsin Inhibitors (ATI) are found in all gluten containing grains and are able to elicit immune-mediated inflammatory responses. Murine models have shown that high intake of ATIs are associated to the development of Non-Alcoholic Steatohepatitis (NASH) and metabolic syndrome, independently of calorie intake.
We performed a randomized controlled trial of ATI/gluten-free (>95%) diet in patients with biopsy-proven NASH. The control arm was represented by recommendations of the German Association of Nutrition (Deutschen Gesellschaft für Ernährung [DGE]). Primary end points were improvement in Controlled Attenuated Parameter (CAP), liver stiffness, Body Mass Index (BMI) and liver aminotransferases.

Method: Patients with biopsy-proven NASH were consecutively enrolled and randomly assigned (ratio 1:1) to either ATI-free diet or DGE for 6 weeks. Clinical and biochemical parameters were collected at baseline and at end of treatment. Liver stiffness and CAP were measured using Fibroscan F530.

Results: A total of 47 patients were included, of which 22 underwent DGE and 25 ATI-free diet. Median age was 53 [47.5-60.7] years and 55.3% was male. Median BMI was 30.9 [27.9-36.6] kg/m2, without differences between groups. Median waist circumference (WC) was 108 [99-121] cm, with higher values in the DGE group (p = 0.02). Median alanine and aspartate aminotransferases were 63 [46.5-115.5] and 43 [30, 2-63, 7] IU/l. Liver stiffness and CAP were equally distributed, with overall median value of 5.9 [4.6-9.7] kPa and 325 [303.5-345.5] dB/m, respectively.
CAP showed a positive correlation with BMI (Spearman's rho = 0.58, p <0.0001) and with WC (Spearman's rho = 0.56, p = 0.0001). 88% of DGE group and 86.3% of ATI-free group completed the study. At intention to treat analysis using Wilcoxon rank test, ATI-free diet provided a significant reduction in CAP (Hodges-Lehmann median difference -30 [95% CI -67.5 to -3], p = 0.03) and in BMI (Hodges-Lehmann median difference -0, 38 [95% CI -0.69 to -0.05], p = 0.02). Conversely, no differences were observed in the DGE group, with respect to CAP (Hodges-Lehmann median difference -8, 5 [95% CI -27, 5 to 9], p = 0.20) and to BMI. Overall, liver stiffness and transaminase levels did not significantly vary in both groups.

Conclusion: CAP shows a positive correlation with both BMI and WC. Short-term treatment with ATI/gluten-free diet is effective in reducing CAP and BMI in patients with NASH.

PO-197
Menopausal women with NAFLD show impaired metabolism of branched chain amino-acids

Roberta Forlano[1], Benjamin H. Mullish[1], Chioma Izzi-Engbeaya[2], Michael Yee[3], Waljit Dhillo[2], Mark Thursz[1], Pinelopi Manousou[1]

[1]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, Imperial college London, London, United Kingdom, *[2]Division of Diabetes, Endocrinology and Metabolism, Department of Metabolism, Digestion and Reproduction, Imperial college London, United Kingdom*, *[3]Department of Endocrinology, Imperial college london, United Kingdom*
Email: r.forlano@imperial.ac.uk

Background and aims: Recent evidence suggests that menopause and related-hormonal changes may increase the risk for developing Non-alcoholic fatty live disease (NAFLD) in women. However, there is little knowledge on how menopause may influence metabolic status in women with specific regards to NAFLD. The aim of this study was to identify serum metabonomic profiles of menopausal women with NAFLD and compare them to younger women and men with NAFLD in a cohort of diabetics.

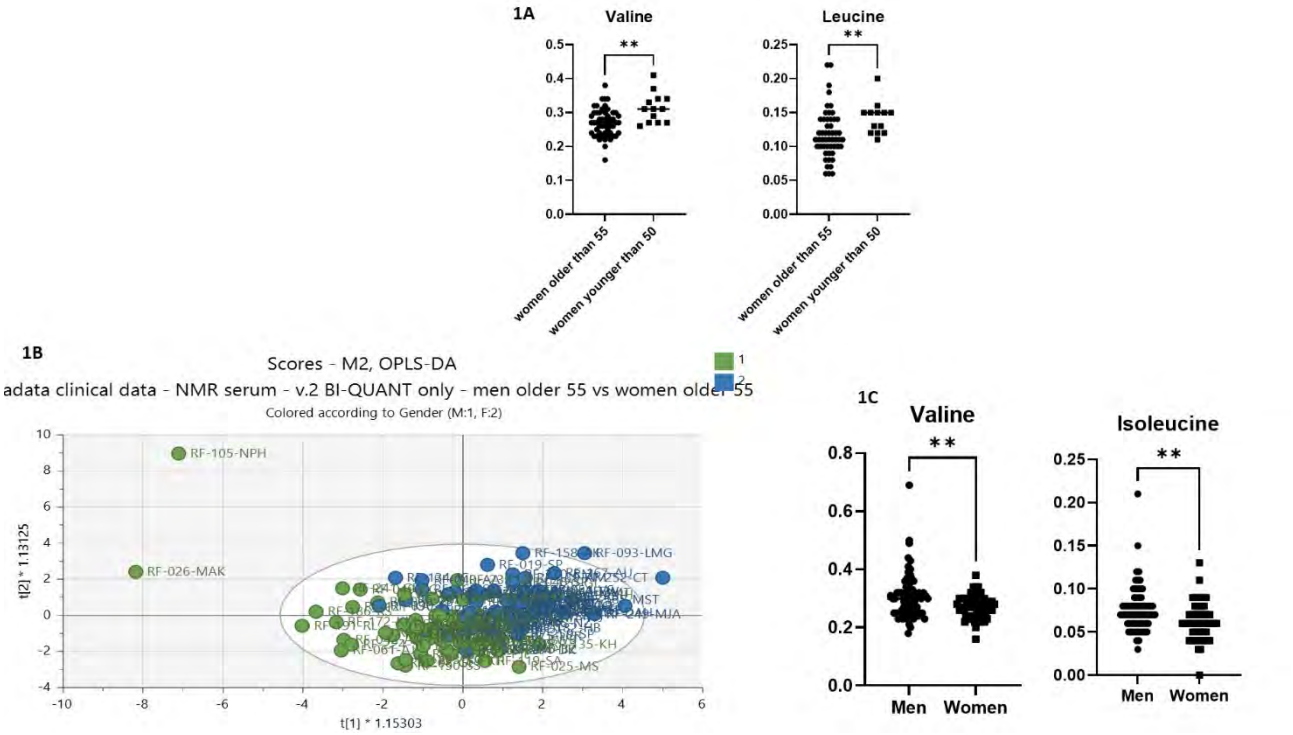
Method: Fasting serum samples obtained from patients with type-2 diabetes mellitus (T2DM) who were screened for liver disease by blood tests, ultrasound (US), fasting liver stiffness measurement (LSM) and controlled attuantion parameter (CAP) score. Women were stratified for age older than 55 years, as a presumed diagnosis of menopause. Serum samples were analysed using a non-targeted approach with Proton-Nuclear Magnetic Resonance (H1-NMR)-based metabolomics. Results were explored by performing multivariate statistical analysis using SIMCA and SPSS®.

Results: Of 300 patients enrolled, serum samples were collected from 254 subjects. Overall, 167 (65%) had NAFLD, 20 (7%) other causes of liver disease (BAFLD and HBV) and 67 (26%) no liver disease.
Among those with NAFLD, 53 (31%) were women ≥55 years-old, while 14 (8%) were ≤50 years old. A range of serum metabolites-including amino acids, lipoproteins and other small molecules-were identified and quantified in all samples. A valid model separated the serum metabolite profile of NAFLD women ≥55 years-old demonstrating lower serum valine and leucine levels compared to NAFLD women ≤50 years old (cross-validated residuals (CV) ANOVA, $p < 0.0001$) (Figure 1A). Similarly, NAFLD women ≥55 years-old demonstrating lower serum valine and leucine levels compared to NAFLD men ≥55 years-old ($n = 63$, 37%) (CV-ANOVA, $p < 0.0001$) (Figure 1B and 1C). There was no difference in terms of metabolites between women ≥55 years-old with and without NAFLD.

Conclusion: In T2DM patients, menopausal women with NAFLD show lower levels of some branched chain amino-acids (valine, leucine) compared to younger women and to men of the same age. Lower levels of branched chain amino-acids may reflect changes in muscle mass and/or insulin resistance in this population. Further studies are required to ascertain whether these changes may be associated to higher risk for advanced liver disease in this population.

Figure:

Figure 1A) Differences in metabolites between NAFLD women ≥55 years-old and NAFLD women ≤50 years old. 1B) Multivariate model differentiating women vs men ≥55 years-old 1C) Differences in metabolites between NAFLD men and women ≥55 years-old.



PO-207
Non-alcoholic steatohepatitis modifies liver and plasma phospholipids' profile from patients with morbid obesity

Helena Castañé[1 2], Julia Martí-Clofent[1], Andrea Jiménez-Franco[1], Elisabet Rodríguez-Tomàs[1 2], Gerard Baiges Gaya[1 2], Anna Hernández-Aguilera[1 3], Salvador Fernández-Arroyo[1 4], Jordi Camps[1 3], Jorge Joven[1 3]

[1]Institut d'Investigació Sanitària Pere Virgili, Unitat de Recerca Biomèdica, Tarragona, Spain, *[2]Rovira i Virgili University, Medicine and Surgery, Reus, [3]Hospital Universitari Sant Joan de Reus, Reus, Spain*, *[4]Eurecat, Centre of Omic Sciences, Reus (Tarragona), Spain*
Email: helena.castanev@gmail.com

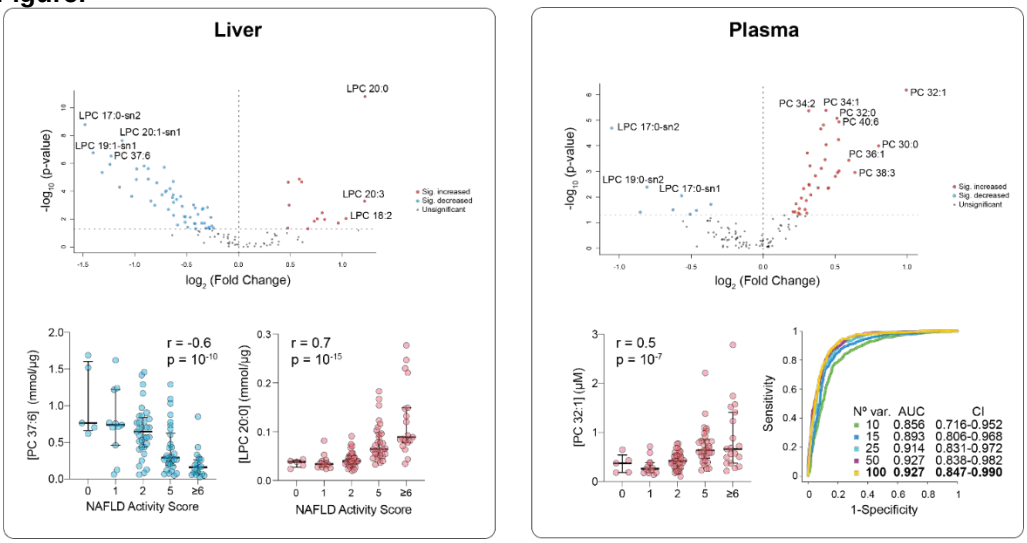
Background and aims: By 2021, NASH is already the most prevalent liver disease in human history and is commonly left untreated as it is hard to diagnose. Because of its lipid's metabolism deregulation, NASH is closely related with obesity, and due to the mitochondrial dysfunction in the former, phospholipid (PL) metabolism may be disturbed. We sought to distinguish patients with NASH and obesity from patients without NASH and with obesity by measuring their phospholipid profile in both plasma and liver.

Method: PL profile was measured by shot-gun lipidomics in liver and plasma samples from patients that underwent bariatric surgery. Among them, n = 51 were diagnosed as non-NASH patients, and n = 49 as NASH patients, both groups also suffered from morbid obesity.

Results: Liver PL profile was markedly different between NASH and non-NASH patients. PL were mostly decreased in NASH. Phosphatidylcholines (PC) were all decreased, and LysoPC (LPC) and Lysophosphatidylethanolamines (LPE) species concentrations were altered in NASH. We could correlate different PL species concentration with the severity of the liver status using Non-alcoholic fatty liver disease (NAFLD) Activity Score (NAS). Plasma PL concentrations were also significantly different between NASH and non-NASH patients, we observed that mostly all PL were increased in NASH, and more specifically, PC species were all increased in NASH. Some species correlated with the NAS evaluation. With machine learning algorithms, plasma PL profile allowed us to diagnose NASH obese patients from non-NASH obese patients with and area under the curve (AUC) of 0.927 (0.847-0.990).

