INFLAMMATORY STATUS, LIPID METABOLISM AND GUT MICROBIOTA AS TARGETS OF THE PROTECTIVE EFFECT OF QUERCETIN AND A. MUCINIPHILA COMBINATION ON OBESITY AND MAFLD

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Background and Aims: Obesity is a health concern linked to MAFLD and gut microbiota disturbances. Nowadays, the protective role of *Akkermansia muciniphila* and the prebiotic effect of quercetin are well-known against obesity and MAFLD, respectively. Our aim was to determine the benefits of the combination of *A. muciniphila* and quercetin with a nutritional intervention in an *in vivo* model of early obesity and MAFLD.

Methods: 21-days-old Wistar rats were fed with control diet (CD) or high fat diet (HFD) for 6 weeks. Later, every animal was given CD supplemented or not with the symbiotic for 3 weeks. Plasmatic and hepatic parameters were determined. Faecal samples collected the 6th and 9th week were analyzed using *Illumina* Myseq system.

Results: HFD rats showed alterations compatible with obesity and MAFLD development, and an associated intestinal dysbiosis. After 3 weeks under symbiotic administration, steatosis was improved, triglycerides were reduced and a lipid metabolism modulation was observed (lower hepatic expression of CEBP/a, DGAT2 and SREBP). Liver inflammatory status was enhanced by the symbiotic, showing a reduced expression of proinflammatory cytokines. Moreover, the HOMA-IR index, as well as plasmatic leptin and triglycerides concentration were improved with the symbiotic. Regarding gut microbiota composition, the combination reversed dysbiosis showing a specific profile characterized and incremented on *Cyanobacteria* and *Oscillospira* taxa, and a reduction of *Actinobacteria, Lactococcus, Lactobacillus* and *Roseburia.*

Conclusions: The symbiotic could counteract obesity and MAFLD development in an *in vivo* model, reversing inflammatory and metabolic alterations and modulating intestinal dysbiosis.

Topic: *AS01 Anchoring NAFLD management on lifestyle*

ASSOCIATION BETWEEN PHYSICAL ACTIVITY, INSULIN RESISTANCE AND NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH TYPE 1 DIABETES.

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Background and Aims: *Background*: Current guidelines on non-alcoholic fatty liver disease (NAFLD) recommend lifestyle modification - consisting of weight reduction, diet and physical activity (PA) - as first line treatment. *Objectives*: To evaluate the association between PA and insulin resistance (IR) and between PA and NAFLD in patients with type 1 diabetes (T1D).

Methods: *Methods*: In this cross-sectional study, patients with T1D from two cohorts from a secondary and tertiary care centre were included. PA was evaluated by a validated questionnaire concerning PA from sports and PA from other activities (walking, cycling, gardening) in the past 12 months. PA was expressed in metabolic equivalent of a task (MET) hours per week. Insulin sensitivity was calculated with the estimated glucose disposal rate (eGDR). NAFLD status was assessed by transient elastography (TE). Associations between PA and IR and between PA and NAFLD were explored by multivariate linear and logistic regression models, adjusted for age, sex and diabetes duration.

Results: *Results*: In total, 254 patients were included (male 56%, mean age 44 years, mean diabetes duration 24 years, median BMI 24.8kg/m^2), of which 150 patients underwent TE. Total PA (from sports and other activities combined, median 50.3METh/week) was borderline significantly associated with IR (median eGDR 7.31mg/kg/min) (beta -0.01, p=0.044). Total PA was not significantly associated with the presence of NAFLD (OR 1.01, 95%CI 1.00-1.02).

Conclusions: *Conclusions*: In our T1D population we could not replicate previous findings from the general population; a higher degree of PA was not associated with decreased IR or a lower odds for NAFLD.

Topic: *AS02 Optimising cardio metabolic outcomes*

CARDIOVASCULAR RISK ASSESSMENT IN A NON-ALCOHOLIC FATTY LIVER DISEASE GROUP OF ROMANIAN PATIENTS

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Background and Aims: Increasing prevalence of metabolic syndrome and type 2 diabetes among patients in conjunction with the close link between the 2 diseases and non-alcoholic fatty liver may presume the existence of a fairy large number of undiagnosed/unnderevaluated patients.We aimed to evaluate the cardio-vascular risk of 125 patients with non-alcoholic fatty liver disease and compared it with a control group.