Conclusion: PL profile in NASH was altered both in liver and plasma from patients with morbid obesity, and the concentration of these species were correlated with the liver severity status. PL profile in plasma could be a diagnostic tool for NASH.

Figure:


Liver
LPC 20:0
LPC 17:0-sn2
LPC 20:1-sn1
LPC 19:1-sn1
PC 37:6
LPC 20:3
LPC 18:2
-log10 (p-value)
log2 (Fold Change)
Sig. increased
Sig. decreased
Unsignificant
[PC 37:6] (mmol/µg)
r = -0.6
p = 10-10
NAFLD Activity Score
[LPC 20:0] (mmol/µg)
r = 0.7
p = 10-15
NAFLD Activity Score
Plasma
PC 32:1
PC 34:2
PC 34:1
PC 32:0
PC 40:6
PC 30:0
PC 36:1
PC 38:3
LPC 17:0-sn2
LPC 19:0-sn2
LPC 17:0-sn1
-log10 (p-value)
log2 (Fold Change)
Sig. increased
Sig. decreased
Unsignificant
[PC 32:1] (µM)
r = 0.5
p = 10-7
Sensitivity
1-Specificity
Nº var. AUC CI
10 0.856 0.716-0.952
15 0.893 0.806-0.968
25 0.914 0.831-0.972
50 0.927 0.838-0.982
100 0.927 0.847-0.990

PO-208
A little is better than nothing: very low alcohol consumption is associated with lower prevalence of cirrhosis and HCC in patients with NAFLD

Silvia Ferri[1], Lorenzo Mulazzani[1 2], Margherita Alvisi[1 2], Francesco Tovoli[1], Simona Leoni[1], Luca Muratori[1 2], Bernardo Stefanini[1 2], Elisabetta Goio[1 2], Fabio Piscaglia[1 2]

[1]Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, *[2]Department of Medical and Surgical Sciences, University of Bologna, Italy*
Email: silvia.ferri@aosp.bo.it

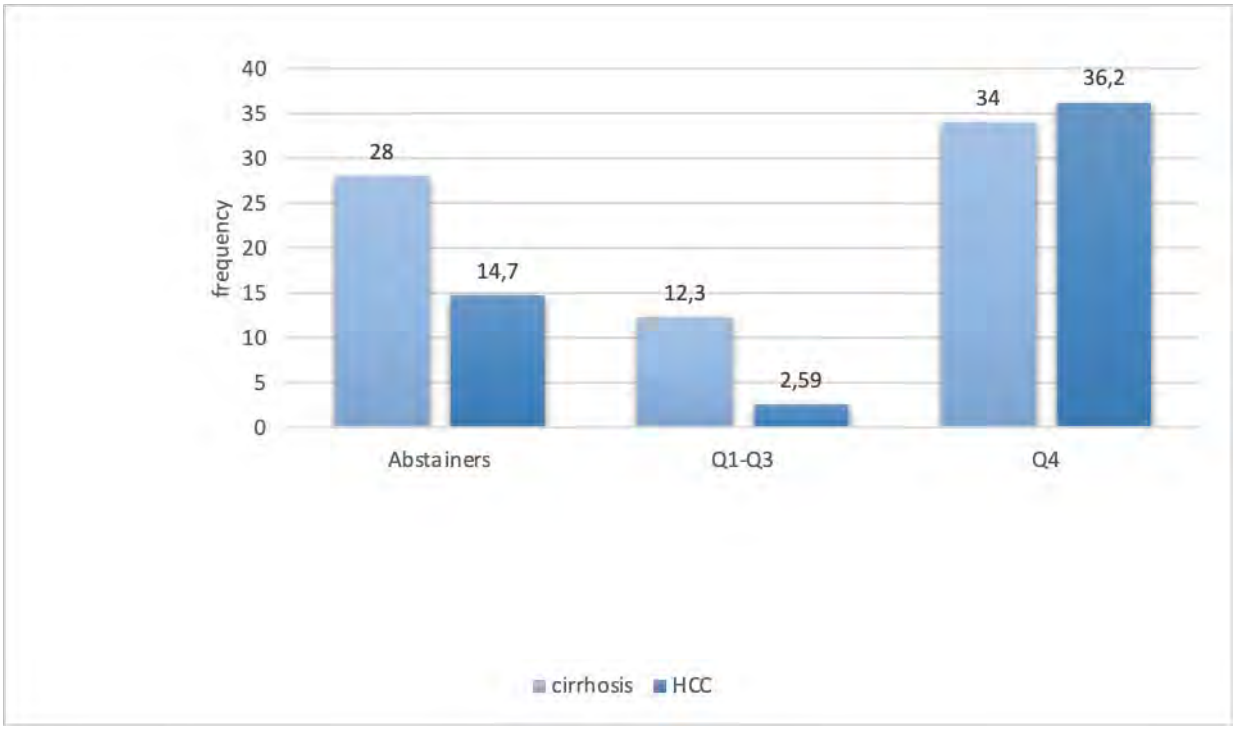
Background and aims: The role of moderate degrees of alcohol use in the evolution of NAFLD is still debated, as most studies evaluated ongoing drinking habits but did not consider lifetime drinking histories.
The aim of this study is to evaluate the impact on natural history of liver disease of both current and lifelong alcohol consumption in a cohort of outpatients with NAFLD. We created a new tool, called LACU (lifetime alcohol consuming unit) to estimate the amount of alcohol consumed in lifetime: 1 LACU was defined as 7 alcohol units per week for 1 drinking year.

Method: From 1 March 2015 to 1 February 2020, we enrolled 276 consecutive patients fulfilling criteria of NAFLD: fatty liver at ultrasound, HSI (hepatic steatosis index) >30 and exclusion of other well-known causes of fatty liver. A Physician performed an interview at baseline and after 2 years regarding alcohol consumption including questions about current and lifetime alcohol consumption.
According to their current alcohol intake per week, 276 patients were divided in: abstainers, current low consumers (C1, 1-70 grams per week) and moderate consumers (C2, 71-140 grams per week for women and 71-210 grams for men). In a different analysis patients were divided according to lifetime exposure to alcohol in abstainers and consumers. The latter were furtherly divided into quartiles: Q1 (0.1-4.29 LACU), Q2 (4.30-12.85 LACU), Q3 (12.86-40.00 LACU), and Q4 (>40.01 LACU).

Results: Age, diabetes, hypertension and alcohol consumption were independent predictors of cirrhosis and hepatocellular carcinoma (HCC) in the multivariable models. Stratification according to alcohol intake showed that a lower current alcohol consumption (C1) was associated with a decreased risk of cirrhosis compared to abstinence and a decreased risk of both cirrhosis and HCC compared to moderate alcohol consumption (C2).
Patients included in the quartiles from Q1 to Q3 displayed highly homogeneous characteristics concerning demography, laboratory and clinical features, therefore we decided to consider them altogether in our analysis. Low alcohol consumers attested by LACU, specifically subgroup composed by patients in Q1, Q2 and Q3, had a decreased risk of cirrhosis and HCC compared to abstainers ($p = 0.043$ and $p = 0.015$, respectively) and moderate consumers (Q4) ($p = 0.010$ for cirrhosis and $p < 0.001$ for HCC).

Conclusion: Low alcohol consumption, both evaluated as current and lifelong exposure, is associated with a decreased frequency of cirrhosis and HCC compared to abstainers and moderate alcohol consumers in patients with NAFLD.

Figure:


40
35
30
25
20
15
10
5
0
frequency
28
14,7
12,3
2,59
34
36,2
Abstainers
Q1-Q3
Q4
cirrhosis
HCC

PO-220
Rising indication of non-alcoholic steatohepatitis as transplant indication in historically low risk areas

Laura Martínez Arenas[1], Marina Berenguer Haym[1 2 3 4]

[1]Instituto de Investigación Sanitaria La Fe, Liver Transplantation and Hepatology Laboratory, Valencia, Spain, *[2]Hospital Universitario y Politécnico La Fe, Liver Transplantation and Hepatology Unit, Valencia, Spain*, *[3]University of Valencia, Faculty of Medicine, Valencia, Spain*, *[4]Instituto de Salud Carlos III, CIBERehd, Madrid, Spain*
Email: laura_munera15@hotmail.com

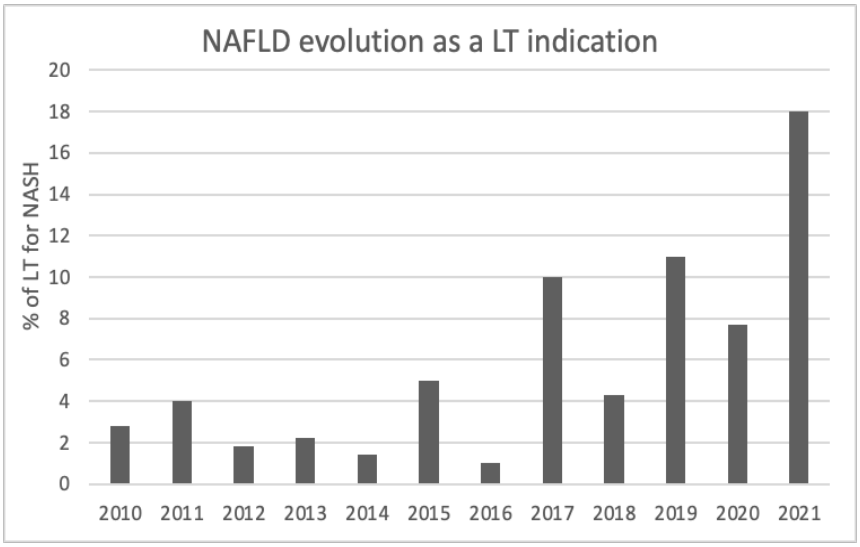
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is becoming one of the most common chronic liver disease in Spain, particularly in individuals with features of metabolic syndrome, yet its exact prevalence and incidence is not completely known. In fact, non-alcoholic steatohepatitis (NASH) is a growing indication for liver transplantation (LT) in our setting. Our aim was to describe NAFLD evolution as a LT indication and the most frequently found features associated with this indication.

Method: Patients undergoing LT for NASH-related cirrhosis from 2010 to 2021 June 30th in a reference LT center in Spain were included in the analysis. The medical records of all these patients were reviewed for determining NASH-associated comorbidities.

Results: NASH-related cirrhosis was the LT indication in 60 patients from 2010 to 2021 June 30th. The percentage of LT for NASH increased 6.4-fold between 2010-2021, from 2.8% to 18.0%. The lowest percentage (1.0%) was registered in 2016 and the highest percentage in 2021 (18.0%). Furthermore, the number of LT for NASH throughout the first six months of 2021 (n = 9) is greater than the rest of the years, except 2017 (n = 10) and 2019 (n = 12). Comorbid conditions were found in most patients; 78.3% had obesity, 60.0% T2DM, 58.3% hypertension (HTN), 35.0% dyslipidemia (DL) and 28.4% cardiovascular disease (CVD). While posttransplant complications were frequent, survival was similar to that of other indications with 10% mortality and only 1 case of graft loss due to recurrence of primary disease.

Conclusion: NAFLD is an increasingly common indication for LT in our country. Yet, the incidence is still far from that reported in countries like the US. As reported, most of these transplant candidates have significant comorbid conditions associated with posttransplant complications and poorer long-term outcome such as obesity, T2DM, HTN, DL and CVD. Yet, in the short-mid term transplant survival is similar to that of other indications.

Figure:


NAFLD evolution as a LT indication
% of LT for NASH
20
18
16
14
12
10
8
6
4
2
0
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021

PO-222
The I148M PNPLA3 variant mitigates Niacin beneficial effects: how the genetic screening in non-alcoholic fatty liver disease patients gains value

Erika Paolini[1 2], Miriam Longo[1 3], Marica Meroni[1], Annalisa Cespiati[1 4], Rosa Lombardi[1 4], Anna Ludovica Fracanzani[1 4], Paola Dongiovanni[1]

[1]General medicine and metabolic diseases; Fondazione IRCCS Cà Granda ospedale maggiore policlinico, Milan, Italy, *[2], Department of pharmacological and biomolecular sciences, Università degli sudi di Milano, Milan, Italy*, *[3], Department of clinical sciences and community health, Università degli studi di Milano, milan, Italy*, *[4], Department of pathophysiology and transplantation, Università degli studi di Milano, Fondazione IRCCS Cà Granda ospedale maggiore policlinico, milan, Italy*
Email: erika.paolini@unimi.it

Background and aims: The I148M polymorphism in the *PNPLA3* gene is the main genetic predictor of non-alcoholic fatty liver disease (NAFLD) and its impact on fat accumulation can be modulated by nutrients. Niacin (NIA) supplementation was proposed for NAFLD management, as it reduces triglycerides (TG) synthesis. However, the interplay between NIA and I148M genotype in NAFLD has not been explored yet. This study aims to:1) assess the dietary and circulating levels of NIA in NAFLD patients stratified according to the presence of the I148M variant; 2) examine the efficacy of NIA in Hep3B and HepG2 cells, which are wild-type and homozygous for the I148M mutation, respectively.

Method: We enrolled 172 NAFLD patients (Discovery cohort) in which fatty liver was non-invasively assessed. Dietary NIA was collected from food diary, while serum NIA was quantified by ELISA. The hepatic expression of enzymes involved in NAD metabolism were analyzed in obese NAFLD patients (n = 125; Transcriptomic cohort) through RNA-seq. HepG2 and Hep3B cells were transfected with a siRNA targeting PNPLA3 and treated with palmitic acid (PA) 0.25mM alone or plus NIA 0.5mM for 24hrs

Results: At bivariate analysis dietary NIA was reduced in NAFLD patients with steatosis >2 (p = 0.01). Both alimentary and circulating NIA levels were lower in NAFLD patients carrying the I148M variant than non-carriers (p = 0.01 and p = 0.03, respectively). At multivariate analysis, adjusted for sex, BMI, and alimentary NIA, the presence of I148M genotype was associated with lower serum NIA levels (β = -18, 01; CI: -35, 6--0, 57; p = 0.04), suggesting that it may independently modulate NIA availability. In the Transcriptomic cohort, the hepatic expression of the first catalyzer of NAD biosynthesis and enzymes consuming NAD was decreased in I148M carriers (p = 0.006), according to NIA reduction observed in the Discovery cohort. In HepG2 and Hep3B cells, NIA administration reduced TG accumulation and synthesis alongside hydrogen peroxide and malondialdehyde (p < 0.05 vs PA), markers of oxidative injury. The efficacy of NIA in reducing TG content was
even enhanced when the *PNPLA3* gene was silenced in HepG2 and Hep3B cells, thus supporting the possible interplay between *PNPLA3* and NIA metabolism.

Conclusion: NIA levels are reduced in patients carrying the I148M variant. The *PNPLA3* silencing in hepatoma cells emphasized the efficacy of NIA, thus suggesting its supplementation in NAFLD subjects with a predisposing genetic background.

Figure:

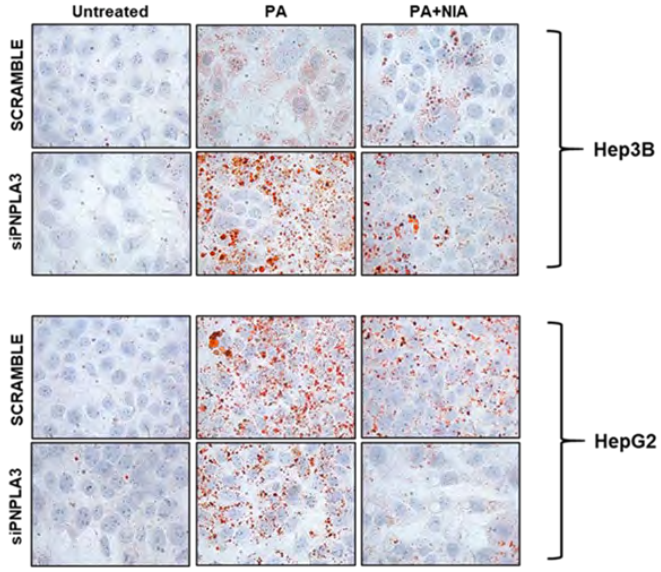


Figure: At Oil Red O (ORO) staining, the silencing of PNPLA3 gene exacerbated the lipid overload in Hep3B wild-type cells, while reduced its up-take in HepG2 ones, which are homozygous for the I148M variant. In both cases, NIA administration significantly decreased the hepatic fat accumulation.

PO-223
MAFLD definition underestimates the risk to develop HCC in genetically predisposed patients

Marica Meroni[1], Miriam Longo[1 2], Erika Paolini[1 3], Paolo Francione[1], Sara Badiali[4], Marco Maggioni[5], Anna Ludovica Fracanzani[1], Paola Dongiovanni[1]

[1]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy;, General Medicine and Metabolic Diseases;, Milano, Italy, *[2]University of Milan, Department of Clinical Sciences and Community Health, Milano, Italy*, *[3]University of Milan, Department of Pharmacological and Biomolecular Sciences, Milano, Italy*, *[4]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy;, Surgery, Milano, Italy*, *[5]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy;, Pathology, Milano, Italy*
Email: maricameroni11@gmail.com

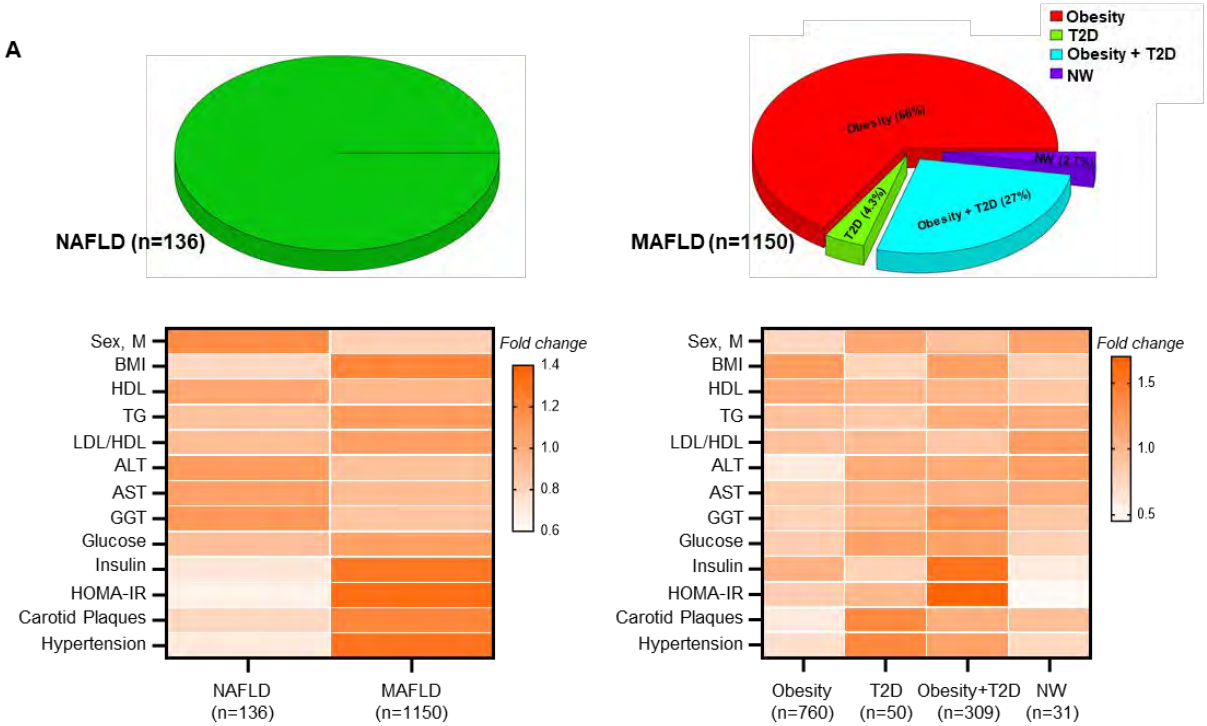
Background and aims: Non-alcoholic fatty liver disease (NAFLD) has been recently re-defined metabolic dysfunction-associated fatty liver disease (MAFLD), which is diagnosed in patients with hepatic steatosis in addition to obesity, type 2 diabetes (T2D) or metabolic dysregulations. We aimed to compare the histological features of MAFLD and NAFLD and to assess the contribution of NAFLD-related genetic variants on MAFLD.

Method: We enrolled 1286 individuals with biopsy-proven steatosis of whom 136 had NAFLD and 1150 MAFLD, stratified by obesity (66%), T2D (4.3%), obesity+T2D (27%) and normal weight with metabolic dysfunction (NW, 2.7%). A schematic representation of the entire cohort, subdivided according to NAFLD and MAFLD diagnosis is represented in Figure 1.

Results: MAFLD patients had more severe steatosis, necroinflammation and NASH ($p < 0.001$). Conversely, the risk of advanced fibrosis and HCC tended to be lower in MAFLD compared to NAFLD. Among MAFLD subjects, T2D was associated with the entire spectrum of the disease whereas obesity with early stages. In MAFLD, the effect of I148M PNPLA3, E167K TM6SF2 variants and their combination in polygenic risk score (PRS) was amplified by the presence of metabolic dysfunctions ($p < 0.05$). However, the PRS was associated with higher risk of cirrhosis and HCC in NAFLD ($p < 0.05$). Finally, the diagnostic accuracy of genetics in predicting steatosis and NASH was higher in MAFLD ($p < 0.0001$ vs NAFLD), whereas the ability of PRS to determine advanced fibrosis and HCC was comparable between the two conditions.

Conclusion: MAFLD definition is useful in identifying patients with severe steatosis, NASH and advanced liver damage. Genetics may be relevant in both MAFLD and NAFLD in predicting the progression of the disease and more so in the latter.

Figure 1: Clinical features of patients enrolled in NAFLD and MAFLD cohorts.



Pie charts display the distribution of the entire cohort (n = 1286) subdivided in NAFLD (n = 136) and MAFLD (N = 1150). MAFLD cohort was stratified according to the diagnostic criteria in: obesity (n = 760, 66%), T2D (n = 50, 4.3%), obesity+T2D (n = 309, 27%) and NW (n = 31, 2, 7%) (upper panel). Heatmaps represent clinical characteristics of NAFLD and MAFLD patients and were generated calculating the fold change of each clinical feature in both groups compared its main value in the entire cohort. The intensity of orange indicates the enrichment of the trait in each group (lower panel).

PO-225
Outcomes of postmenopausal women with non-alcoholic fatty liver disease (NAFLD)

Chioma Izzi-Engbeaya[1 2], Roberta Forlano[3 4], Benjamin H. Mullish[3 4], Tricia Tan[1 2], Michael Yee[2], Pinelopi Manousou[3], Waljit Dhillo[1 2]

[1]Imperial College London, Section of Endocrinology and Investigative Medicine, Department of Metabolism, Digestion and Reproduction, United Kingdom, *[2]Imperial College Healthcare NHS Trust, Department of Diabetes and Endocrinology, London, United Kingdom*, *[3]Imperial College London, Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, London, United Kingdom*, *[4]Imperial College Healthcare NHS Trust, Department of Hepatology, London, United Kingdom*
Email: w.dhillo@imperial.ac.uk

Background and aims: Non-alcoholic fatty liver disease (NAFLD), encompasses hepatic steatosis alone (NAFL), steatohepatitis (NASH), NAFL/NASH with fibrosis and cirrhosis. Severe fibrosis and cirrhosis are associated with increased risk of morbidity and mortality (due to cardiovascular events, end-stage liver failure and cancer). Postmenopausal women are a high-risk group of patients that have worse outcomes, but the specific factors that place them at higher risk are incompletely understood.

Method: We performed a retrospective analysis of patients with clinically or histologically diagnosed NAFLD, followed-up in the multi-disciplinary Metabolic Hepatology Clinic at Imperial College Healthcare NHS Trust (London), with an initial clinic visit between 2010 and 2017.

Results: Within this cohort of 220 patients, 34% were women ≥55 years (presumed to be postmenopausal), 31% were men ≥55 years, 45% were White. In terms of outcomes, 22% had cirrhosis and 8% died during the follow-up period (up to 11 years). 11 (65%) of the patients who died and 11 (23%) of the patients with cirrhosis were postmenopausal women. There was a significantly higher proportion of women ≥55 years who had a baseline FibroScan liver stiffness measurement (LSM) ≥8kPa (increased risk of advanced fibrosis) compared to men ≥55 years, (59% vs 41%, p = 0.032, using chi-squared tests). Multivariate logistic regression demonstrated that women ≥55 years were more likely to have follow-up LSM ≥8Pa (OR 2.5 [1.1-5.8], p = 0.019) and type 2 diabetes (OR 2.4 [1.0-5.5], p = 0.026), whilst men ≥55 years were more likely to have ischaemic heart disease/stroke (p = 0.032).

Conclusion: Postmenopausal women represent a significant proportion of NAFLD patients referred to specialist care, and they may be more likely to have advanced fibrosis when they are initially reviewed. Some co-morbidities may be more prevalent in postmenopausal women compared to other groups. Further work is required to fully phenotype these patients, so that modifiable factors can be targeted to improve outcomes.