Methods: We used *Hypertriglyceridemic waist, Hipertensive waist, Framingham core* and *SCORE Heart Score* to evaluate lots.

Results: In fatty liver patients the hypertrigliceridemic waist prevalence was 43.2% in men and 46.6% in women, with a total prevalence of 45.5%.We calculated the Framingham score risk in fatty liver patients with an average risk of 12.21239%, the risk in the control group being 3.4. A total of 20 fatty liver patients(mostly men) showed an increased cardiovascular risk (≥ 5) with the SCORE system. We also found weak positive linear correlation between waist circumference ($r = 0.137$), respectively average blood pressure ($r = 0.238$) and Framingham risk.

Conclusions: Estimation of Framingham and SCORE cardiovascular risk proved an increased risk with age(Spearman coefficient $r = 0.64$, respectively $r = 0.47$). The cardiovascular risk was lower in females and higher in those presenting obesity, hypertensive waist or metabolic syndrome.The clinical diagnosis of metabolic syndrome is not sufficient to assess the risk of cardiovascular disease. In order to appropriate assessment and management of overall cardiovascular risk in clinical practice, is important to take into account the traditional risk factors and the additional contribution brought by obesity/insulin resistance and their related complications.

REGISTRE-NASH- A MULTIDISCIPLINARY NAFLD/NASH CLINIQUE AT CHU DE QUÉBEC-UNIVERSITÉ LAVAL

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Background and Aims: The natural history of NAFLD according to comorbidities severities and treatments is still poorly documented. **Objective.** REGISTRE-NASH is a prospective 5-years observational study to describe NAFLD progression, comorbidities, and lifestyle habits in patients followed at our multidisciplinary NAFLD/NASH clinic in Québec, Canada.

Methods: Questionnaires were conducted to collect personal and family medical history; web questionnaires on alcohol consumption and physical activity were collected; medical comorbidities, biochemical and imaging results were collected from medical files.

Results: 53 participants have completed the initial visit of the registry; 17% with cirrhosis, 62% with NASH and 21% with simple hepatic steatosis. Median age was 56 (range 21-77 years-old); median BMI 34 (range 22-52 kg/m^2); 47% were women. 60% had diabetes, 60% hypertension, 47% dyslipidemia. After their initial visit, 81% of participants with diabetes were treated with metformin and 60% with a GLP-1 agonist, 22% with insulin therapy, whereas 28% were treated with insulin therapy before their initial visit. 53% of participants with dyslipidemia were treated with a statin at the diagnosis of NAFLD, while 87% of participants with dyslipidemia were treated with a statin after their initial visit. 65% were consuming soft drinks at the diagnosis of NAFLD, while 48% were still consuming soft drinks after initial visit.

Conclusions: Preliminary results from our registry shows a high prevalence of metabolic comorbidities in patients with NAFLD and that multidisciplinary care may improve patients care. Results over a 5-year period will be necessary to evaluate the impact of the multidisciplinary care and comorbidities on NAFLD progression.

Topic: *AS02 Optimising cardio metabolic outcomes*

DO REAL-WORLD DIAGNOSTIC, MANAGEMENT AND SPECIALITY APPROACHES DIFFER IN NASH PATIENTS WITH/WITHOUT CARDIOVASCULAR-METABOLIC COMORBIDITIES?

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Background and Aims: Non-alcoholic steatohepatisis (NASH) is associated with comorbidities such as type 2 diabetes mellitus (T2DM), obesity and atherosclerotic cardiovascular disease (ASCVD). This analysis aimed to describe the impact of these cardiovascular-metabolic conditions (CVM) on NASH patient characteristics, diagnosis and management.

Methods: Data from 2018/19 Adelphi NASH Disease Specific Programme™, a 12-country real-world survey, included physicians completing questionnaires describing up to 8 patients with NASH, covering patient demographics, fibrosis stage, consultation history, and comorbidities. 8 patient groups were assessed, defined by presence of obesity, T2DM and/or ASCVD alongside their NASH.