PO-234
Assessment of systemic inflammation by 3D MR Elastography in obese patients treated with bariatric surgery

Jiahui Li[1], Marzanna Obrzut[1], Xin Lu[1], Kevin Glaser[1], Alina Allen[2], Sudhakar VENKATESH[1], Taofic Mounajjed[3], Jun Chen[1], Armando Manduca[1], Vijay Shah[2], Richard L. Ehman[1], Meng Yin[1]
[1]Mayo Clinic, Radiology, Rochester, United States, *[2]Mayo Clinic, Gastroenterology, Rochester, United States*, *[3]Mayo Clinic, Anatomic Pathology, Rochester, United States*
Email: yin.meng@mayo.edu

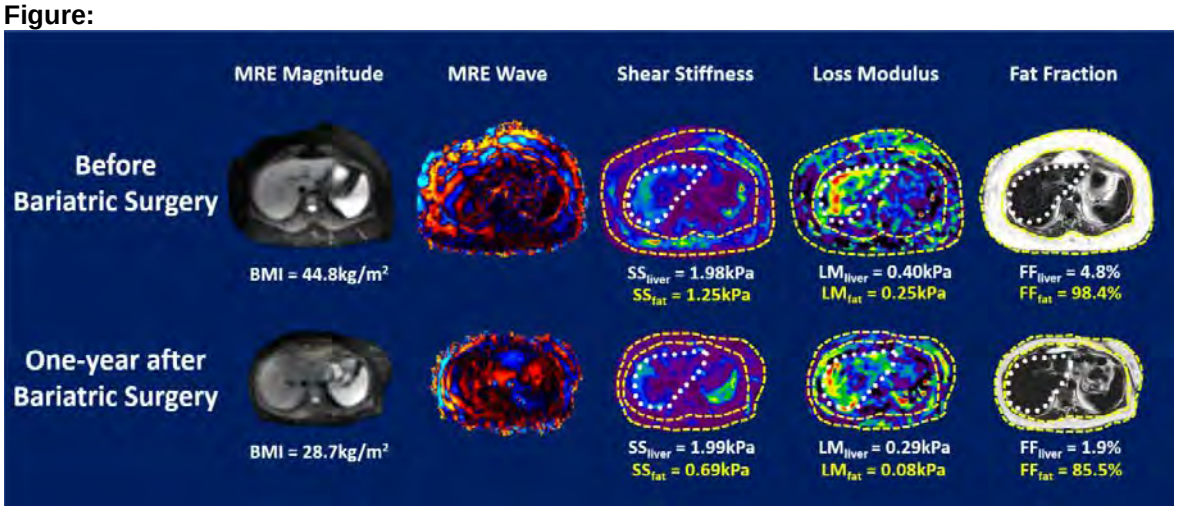
Background and aims: Metabolic-associated fatty liver disease (MAFLD) is a heterogeneous disease, representing hepatic manifestations of a multisystem disorder. Diagnostic criteria are based on evidence of steatosis, presence of obesity, type 2 diabetes mellitus, or metabolic abnormalities. There is no single test to predict, diagnose and characterize MAFLD completely. Prior preclinical and clinical studies have shown that multiparametric magnetic resonance elastography (MRE) is a promising non-invasive technique for detecting non-alcoholic steatohepatitis (NASH) and predicting the NAFLD activity score (NAS). MRE-assessed loss modulus has emerged as a potential quantitative biomarker for hepatic inflammation. This study aimed to evaluate loss modulus as a biomarker for systemic inflammation in both liver and subcutaneous fat in obese patients with a high risk of developing MAFLD.

Method: We performed 6-point Dixon MRI and 3D vector MRE at 30Hz on 34 subjects, including 10 non-obese healthy subjects and 24 obese patients with intraoperative biopsy-proven non-MAFLD (i.e., no steatosis). 12 of 24 patients had one-year post-operative exams and biopsies. We calculated shear stiffness (SS), loss modulus (LM), and proton density fat fraction (PDFF) in liver and subcutaneous fat and evaluated multiple imaging parameters between the groups and at different time points with nonparametric comparisons.

Results: When compared with controls, obese patients had significantly increased $PDFF_{liver}$ (2.0 ± 0.9 vs. 5.3 ± 1.8%), SS_{liver} (1.12 ± 0.17 vs. 1.46 ± 0.18kPa), and LM_{liver} (0.23 ± 0.08 vs. 0.36 ± 0.07kPa) before treatment ($p < 0.001$ for all). All these parameters in the liver and fat decreased significantly after treatment ($PDFF_{liver}$: 5.3 ± 1.8 vs. 2.7 ± 1.8%; SS_{liver}: 1.46 ± 0.18 vs. 1.26 ± 0.18kPa; LM_{liver}: 0.36 ± 0.07 vs. 0.27 ± 0.08kPa; $PDFF_{fat}$: 94.8 ± 1.8 vs. 87.9 ± 4.5%; SS_{fat}: 0.92 ± 0.04 vs. 0.66 ± 0.05kPa; LM_{fat}: 0.16 ± 0.06 vs. 0.10 ± 0.03kPa, $p < 0.01$ for all). Figure 1 shows one example patient.

Conclusion: Our results suggest that MRE-assessed loss modulus can distinguish early low-grade systemic inflammation in the liver and subcutaneous fat, even before histologic evidence of MAFLD. Multiparametric MRE provides a promising metric for assessing therapeutic response to weight loss in obese patients.

Figure:


MRE Magnitude
MRE Wave
Shear Stiffness
Loss Modulus
Fat Fraction
Before Bariatric Surgery
BMI = 44.8kg/m²
SS$_{liver}$ = 1.98kPa
SS$_{fat}$ = 1.25kPa
LM$_{liver}$ = 0.40kPa
LM$_{fat}$ = 0.25kPa
FF$_{liver}$ = 4.8%
FF$_{fat}$ = 98.4%
One-year after Bariatric Surgery
BMI = 28.7kg/m²
SS$_{liver}$ = 1.99kPa
SS$_{fat}$ = 0.69kPa
LM$_{liver}$ = 0.29kPa
LM$_{fat}$ = 0.08kPa
FF$_{liver}$ = 1.9%
FF$_{fat}$ = 85.5%

PO-237
Gender differences in characteristics of adolescents with non-obese non-alcoholic fatty liver disease

Oyekoya Ayonrinde[1], Phillip Melton[2], Caryn Elsegood[3], Leon Adams[4], Trevor Mori[4], Lawrence J. Beilin[5], Niwansa Adris[1], John Olynyk[2]

[1]Fiona Stanley Hospital, Gastroenterology and Hepatology, MURDOCH, Australia, *[2]University of Tasmania, Australia*, *[3]Curtin University, Australia*, *[4]The University of Western Australia, Australia*, *[5]The University of Western Australia, Perth, Australia*
Email: oyekoya.ayonrinde@uwa.edu.au

Background and aims: Non-alcoholic fatty liver disease (NAFLD) in people who are neither overweight nor obese using body mass index (BMI) criteria has been termed lean NAFLD. Up to 20% of adults with NAFLD have lean NAFLD. We aimed to examine features associated with NAFLD in non-obese community-based adolescents.

Method: Anthropometric, cardiovascular, blood tests and abdominal ultrasound assessments were performed on 1, 170 adolescents aged seventeen years, participating in a cross-sectional follow-up of the Raine Study. Medical history, medications and alcohol use data were recorded. Central obesity in the adolescents was defined using the International Diabetes Federation waist circumference criteria. The data were used to establish a diagnosis of NAFLD and compare the metabolic phenotype in non-obese adolescents with NAFLD versus those without NAFLD. Sex-specific analyses were performed. Multivariate analysis was used to determine adolescent characteristics that were independently associated with NAFLD.

Results: Amongst the adolescents, 79% were non-obese. NAFLD was diagnosed in 7.3% of non-obese adolescents (11.0% females vs. 4.7% males). Non-obese females with NAFLD had higher mean[SD] suprailiac skinfold thickness or SFT (18.1[7.0] vs. 15.2[5.8] and serum leptin (27.0[15.4-35.1] vs. 18.6[12.0-28.0]) compared with females without NAFLD. By contrast, non-obese males with NAFLD had higher body weight (90.3[22.6] vs. 69.4[11.0] kg), BMI (28.9[7.1] vs. 21.9[3.1]) kg/m2), waist circumference (83.7 [7.8] vs. 77.3[6.3]) cm, SFT (25.1[11.5] vs. 11.5[7.1] mm), systolic blood pressure (125.4[9.2] vs. 119.3[10.1] mmHg, fasting serum leptin (10.3[4.9-29.1] vs. 2.3[1.4-5.2] microgram/L), ALT (38.2 [23.0] vs. 22.1 [10.0] U/L), AST (31.4 [17.7] vs. 26.9[8.3] U/L), GGT (21.4[9.6) vs. 15.4[7.4] U/L), high median[IQR] serum high sensitivity C-reactive protein (1.2[0.6-2.7] vs. 0.4 [0.2-0.8] microgram/L), insulin (14.0 [8.2] vs. 8.1 [6.0] mU/L) and homeostasis model assessment for insulin resistance (HOMA-IR) (2.8 [1.5-4.8] vs. 1.4[0.9-2.2]), but lower mean serum HDL-cholesterol (1.1[0.2] vs. 1.2 [0.2]mmol/L) than those without NAFLD ($p < 0.05$). Using multivariate logistic regression analysis, serum ALT (OR 1.034, 95% CI 1.001-1.069) and SFT (OR, 1.13, 95% CI 1.08-1.19) in males, and suprailiac skinfold thickness alone in females (OR 1.08, 95% CI 1.02-1.13), were associated with non-obese NAFLD after adjusting for other covariates.

Conclusion: There was a sexual dimorphism in the phenotype of non-obese NAFLD. Non-obese males with NAFLD had a more adverse metabolic phenotype, with increased adiposity, dyslipidaemia, insulin resistance and raised liver transaminases, compared with males without NAFLD. SFT and serum ALT in non-obese male adolescents, and SFT alone in non-obese females were independently associated with a NAFLD diagnosis.

PO-239
Ankle brachial index and all-cause and cardiovascular mortality in patients with NAFLD

Stefano Ciardullo[1 2], Eleonora Bianconi[2], Rosa Cannistraci[2 3], Paola Parmeggiani[2], Enrico Maria Marone[4 5], Gianluca Perseghin[2 6]

[1]University of Milano Bicocca, Medicine and Surgery, Italy, *[2]Policlinico di Monza, Medicine and Rehabilitation*, *[1]University of Milano Bicocca, Medicine and Surgery, Italy*, *[4]University of Pavia, Clinical-Surgical, Diagnostic and Pediatric Sciences*, *[5]Policlinico di Monza, Vascular Surgery*, *[6]University of Milano Bicocca, Medicine and Surgery*
Email: stefanociardullo2@gmail.com

Background and aims: The present analysis was undertaken to assess the prevalence of peripheral arterial disease (PAD) and its association with all-cause and cardiovascular mortality in patients with NAFLD.

Method: 9145 participants 40 years or older attended a mobile examination center visit in the 1999-2004 cycles of the National Health and Nutrition Examination Survey. PAD was defined as an ankle brachial index (ABI) <0.90 in either leg and mortality data through December 2015 were obtained from the National Death Index. We excluded individuals without an available ABI measurement (n = 1574), and those with evidence of viral hepatitis, significant alcohol consumption or missing data on these variables (n = 673). NAFLD was defined by a fatty liver index ≥60, leading to a final sample of 3094 participants.

Results: The overall weighted prevalence of PAD was 5.9% (95% CI 5.0-6.9). Over a median follow-up of 13 years, 876 participants died, 208 of cardiovascular causes. Incidence rates of all-cause mortality (for 1000 person-years) were 20.15 (95% CI 18.72-21.69) and 70.02 (95% CI 60.08-81.60) for participants without and with PAD, respectively. Multivariable-adjusted Cox proportional hazard models showed that PAD was associated with a higher risk of all-cause (HR 2.2, 95% CI 1.7-2.8) and cardiovascular mortality (HR 3.3, 95% CI 2.1-5.3) after adjustment for age, race-ethnicity, education, BMI, cigarette smoke, diabetes, hypertension and prevalent cardiovascular disease.

Conclusion: Based on an ABI<0.9, ~ 1 in 20 patients with NAFLD from the general US population has PAD, which was associated with an increased incidence of all-cause and CVD mortality. Current guidelines strongly encourage the screening of CVD in patients with NAFLD and the use of the simple and inexpensive measurement of ABI in routine clinical practice may find indication.

PO-240
Intestinal microbiota in obese children with non-alcoholic fatty liver disease depending on the gallbladder function

Nataliya Zavhorodnia[1], Oksana Tatatrchuk[2], Inna Klenina[2], Olena Hrabovskaya[2], Elvira Zygalo[3], oksana petishko[2]

[1]SI "Institute Gastroenterology of the NAMS of Ukraine", Pediatric Gastroenterology, Dnipro, Ukraine, *[2]SI "Institute Gastroenterology of the NAMS of Ukraine", Research Department, Dnipro, Ukraine*, *[3]SI "Institute Gastroenterology of the NAMS of Ukraine", Mini-invasive endoscopic interventions and instrumental diagnostics Department, Dnipro, Ukraine*
Email: nzavgorodni75@gmail.com

Background and aims: Non-alcoholic fatty liver (NAFLD) pathophysiology has been associated with altered gut microbiota, lower microbial diversity, and a weakened intestinal barrier which promote immune defense pathways and inflammation. Impaired gallbladder function is considered to be associated with bile lipid abnormalities so may predispose to NAFLD progression. The aim of our study was to determine the features of the intestinal microbiota in obese children with NAFLD depending on the functional state of the gallbladder.

Method: A survey included 73 children aged 10 to 17 years (mean age-12.15 ± 2.51 years). According to body mass index, transient elastography (Fibroscan®), and ultrasound data patients were divided into 3 groups: I group-35 obese children with NAFLD and gallbladder hypokinesia, II group-30 obese children with NAFLD and gallbladder normokinesia, III group (control)-8 healthy children with normal weight and gallbladder normokinesia. Contractile gallbladder function was assessed by ultrasound examination after physiological nutritional load. Small intestine bacterial overgrowth (SIBO) and lactose absorption were assessed with the hydrogen breath test (HBT) with glucose and lactose loading using a Gastrolyzer (Bedfont Scientific Ltd, UK). Qualitative and quantitative intestinal microbiome composition were investigated by bacterial culture method using ten-fold dilutions (10^{-1}-10^{-9}) on a standard set of selective and differential diagnostic culture media for the aerobic and anaerobic microbes isolation. Fecal short-chain fatty acid (SCFA) content was evaluated with gas chromatography (Chromatec-Crystal-5000).

Results: Lactose-dependent SIBO was observed in almost half of NAFLD patients (49, 2%) without significant differences depending on functional activity of the gallbladder.
Patients with decompensated dysbiosis predominated in I group children (37, 1% of patients). In II group children the subcompensated dysbiosis was more common (36, 7% of patients). The concentration of *Lactobacillus* and *Enterococcus* in I group patients was significantly lower than in II group patients: in 1, 9 times (p <0, 05) and in 1, 4 times (p <0.05), respectively.
The level of fecal acetic acid in I group children increased in 6, 9 times (p <0, 05), butyric acid-in 2.0 times compared to other groups assuming participation of SCFA in the regulation of the microbiome composition.

Conclusion: Impaired contractile function of the gallbladder in NAFLD children is associated with a sharp decrease in the number of major symbionts of the intestinal microbiota as well as increased production of acetic and butyric SCFA.

PO-242
Reduction in fibrosis and steatohepatitis imaging and biomarkers in 52-week resmetirom non-alcoholic steatohepatitis trial

Stephen Harrison[1], Naim Alkhouri[2], Rebecca Taub[3], Ziad H. Younes[4], Guy Neff[5], Mustafa Bashir[6]
[1]Oxford, Hepatology, Oxford, United Kingdom, *[2]Arizona Liver Institute, Phoenix, United States*, *[3]Madrigal Pharmaceuticals, RandD, Conshohocken, United States*, *[4]Gstro One, Tennessee, United States*, *[5]Covenant Research, Sarasota, United States*, *[6]Duke University, Raleigh, United States*
Email: rebeccataub@yahoo.com

Background and aims: MAESTRO-NASH NCT03900429 and MAESTRO-NAFLD-1 NCT04197479 are 52 week Phase 3 registrational double blind placebo controlled NASH clinical trials to study the effect of resmetirom in more than 2000 NASH patients. A goal of MAESTRO-NAFLD-1, a fully enrolled (n = ~1200) "real life" NASH study is to identify non-invasive markers that correlate with patient response to resmetirom treatment.

Method: An exploratory evaluation of safety, imaging and biomarkers was conducted in >150 patients enrolled in the open label 100 mg daily dose active treatment arm of MAESTRO-NAFLD-1. Eligibility required at least 3 metabolic risk factors (Metabolic syndrome), fibroscan kiloPascals (kPa) consistent with ≥F1 fibrosis stage, and MRI-PDFF≥8%. The primary and key secondary end points of MAESTRO-NAFLD-1 include safety, relative percent reduction of MRI-PDFF (week 16), LDL cholesterol (LDL-C) (week 24), Apolipoprotein B and triglycerides.

Results: At the time of this abstract 115 patients had completed Week 52 including laboratory tests, safety analyses, MRI-PDFF, MRE, and FibroScan (VCTE). Demographics include mean age 55.7 (11.3 (SD)), female 71%, BMI 36.2 (6.2), diabetes 41%, hypertension 64%, dyslipidemia >70%, mean ASCVD score 11.1%. Fibroscan (kPa 7.4 (2.9)), and mean MRI-PDFF 18% (6.9%) are consistent with F2 stage NASH. Statistically significant ($p < 0.0001$) MRI-PDFF reduction of 53.4% fat fraction was observed at week 52. MRE was statistically significantly reduced at week 52 (Figure). At week 52 Fibroscan CAP and kPa were reduced relative to baseline. LDL-C (-25% (2.3% (SE))), apolipoprotein-B (-24% (2.3%)), triglycerides (med, -56% (7.8) mg/dL), and lipoprotein (a) (-34% (4.6%)) were statistically significantly reduced compared to baseline. Decreases from baseline in ALT -20.4 IU, AST -10.2 IU, GGT -28.5 IU ($p <0.0001$). Statistically significant reductions in inflammatory and fibrosis biomarkers hsCRP, reverse T3, ELF and M30 were observed. No safety issues were identified.

Conclusion: In this 52 week Phase 3 open label study 100 mg resmetirom demonstrated rapid and sustained reduction in 1-hepatic fat; 2-fibrosis stage as assessed by biomarkers, MRE and fibroscan; 3-LDL-C and atherogenic lipids, 4-liver enzymes and inflammatory biomarkers, providing support for the use of non-invasive tests to monitor individual NASH patient response to resmetirom treatment.

Figure:	***WEEK 52***
MRI-PDFF	
Relative %change	-53.4
p- value	<0.0001
Fibroscan[1] CAP	
Change (db/M)	-45.2
p value	<0.0001
Fibroscan[1] VCTE *(Baseline≥7.4)*	
Change (kPa)	-2.8
p value	0.0006
MRE *(Baseline≥2.9)*	
Change (kPa)	-0.43
p value	0.01

PO-244
Intestinal microbiota features in obese children with non-alcoholic fatty liver disease depending on the grade of steatosis

Nataliya Zavhorodnia[1], Natalia Zhigir[1], Olena Hrabovskaya[2], Elvira Zygalo[3], oksana petishko[2]

[1]SI "Institute Gastroenterology of the NAMS of Ukraine", Pediatric Gastroenterology, Dnipro, Ukraine, *[2]SI "Institute Gastroenterology of the NAMS of Ukraine", Research Department, Dnipro, Ukraine*, *[3]SI "Institute Gastroenterology of the NAMS of Ukraine", Mini-invasive endoscopic interventions and instrumental diagnostics Department, Dnipro, Ukraine*
Email: nzavgorodni75@gmail.com

Background and aims: Since intestinal microflora metabolites are a source of additional energy, increase production of short-chain fatty acids (SCFA) can lead to the non-alcoholic fatty liver (NAFLD) progression. The aim of our study was to investigate the intestinal microbiota by determining the small intestine bacterial overgrowth (SIBO) and the fecal short-chain fatty acids (SCFA) content in obese children with NAFLD depending on different steatosis degrees.

Method: 69 obese children aged 10 to 17 years were examined and divided into 3 groups according to Fibroscan data: 1 group -19 NAFLD children with 1 grade of the steatosis; 2 group-28 NAFLD children with 2 grade of the steatosis; 3 group-22 NAFLD children with 3 grade of the steatosis. 4 group (control) consisted of 9 normal-weight children without NAFLD, SIBO. A hydrogen breath test was performed to diagnose SIBO and lactose malabsorption using a Gastrolyzer (Bedfont Scientific Ltd, UK). Fecal short-chain fatty acid (SCFA) content was evaluated with gas chromatography (Chromatec-Crystal-5000).

Results: The frequency of lactose-dependent SIBO detection in children with NAFLD was in 1 group -47.4%, in 2 group -46.4%, in 3 group-40.9%. In NAFLD children SIBO was observed more often than in the control group without significant differences between groups with varying degrees of steatosis. Fecal acetic acid levels increased significantly in parallel with an increase in the degree of hepatic steatosis: concentration of fecal acetic acid in 1 group was 0, 042 ± 0, 012 μg/ml, in 2 group-0, 075 ± 0, 028 μg/ml, in 3 group-0, 105 ± 0, 032 μg/ml (p <0, 05). It was also observed a significant decrease in the concentration of propionic acid in NAFLD children compared to the control group: in 1 group mean level of propionic acid was 0, 025 ± 0, 007 μg/ml, in 2 group-0, 028 ± 0, 009 μg/ml, in 3 group-0, 059 ± 0, 014 μg/ml (p <0, 05). An increase in the fecal butyric acid content in NAFLD children was observed compared to the control group: in 1 group mean level of fecal butyric acid was 0, 098 ± 0, 040 μg/ml, in 2 group-0, 091 ± 0, 030 μg/ml, in 3 group-0, 096 ± 0, 024 μg/ml (p >0, 05). The total amount of SCFA increased significantly in 1 group to 0, 166 ± 0, 048 μg/ml, in 2 group to 0, 190 ± 0, 051 μg/ml, in 3 group to 0, 253 ± 0, 051 μg/ml in comparison with control group. Anaerobic index in 1 group decreased to 2, 419 ± 0, 821, in 2 group to 2, 086 ± 0, 498, in 3 group to 2, 549 ± 1, 344 in comparison with control group regardless of the steatosis grade.

Conclusion: Obese children with NAFLD demonstrated a significant increase in the incidence of lactose-dependent SIBO that was not associated with the grade of steatosis. Intestinal microbiome disturbances in NAFLD children are associated with an increase in the total amount of SCFA, an increase in acetic acid content which occurs in parallel with the liver steatosis raising, a decrease of propionic acid content and anaerobic index.

PO-249
Identification of new potential biomarkers to follow steatohepatitis in patients with non-alcoholic/metabolic associated fatty liver disease

Douglas Maya[1 2 3], Juan Antonio Cordero Varela[4], María Peña Chilet[5 6], Rocío Montero-Vallejo[1 2 3], Sheila Gato Zambrano[1 2], Rocío Gallego-Durán[1 2 3], Angela ROJAS ALVAREZ-OSSORIO[1 2 3], Rocio Munoz Hernandez[1 2 3 7], Joaquín Dopazo[5 6], Antonio Gil-Gomez[1 2 3], Manuel Romero Gomez[1 2 3 7], javier ampuero[1 2 3 7]

[1]UGC Ap. Digestivo. Hospital Universitario Virgen del Rocío, Spain, *[2]SeLiver Group. Instituto de Biomedicina de Sevilla, Sevilla*, *[3]Centro de Investigación Biomédica en Red. Área de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain*, *[4]Unidad de Bioinformática y Biología Computacional. Instituto de Biomedicina de Sevilla. Universidad de Sevilla. Sevilla, Spain*, *[5]Área de Bioinformática. Fundación progreso y Salud. Junta de Andalucía, Spain*, *[6]Centro de Investigación Biomédica en Red. Área de Enfermedades Raras (CIBERER), Spain*, *[7]Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain*
Email: mromerogomez@us.es

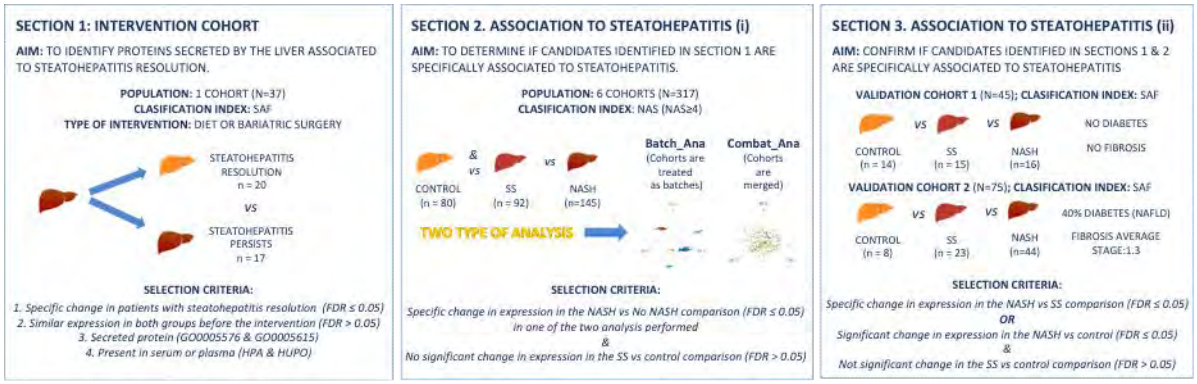
Background and aims: To integrate different liver gene expression datasets to identify novel potential serological biomarkers to follow-up steatohepatitis in MAFLD patients.

Method: Candidates were obtained by comparing the gene expression of paired liver biopsies coming from MAFLD patients that were able (n = 20) or not (n = 17) to resolve steatohepatitis at the end of a dietary or surgical intervention of 1 year (section 1). Association of candidates to steatohepatitis was explored in 6 microarray gene expression datasets (n = 317) analysed as a single cohort after Combat (Combat_Ana) or batch (Batch_Ana) normalization, performing NASH vs No-NASH comparisons as well as pairwised comparisons between healthy individuals (C; n = 82), patients with Bland Steatosis (SS; n = 90) and patients with steatohepatitis (NASH; n = 145; NAS ≥4) (Section 2). Selected candidates where finally analised in two additional datasets (N = 45 14 C/15 SS/16 NASH) (N = 75 8 C/23 SS/44 NASH) in which gene expression was analyzed by RNA-seq (section 3).

Results: Paired liver biopsy analysis identified 65 potential candidates. 12 of them showed an association to steatohepatitis in at least one of the two analyses (Combat_Ana, Batch_Ana) performed with the microarray multicohort dataset (Figures 1 and 2) and were further explored in the two cohorts in which gene expression was analyzed by RNA-seq. 6 of them replicated in one of these cohorts, and specifically enhanced (INHBE Fold Change-FC: 1.65 False Discovery Rate-FDR: 1.68E-02; LYZ FC: 1.71 FDR: 4.46E-02 ; LGALS3 FC: 1.89 FDR: 9.10E-03, PDGFA FC: 1.88 FDR: 3.31 E-03; LECT2 FC: 2.02 FDR: 3.01E-06) or decreased (IGFBP2 -3.55 6.32E-03) their expression in patients with steatohepatitis. Two of them, the hepatokine INHBE and the antimicrobial enzyme LYZ, replicated in the second validation cohort (INHBE FC: 1.88 FDR: 2.07E-03; LYZ FC: 1.85 FDR: 3.70E-06) (Figure 3).

Conclusion: LYZ and INHBE expression in the liver show a robust and dynamic association to steatohepatitis in this organ. Serum analysis will be required to determine their usefulness as biomarkers to monitor steatohepatitis.

Figure:


SECTION 1: INTERVENTION COHORT
AIM: TO IDENTIFY PROTEINS SECRETED BY THE LIVER ASSOCIATED TO STEATOHEPATITIS RESOLUTION.
POPULATION: 1 COHORT (N=37)
CLASIFICATION INDEX: SAF
TYPE OF INTERVENTION: DIET OR BARIATRIC SURGERY
STEATOHEPATITIS RESOLUTION n = 20
vs
STEATOHEPATITIS PERSISTS n = 17
SELECTION CRITERIA:
1. Specific change in patients with steatohepatitis resolution (FDR ≤ 0.05)
2. Similar expression in both groups before the intervention (FDR > 0.05)
3. Secreted protein (GO0005576 & GO0005615)
4. Present in serum or plasma (HPA & HUPO)
SECTION 2. ASSOCIATION TO STEATOHEPATITIS (i)
AIM: TO DETERMINE IF CANDIDATES IDENTIFIED IN SECTION 1 ARE SPECIFICALLY ASSOCIATED TO STEATOHEPATITIS.
POPULATION: 6 COHORTS (N=317)
CLASIFICATION INDEX: NAS (NAS≥4)
& vs
vs
CONTROL (n = 80)
SS (n = 92)
NASH (n=145)
Batch_Ana (Cohorts are treated as batches)
Combat_Ana (Cohorts are merged)
TWO TYPE OF ANALYSIS
SELECTION CRITERIA:
Specific change in expression in the NASH vs No NASH comparison (FDR ≤ 0.05) in one of the two analysis performed
&
No significant change in expression in the SS vs control comparison (FDR > 0.05)
SECTION 3. ASSOCIATION TO STEATOHEPATITIS (ii)
AIM: CONFIRM IF CANDIDATES IDENTIFIED IN SECTIONS 1 & 2 ARE SPECIFICALLY ASSOCIATED TO STEATOHEPATITIS
VALIDATION COHORT 1 (N=45); CLASIFICATION INDEX: SAF
vs
vs
NO DIABETES
NO FIBROSIS
CONTROL (n = 14)
SS (n = 15)
NASH (n=16)
VALIDATION COHORT 2 (N=75); CLASIFICATION INDEX: SAF
vs
vs
40% DIABETES (NAFLD)
FIBROSIS AVERAGE STAGE:1.3
CONTROL (n = 8)
SS (n = 23)
NASH (n=44)
SELECTION CRITERIA:
Specific change in expression in the NASH vs SS comparison (FDR ≤ 0.05)
OR
Significant change in expression in the NASH vs control (FDR ≤ 0.05)
&
Not significant change in the SS vs control comparison (FDR > 0.05)

PO-251
Increase metabolic dysfunction fatty liver disease related hepatocellular carcinoma in Egypt over a decade of period

Mohammed Eslam[1], Yasser Fouad[2], Yasmin Mohamed[3], Mohamed AbdAllah[4], SHAMARDAN Bazeed[5], Asmaa Salama[6], Ebada Said[7], ahmed ali[8], Dina Attia[6]

[1]Sydney University, *[2]Minia University, Gastroenterology, Hepatology and Endemic Medicine, Egypt*, *[3]Cairo University, Gastroenterology, Hepatology and Endemic Medicine, Egypt*, *[4]National research institute, Gastroenterology, Hepatology and Endemic Medicine, dokki, Egypt*, *[5]Qena University, Gastroenterology, Hepatology and Endemic Medicine, Egypt*, *[6]Beni-Suef University, Gastroenterology, Hepatology and Endemic Medicine, Beni-suef, Egypt*, *[7]Benha University, Gastroenterology, Hepatology and Endemic Medicine, Egypt*, *[8]Fayoum University, Gastroenterology, Hepatology and Endemic Medicine, Fayoum, Egypt*
Email: dinaattia14@yahoo.com

Background and aims: Although, the Middle East region, including Egypt has the highest prevalence of metabolic (dysfunction) associated fatty liver disease (MAFLD) globally, the total burden of MAFLD related hepatocellular carcinoma (HCC) in Egypt is unknown. Aim**:** To determine the temporal trends in the etiologies of hepatocellular carcinoma-in Egypt over a decade

Method: We retrospectively analyzed data from consecutive patients who were diagnosed and treated HCC over a 10-year period (2010-2020) in a large centre in upper Egypt. Hepatitis C virus (HCV), hepatitis B virus (HBV), alcoholic liver disease (ALD), and other liver disease were diagnosed using the standard tests. MAFLD was diagnosed based on past or present exposure to obesity or diabetes without other causes of chronic liver disease or by clinical diagnosis of cryptogenic cirrhosis.

Results: A total of 1538 HCC patients were included, 14% with MAFLD. From 2010-2015 to 2016-2020, the prevalence of MAFLD-HCC increased from (8% to 19%, respectively, p = 0.0001), and accompanied with the temporal trends in the prevalence of metabolic risk factors (such as diabetes) (56% vs 60.5%, p = .06). While HCV-HCC decreased (from 90% to 79%, p = 0.0001) and consistently the history of receiving antiviral therapy increased from (36% to 67%, 0.0001). There is no difference in age, gender, and history of smoking during these duration periods.

Conclusion: With the declining of hepatitis C, MAFLD is becoming a major cause of HCC in Egypt. MAFLD related HCC increased substantially over the past 10 years, and at least 1 to 5 individuals with HCC are attributed to MAFLD.

PO-253
A cholestatic pattern predicts liver-related events in patients with non-alcoholic fatty liver disease

Grazia Pennisi[1], Rosaria Maria Pipitone[1], Daniela Cabibi[1], Marco Enea[1], Claudia La Mantia[1], Federica Vernuccio[1], Rossella Zito[1], Vito Di Marco[1], Calogero Camma[1], Antonio Craxi[1], Stefania Grimaudo[1], Salvatore Petta[1]

[1]*Università di Palermo, Dipartimento Di Promozione Della Salute, Materno Infantile, Medicina Interna e Specialistica Di Eccellenza (PROMISE), Palermo, Italy*
Email: graziapennisi901@gmail.com

Background and aims: NAFLD (non-alcoholic fatty liver disease) has usually a biochemical "hepatocellular" pattern, but a "cholestatic" pattern can also be observed. We aimed to assess the impact of the "cholestatic" pattern in subjects with NAFLD on development of liver-related events (LRE).

Method: A cohort of consecutive NAFLD patients-diagnosed by biopsy or, in case of cirrhosis, by transient elastography, were divided into 3 groups, by using the formula (ALT/ALTULN)/ (ALP/ALPULN), based on the pattern of elevated liver enzymes: predominantly cholestatic pattern (C) with a ratio of less than 2, predominantly hepatocellular pattern (H) with a ratio of more than 5 and mixed (M) with a ratio between 2-5. LRE-liver decompensation (LD) defined as: ascites, encephalopathy, variceal bleeding, jaundice; and hepatocellular carcinoma (HCC)-were recorded during follow-up.

Results: 582 patients were enrolled; H, M and C patterns were found in 153 (26.3%), 272 (46.7%) and 157 (27%), respectively; F3-F4 fibrosis was more prevalent in C pattern (55%), compared to the others. During follow-up, only 1 patient with H pattern experience LRE; while 15 and 38 patients had LRE in M and C group, respectively. At multivariate Cox Regression analysis, age>55 years (HR 2.55, 95% C.I. 1.17-5.54; p = 0.01), platelets<150, 000/mmc (HR 0.14, 95% C.I. 0.06-0.32; p < 0.001), albumin <4 g/L (HR 0.62, 95% C.I. 0.35-1.08; p = 0.09), C vs M pattern (HR 7.86, 95% C.I. 1.03-60.1; p = 0.04), C vs H pattern (HR 12.1, 95% C.I. 1.61-90.9; p = 0.01) and fibrosis F3-F4 (HR 35.8, 95% C.I. 4.65-275.2; p < 0.001) were independent risk factors for LRE occurrence. The observed clinical results were validated in a multicentric cohort with histological diagnosis of NAFLD. Immunohystochemical analysis found a significant higher prevalence of moderate-high grade ductular metaplasia combined with low grade ductular proliferation in C pattern when compared with biochemical H pattern (58.3% vs 11.5%, p = 0.002). Gene expression analysis showed a difference expression of NR1H3, RXRα, VCAM1 among biochemical patterns.

Conclusion: Patients with NAFLD and fibrosis F3-F4 have frequently a cholestatic pattern. This is associated to a higher risk of LRE and with different gene expression pathways and liver histological changes.

PO-258
Hepatologists 'awareness and knowledge towards NAFLD and familiarity with renaming from NAFLD to MAFLD

Yasser Fouad[1], Shereen Abdel Alem[2], Mohamed AbdAllah[3], Yasmine Gaber[2], Ebada Said[4], Inas Kamal[1], ahmed ali[5], Dina Attia[6 6]
[1]Minia University, Egypt, *[2]Cairo University, Egypt*, *[3]National Research center, Medical Research Division, Cairo, Egypt*, *[4]Benha University, Egypt*, *[5]Fayoum University, Egypt*, *[6]Beni-Suef University, Egypt*
Email: yasserfouad10@yahoo.com

Background and aims: non-alcoholic fatty liver disease (NAFLD) is an emerging health issue, and its increased prevalence parallels the increasing prevalence of diabetes mellitus, obesity, and hypertension. Given the growing significance of the problem, the data on attitudes and knowledge regarding NAFLD among hepatologists are scarce. We performed this study to assess hepatologists' awareness and knowledge of screening, diagnosis and management strategies regarding NAFLD and a reorientation of the NAFLD name to MAFLD and the impact of changing nomenclature on the awareness of fatty liver disease (FLD)

Method: Hepatologists were asked to participate in a multicenter online, 36-question survey, to determine the current level of hepatologists' awareness, familiarity, and practices in the screening, diagnosis and management of NAFLD/MAFLD, together with their familiarity with changing the name from NAFLD to MAFLD and the impact of this change on the awareness of FLD

Results: This study included 208 hepatologists, 107 (51.4%) were males, with a mean age of 36.43 ± 8.72 years. About 50.2% (n = 104) of the physicians were familiar with NAFLD. Only 41 (19.8%) thought that NAFLD may frequently result in severe hepatic disease. Most of the participating hepatologists rarely screen for NAFLD (118, 57%) and sometimes they use liver biopsy for diagnosis of NAFLD (135, 65.2%). Changing the nomenclature was somewhat familiar in (104, 50.2%) of physicians. Additionally, 149 physicians thought that the new name gave a better awareness of fatty liver

Conclusion: the majority of hepatologists accepted that NAFLD sometimes results in severe liver disease and may be associated with metabolic risk factors that require a multidisciplinary approach in the management. Awareness of the management of NAFLD is low among hepatologists. Changing nomenclature from NAFLD to MAFLD will be more familiar among hepatologists and that change may aid in increasing awareness of FLD.

PO-259
Eating habits in patients affected by non-alcoholic steatohepatitis and type 2 diabetes mellitus

Lucia Brodosi[1], Dorina Mita[1], Federico Ravaioli[1], Giulia Simonetti[1], Giulia Bocedi[1], Bianca Baracco[1], Michela Genovese[1], Giovanni Vitale[2], Maria Cristina Morelli[2], Loris Pironi[1]

[1]Alma Mater Studiorum-Università di Bologna, Bologna, Italy, *[2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*
Email: lucia.brodosi2@unibo.it

Background and aims: Dietary problems in diabetic patients are well known in the literature, especially regarding adhesion to dietary regimens in a restrictive sense. Obesity and type 2 diabetes mellitus (T2DM) boost the progression of liver disease to liver cirrhosis. These conditions make lifestyle changes aiming at weight loss crucial for the prevention and treatment of non-alcoholic steatohepatitis (NASH). The Mediterranean Diet (MD) has proved to be the most functional tool for losing and maintaining weight, protecting from cardiovascular mortality. Besides, eating behaviors in diabetic patients affected by NASH are not well understood.
We aim to study the presence of Orthorexia Nervosa (ON) in patients suffering from liver disease, sent to our Clinical Nutrition clinic with the question for evaluation in metabolic syndrome. In addition, to evaluate calorie intake (CI) and adherence to MD in patients with BMI and basal metabolic rate (BMR).

Method: We studied all patients affected by T2DM and NASH, referred to the Unit from October 2019 to March 2020 (n = 36), 24% had a concomitant viral etiology. For the stage of disease, 33, 3% had a diagnosis of cirrhosis. The tests used to investigate the ON were the Bratman test consisting of 10 questions with a dichotomous YES/NO answer and the ORTO-15 test, which derives from the previous one, consisting of 15 questions with scaled answers 4-point Likert. CI was determined utilizing an in-house developed questionnaire ("QuantoMangioVeramente"-QMV), measuring the CI based on 20 items; the Mediterranean Diet Score (MDS) was used for evaluating adherence to MD; BMR and body composition were assessed by bioimpedance analysis.

Results: Males (61, 1%), mean age 61 years (± 5), BMI 33, 3 kg/m2 (± 7, 5), Waist Circumference 105cm (± 7, 5) lean mass 59, 5 kg (± 10, 4), total body water 47.2% (± 5.1), fat mass 33, 8% (± 8.1), BMR 1623Kcal (± 272), CI from QMV 1779Kcal (± 272). Adherence to MD was low in 94, 1% of the population. 75% of the sample was at risk for ON considering the Bratman test, while the ORTO-15 test showed that 64% of the sample was orthorexic.

Conclusion: Given the low adherence to MD, it is essential to invest resources in nutritional counseling in patients suffering from NASH. On the other hand, in addition to close medical surveillance, these data, albeit preliminary, also invite attention to the mental health of these patients, always taking into account the biopsychosocial perspective towards an increasingly patient-centered and oriented treatment.

PO-260
EUropean Patient cEntric clinicAl tRial pLatform (EU-PEARL): generating an integrated research platform to revolutionizing drug development in NASH

Juan Manuel Pericàs[1 2], Nicholas Di Prospero[3], Frank Tacke[4], Mette Kjaer[5], Quentin Anstee[6], Peter Mesenbrink[7], Franz Koenig[8], Nathalie De Préville[9], Blaise Kutala[10], Jesús Rivera[1 2], Lada Murcia[2], Vlad Ratziu[10 11]

[1]Vall d'Hebron University Hospital, Vall d'Hebron Barcelona Hospital Campus, Liver Unit, Internal Medicine Department, Barcelona, Spain, *[2]Vall d'Hebron Institute for Research (VHIR), Liver Diseases Group, Barcelona, Spain*, *[3]Janssen Research and Development, LLC, Raritan, United States*, *[4]Charité Universitätsmedizin Berlin, Campus Virchow-Klinikum and Campus Charité Mitte, Department of Hepatology and Gastroenterology, Berlin, Germany*, *[5]Novo Nordisk, Hovedstaden region, Denmark*, *[6]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK; Newcastle NIHR Biomedical Research Centre, Newcastle upon Tyne Hospitals, NHS Foundation Trust, Liver Research Group, Newcastle upon Tyne, United Kingdom*, *[7]Novartis Pharmaceuticals Corporation, East Hanover, United States*, *[8]Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, Austria*, *[9]Servier, Suresnes, France*, *[10]Hospital Pitié-Salpêtrière, Institute of Cardiometabolism and Nutrition, Paris, France*, *[11]Sorbonne Universités, Université Pierre et Marie Curie, Paris, France*
Email: jpericas@vhebron.net

Background and aims: Despite the high unmet needs in NASH and the efforts to develop new therapies, no treatments have so far received regulatory approval in the EU or US. Late-phase trials are slow to recruit, costly with a high screen failure rate while trial participants cannot be reallocated to the most promising agents as each trial is performed in isolation from the others. Innovative methodologies for next-generation therapeutic trials that optimize resources and allow for a more efficient patient participation are needed.

Method: EU-PEARL is an IMI-2 funded project (nº 853966) that was set up to create an integrated research platform (IRP) that uses a common supporting research framework (clinical and data networks, labs, legal and regulatory tools, etc.) and a master protocol which involves testing several treatments in the same overarching platform trial design. This structure will accelerate study conduct while lowering costs and creating more opportunities for patients.

Results: In the first year of EU-PEARL, the NASH working group has thoroughly reviewed the opportunities that arise from the creation of a NASH IRP and has examined the most relevant associated challenges and complexities. Phase design (phase II vs phase III vs seamless design), populations (e.g. non-cirrhotic vs. cirrhotic patients), adaptive methods of the platform trial design including interim analyses, Bayesian vs. frequentist statistical approaches, rules for inclusion, use of a historical control arm, monitoring of lifestyle, and inclusion of biomarkers indicative of treatment response as study end points. We have focused on proposing solutions and strategies to overcome the issues raised by patients, investigators, sponsors and health authorities while developing a draft master protocol trial design that allows the testing of combination therapies in NASH.

Conclusion: EU-PEARL is establishing a framework to advance drug development in the NASH field by developing a cross-company, multi-compound platform trial that optimizes patient participation and multiple operational aspects. This is expected to improve on speed, efficiency and cost of future NASH trials.

PO-261
Liver related and extrahepatic events occurrence in patients with non-alcoholic fatty liver disease: a competing risk analysis

Grazia Pennisi[1], Marco Enea[1], Manuel Romero Gomez[2], Mauro Viganò[3], Elisabetta Bugianesi[4], Vincent Wai-Sun Wong[5], Anna Ludovica Fracanzani[6], Giada Sebastiani[7], Jerome Boursier[8], Annalisa Berzigotti[9], Mohammed Eslam[10], javier ampuero[2], Jacob George[10], Antonio Craxi[1], Calogero Camma[1], Victor de Lédinghen[11], Salvatore Petta[1]

[1]Policlinico Paolo Giaccone, Section of Gastroenterology and Hepatology, Dipartimento Di Promozione Della Salute, Materno Infantile, Medicina Interna e Specialistica Di Eccellenza (PROMISE), Italy, *[2], Digestive Diseases Unit, Hospital Universitario Virgen del Rocío, Biomedical Research Networking Center in Hepatic and Digestive Diseases (CIBEREHD), Institute of Biomedicine of Sevilleo de Biomedicina de Sevilla, University of Seville, Seville, Spain*, *[3]Hepatology Unit, Ospedale San Giuseppe, University of Milan, Milan, Italy.*, *[4]Division of Gastroenterology, Department of Medical Sciences, University of Torino, Turin, Italy, Italy*, *[5]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong*, *[6]Department of Pathophysiology and Transplantation, Ca' Granda IRCCS Foundation, Policlinico Hospital, University of Milan, Milan, Italy*, *[7]Division of Gastroenterology and Hepatology, McGill University Health Centre, Montreal, Quebec, Canada*, *[8]Service d'Hépato-Gastroentérologie et Oncologie Digestive, Centre Hospitalier Universitaire d'Angers, Angers, France*, *[9]Hepatology Group, University Clinic for Visceral Surgery and Medicine, Inselspital, Department for Biomedical Research, University of Bern, Bern, Switzerland*, *[10]Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Sydney, NSW, Australia*, *[11]Centre d'Investigation de la Fibrose Hépatique, INSERM U1053, Hôpital Haut-Lévêque, Bordeaux University Hospital, Pessac, France*
Email: graziapennisi901@gmail.com

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with a high risk of liver-related events (LRE) and also extrahepatic events (EHE). We evaluated the competive risk occurrence of LRE and EHE in a large cohort of biopsy-proven NAFLD stratified according to baseline severity of fibrosis.

Method: Patients with a histological diagnosis of NAFLD were enrolled. During follow-up, LRE and EHE were recorded. Observed cumulative incidence functions (CIFs) were used to evaluate the risk of LRE and EHE occurrence; cause-specific Cox, subdistribution hazard models and predicted CIFs were fitted to identify predictors of LRE and EHE. Models were validated in a replication cohort of NAFLD with non-invasive assessment of liver fibrosis by liver stiffness.

Results: 2135 patients (F0-F1 = 1136; F2 = 362; F3-F4 = 637) with biopsy-proven NAFLD were enrolled. According to the observed CIFs the 60 month probability of LRE and EHE was 0.2% and 3% in F0-F1, 2% and 3.8% in F2, and 9.7% and 6.4% in F3-F4 patients, respectively. In the cause-specific Cox model, in F0-F1 and F2 patients, age>50 years (HR2.7, beta0.99, p = 0.001) was the only one predictor of LRE, while age>50 years (HR2.96, beta1.08, p < 0.001), previous cardiovascular events (CVE, HR2.07, beta0.73, p = 0.03) and previous extra hepatic cancer (EHC, HR2.36, beta0.86, p = 0.003) were independent risk factors for EHE. In F3-F4 patients, age>55 years (HR1.73, beta0.55, p = 0.03), obesity (HR1.52, beta0.42, p = 0.03), PLT<150, 000/mmc (HR3.66, beta1.30, p < 0.001) and GGT (HR1.77, beta0.57, p < 0.001) were associated with LRE, while age>55 years (HR1.74, beta0.55, p = 0.006) and previous CVE (HR2.51, beta0.92, p < 0.001) were independent predictors of EHE. Predicted CIFs for HE and EHE in F0-F1, F2 and F3-F4 patients well stratified the risk of events. The results were confirmed with the FineandGray model, and externally validated.

Conclusion: In NAFLD patients, by using competing risks models, the likelihood of EHE is relevant and raises according to the severity of liver fibrosis, while the risk of LRE is negligible in F0-F1, low but clinically relevant in F2 and high in F3-F4 patients. These data could help to personalize follow-up in NAFLD.

PO-268
Saga on an outlier: lean non-alcoholic fatty liver disease in urban population of Pakistan

Amna Butt[1], Saeed Sadiq Hamid[1], Asma Ahmed[1], Wasim Wasim Memon[2]

[1]Aga khan university hospital, Karachi, Pakistan, Medicine, Karachi, Pakistan, *[2]Aga khan university hospital, Karachi, Pakistan, Radiology, Karachi, Pakistan*
Email: amna.subhan@gmail.com

Background and aims: Lean NAFLD is unique wherein the absence of obesity and unrecognized traditional risk factors, diagnosis is either get delayed or even overlooked; hence resulting in compromised effectiveness or complete absence of required treatment. We aim to investigate the prevalence of lean NAFLD and to compare the clinical, metabolic characteristics of lean and obese NAFLD in the urban, adult population of the largest city of Pakistan.

Method: This was a population-based cross-sectional study piggyback with a large community-based trial "Pakistan Diabetes Prevention Programme" conducted in collaboration with the University of Helsinki in "Karachi", Pakistan during 2013-2016. Approximately 20, 000 residents of Karachi were screened for diabetes using systematic sampling. Individuals aged 35-75 years, having an Indian Diabetes Risk Score (IDRs) score ≥60 were enrolled. Ultrasound liver was performed by an experienced sonologist to identify NAFLD. Anthropometric measurements, laboratory investigations were carried out. Lean NAFLD was defined if BMI was <25 Kg/m^2. Obese NAFLD defined if BMI was ≥25 Kg/m^2. The study was funded by IDF) and URC, AKUH, Pakistan.

Results: Out of 1225 individuals, 741 (60.5%) had NAFLD. Lean NAFLD was found in 128 (17.2%). Comparing lean NAFLD with obese NAFLD higher proportion of males, smaller waist circumferences, and lower ranges of metabolic factors were found in lean NAFLD (Table 1). The risk estimates for lean NAFLD were higher among smokers, subjects having larger waist circumference, HTN, elevated LDL, and ALT (Table 2).

Conclusion: Lean NAFLD is common in the South Asian urban community of Pakistan. In the absence of significant metabolic derangements, early detection of lean NAFLD is challenging.

Figure:

Table 2: Univariatee and multivariate analysis for factors associated with Lean NAFLD

	Univariate analysis		Multivariate analysis	
	OR [95% CI]	*p* value	OR [95% CI]	*p* value
Smoking				
No	1.0		1.0	
Yes	2.09[1.16-3.76]	*0.01*	2.49[1.26-4.91]	*0.008*
Glycemic status				
Normal	1.0			
IGT/DM	1.9[1.29-2.86]	<0.001		
Waist circumference (cm)	11.2[5.8-21.8]	<0.001	10.17[5.15-20.11]	<0.001
Hip circumference (cm)	1.13[1.11-1.16]	<0.001		
HTN				
Normal BP	1.0			
120-139 systolic or 80-89 mmHg diastolic	1.64[1.07-2.50]	0.02	1.0 1.42[1.06-1.89]	0.01
>140 Systolic or >90 mmHg diastolic	2.10[1.20-3.69]	0.009		
RBS	0.99[0.99-1.00]	0.07		
LDL	1.01[1.003-1.015]	0.002	1.01[1.003-1.106]	0.006
ALT	1.01[1.003-1.026]	0.01	1.01[1.005-1.032]	0.007

Table 1: Clinical and Metabolic characteristics of Lean vs. obese NAFLD

	Lean NAFLD(n=128)	Obese NAFLD(n=593)	p value
Age (in years)	45.6 ± 10.3	44.6 ± 8.7	0.29
Gender			
Male	**60(46.9)**	175(29.5)	**<0.001**
Female	68(53.1)	418(70.5)	
Group			
Normal	53(41.4)	159(26.8)	**0.001**
IGT and DM	75(58.6)	**434(73.2)**	
Waist circumference (cm)			
Normal	29(22.7)	15(2.5)	**<0.001**
≥90 men and ≥80 women	99(77.3)	**578(97.5)**	
Waist circumference (cm)	89.9 ± 10.8	**102.7 ± 10.7**	**<0.001**
Waist to hip ratio	0.95 ± 0.09	0.94 ± 0.08	0.35
Severity of NAFLD			
I	90(70.3)	327(55.2)	**0.005**
II	36(28.1)	**239(40.4)**	
III	2(1.6)	**26(4.4)**	
History of HTN	33(25.8)	**254(42.8)**	**<0.001**
History of antihypertensives	22(17.2)	**188(31.7)**	**0.001**
History of impaired blood sugar	**35(27.3)**	107(18)	**0.01**
History of dyslipidemia	23(18)	**167(28.2)**	**0.01**
Any metabolic illness in family	98(76.6)	490(82.6)	0.10
Family history of DM	65(50.8)	307(51.8)	0.83
Family history of BP	63(49.2)	323(54.5)	0.28
Family history of cholesterol	22(17.2)	121(20.4)	0.40
Family history of obesity	28(21.9)	**235(39.6)**	**<0.001**
Family history of IHD	32(25)	**207(34.9)**	**0.03**
Smoker	**18(14.1)**	43(7.3)	**0.01**
HTN			
Normal	61(47.7)	201(33.9)	**0.01**
120-139 systolic or 80-89 mmHg diastolic	48(37.5)	**260(43.8)**	
>140 Systolic or >90 mmHg diastolic	19(14.8)	**132(22.3)**	
Systolic blood pressure	121.1 ± 17.9	**125.6 ± 19.9**	**0.01**
Diastolic BP	71.7 ± 9.8	**74.4 ± 11.3**	**0.007**
FBS	114.1 ± 49.7	107.2 ± 43.1	0.14
RBS	148.2 ± 84.0	136.0 ± 65.1	0.12
TG	187.1 ± 38.0	192.3 ± 38.4	0.55
HDL	181.4 ± 203.0	170.3 ± 118.7	0.87
LDL	109.3 ± 28.6	**119.1 ± 33.6**	**0.001**
ALT	27.4 ±18.3	**32.6 ± 22.7**	**0.01**

POSTER ABSTRACT PRESENTATION

NURSES & ALLIED HEALTH PROFESSIONALS

PO-35
Is it a 'HIIT'? High-Intensity Interval Training (HIIT) for the management of Non-alcoholic Steatohepatitis: patient experiences and perspectives

Shelley Keating[1], Ilaria Croci[2], Matthew Wallen[3], Jeff Coombes[1], Nicola Burton[4], Graeme Macdonald[5 6 7], Ingrid Hickman[7 8]

[1]The University of Queensland, Human Movement and Nutrition Sciences, Brisbane, Australia, *[2]K.G. Jebsen Center of Exercise in Medicine- Norwegian University of Science and Technology, Department of Circulation and Medical Imaging, Faculty of Medicine, Trondheim, Norway*, *[3]Flinders University, College of Nursing and Health Sciences, Adelaide, Australia*, *[4]Griffith University, School of Applied Psychology, Mt Gravatt, Australia*, *[5]Princess Alexandra Hospital, Department of Gastroenterology and Hepatology, Brisbane, Australia*, *[6]Princess Alexandra Hospital, Queensland Liver Transplant Service, Brisbane, Australia*, *[7]The University of Queensland, Faculty of Medicine, Brisbane, Australia*, *[8]Princess Alexandra Hospital, Department of Nutrition and Dietetics, Brisbane, Australia*
Email: s.keating@uq.edu.au

Background and aims: High-intensity Interval Training (HIIT) involves bursts of high intensity exercise interspersed with low intensity exercise recovery and has been shown to be safe, effective and feasible in a broad range of chronic conditions. High-intensity exercise may be beneficial for patients with NASH, however, whether HIIT is enjoyable, acceptable and sustainable for people with NASH is unknown. This study aimed to explore the experience and perceptions of both professionally supervised and self-directed HIIT among people with NASH.

Method: Participants with NASH (n = 12) undertook 12 weeks of supervised HIIT (4 x 4 min at 85-95 % maximal heart rate, interspersed with 3 min active recovery; 3 days/week), followed by 12 weeks of self-directed (unsupervised) HIIT. One-on-one, semi structured interviews with exercise staff were conducted prior to HIIT, and following both supervised and self-directed HIIT, to explore participants' knowledge, feelings, barriers, enablers, perceived outcomes, and intentions at each stage. Interviews were audio recorded, transcribed, coded and thematically analysed by two independent researchers.

Results: Four overarching themes (with subthemes) emerged (Figure 1) including: i) Specialist exercise support was highly valued (HIIT awareness and education, personalised tailoring of exercise, social support, feelings of safety despite apprehension about physical and mental capabilities, and logistical support with specialised equipment) ii) Complex medical and social exercise barriers exist (multi-morbidities, medical appointments and carer obligations) iii) HIIT offers holistic benefits (physiological, mental, social and activities of daily living) and was unanimously enjoyed; iv) Accountability is a critical enabler to HIIT (desire to honour commitments to the project/team facilitated attendance, self-directed HIIT was rarely sustained).

Conclusion: Supervised HIIT was unanimously enjoyed by people with NASH, but rarely sustained when self-directed. Support from exercise professionals, social support from staff and individual accountability are key factors to promote the adoption of, and adherence to, HIIT. These factors need to be addressed in future clinical programs to augment the uptake and long-term sustainability of HIIT for patients with NASH.

Figure:

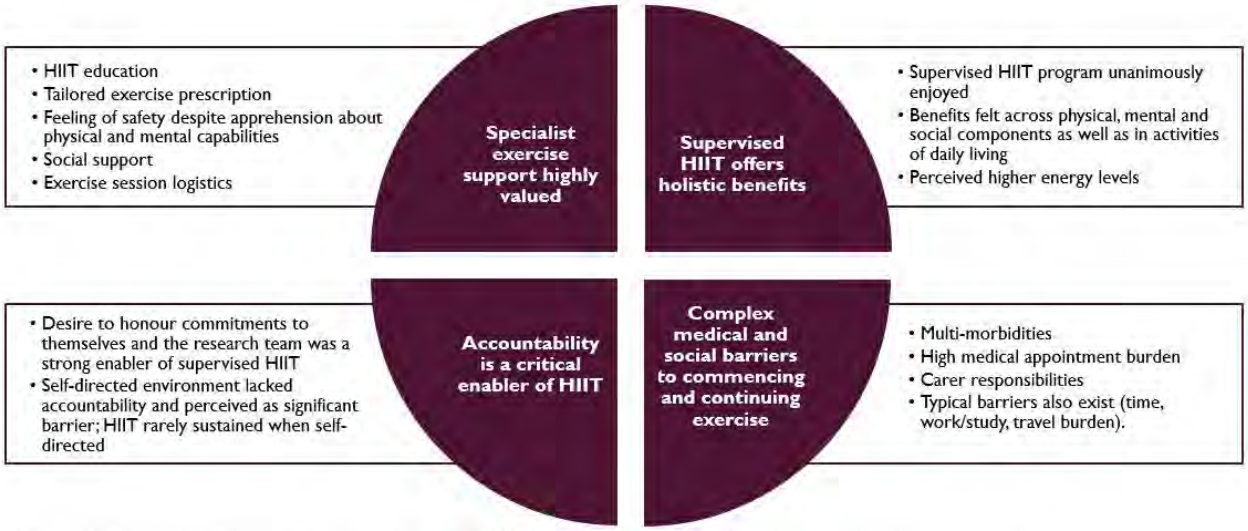


Figure 1. Schematic of themes from interviews with NASH patients. Central circle presents major themes, outer boxes presents subthemes. HIIT, high intensity interval training

PO-269
Hepatoprotective activity of Juglans regia stem bark ethanolic extract on CCl4 induced liver injury rat model

Bipindra Pandey[1], Atisammodavardhana Kaundinnayayana[1], Sushil Panta[1]

[1]*School of Health and Allied Sciences, Pokhara University, Master of Pharmaceutical Sciences, Pokhara, Nepal*
Email: bipindra.p101@gmail.com

Background and aims: *Juglans regia* is common medicinal plant used in folk medicine to treat liver disease in Nepal. The present study was designed to investigate the in vivo hepatoprotective activity of the ethanolic stem bark extract from *J. regia* using a CCl_4 induced liver damage rat model.

Method: Ethanolic plant extract were prepared using cold double maceration methods. The in vivo hepatoprotective activity, rats were randomly divided into six groups six in each: normal control (10 ml/kg distilled water), toxic control (10 ml/kg 5% dimethyl sulfoxide), positive control (Silymarin 100 mg/kg), *J. regia* extract (125, 250, 500 mg/kg) day^{-1} P.O for 7 days followed by a single dose of 50% CCl_4 in olive oil (1.5 ml/kg, i.p.) on 8^{th} day except normal control group. On the 9^{th} day, rats were sacrificed, and blood was withdrawn from retro orbital route. Liver injury was assessment by measuring serum biochemical parameters like AST, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), direct bilirubin (DBI), total bilirubin (TBI), total protein (TP), albumin level and histopathological observation.

Results: *J. regia* ethanolic extract up to 5000 mg/kg did not show any sign of toxicity and mortality in oral acute toxicity study. *J. regia* extract at doses 500 and 250 mg/kg shows significant decreased the liver weight ($p < 0.05$) as compared to the normal, toxic and positive control group. Liver injury test of the rats showed that, *J. regia* extract treated groups significantly decreased ($p < 0.001$) the elevated serum ALT, ALP, DBI, and TBI as compared to the toxic control group. However, *J. regia* extract administered groups significantly increased ($p < 0.001$) the decrease serum albumin level as compared to the toxic control group. Histopathological investigation of liver revealed that *J. regia* stem bark extract attenuating hepatocellular damage by diminishing fatty degeneration and necrosis in CCl_4 induced liver injury rat model.

Conclusion: It is confirmed that ethanolic extract of *J. regia* bark have marked curative effects in CCl_4 induced liver injury rat model. The results suggest that the hepatoprotective activity of *J. regia* extract is maybe due to presence of high phenolic, flavonoid contents and its antioxidant properties.