Results: 654 physicians (29% Hepatologist, 48% Gastroenterologist, 21% Diabetologist, 2% PCP) recruited 3434 patients with NASH; 58% male, mean age 55.1 years, mean BMI 32.3. Largest three patient groups: obesity/T2DM/NASH (35%), obesity/NASH (24%), and NASH-only (18%). NASH-only patients youngest (49.7 years); obesity/T2DM/ASCVD patients oldest (62.0 years). Gastroenterologists most commonly diagnosed NASH irrespective of comorbidity status; T2DM patients more likely to have a diabetologist diagnose NASH. NASH-only patients most likely to be F0-1 at diagnosis; presence of CVM comorbidities associated with later F-stage at diagnosis. Liver biopsy was low among NASH-only patients (29%); higher use among CVM patients. NASH-only patients least likely to be co-managed for NASH by different specialties (37%) vs. CVM patients (45-72%). All results $p < 0.05$.

Conclusions: Presence of CVM comorbidities among NASH patients is associated with increased fibrosis score at diagnosis, level of liver biopsy testing, and physician speciality co-management vs. NASH-only patients. This reflects how collaboration between relevant specialties is necessary in patient management given the comorbid nature of NASH.

Topic: *AS02 Optimising cardio metabolic outcomes*

HBA1C IS SUBOPTIMAL FOR THE SCREENING OF TYPE 2 DIABETES IN INDIVIDUALS WITH NAFLD

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Background and Aims: Individuals with NAFLD are at high risk for developing Type 2 diabetes (T2D). The early screening for T2D is therefore crucial but its accuracy may be impaired because of the specific insulin resistance associated with NAFLD. In a retrospective study we aimed to 1) determine T2D screening accuracy using HbA1c and fasting plasma glucose (FPG) according to Diabetes Canada guidelines and 2) establish optimal screening thresholds in individuals with NAFLD.

Methods: Screening with HbA1c and FPG were compared to the gold standard 75g oral glucose tolerance test (OGTT), retrospectively in patients who had the three tests within three months. ROC curves were used assess the optimal thresholds for HbA1c and FPG.

Results: 50 OGTT were included. Median age and BMI were 48 years-old (36-63) and 34 kg/m^2 (29-38). 32% had NAFL, 58% NASH and 10% cirrhosis. HbA1c at the thresholds of our current guidelines had 35% (20-54) and 7% (0.3-31) sensitivity for the diagnosis of prediabetes and T2D. FPG had 75% (60-90) and 71% (45-89) sensitivity for prediabetes and T2D. Thresholds to optimize Youden index for prediabetes were Hba1c ≥ 5.4% (AUCROC: 0.82) and a FPG ≥ 5.8 mmol/L (AUCROC: 0.92). For T2D, Hba1c ≥ 5.6% (AUCROC: 0.77) and FPG ≥ 6.5 mmol/L (AUCROC: 0.92) were optimal.

Conclusions: HbA1c may be suboptimal for T2D screening in patients with NAFLD. Lower FPG thresholds would be more adequate. OGTT would be indicated for the screening of T2D in the context of NAFLD, because of the low sensitivity of Hba1c and FPG according to current guidelines.

Topic: *AS02 Optimising cardio metabolic outcomes*

CAROTID INTIMAL THICKNESS VERSUS AORTIC INTIMAL THICKNESS IN NAFLD :AN EGYPTIAN PIOLT STUDY

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Background and Aims: An emerging concern is the apparent onset of significant for Non Alcohoic Fatty Disease(NAFLD) in early life at age of 20th even earlier .The major complications hepatic and extrahepatic especially on the CVS with high mortality to youth. Aim of the study to evaluate the presence of abnormal carotid intimal thickness versus abnormal aortic intimal thickness in NAFLD subjects.

Methods: This was a cross sectional study, conducted on 88 Subjects both male and females . aged from 18-70 years old .The patients were evaluated clinically thorough Full medical history**,**Full clinical examination**:** as Blood pressure, body mass index (BMI)= weight (Kgm)/ height (meter)^2 >30 kg/m^2 **,** laboratory Investigations done .Abdominal ultrasound ,plus measurment of CIMT and AIMT byA scanning frequency of 13 MHz was preferred, although scanning frequencies of 11.5 and 10 MHz were also used as necessary .The liver echogenicity was classified into 4 grades

Results: All subjects 80 in 88 have fatty liver with different grades ,that in group subjects not DM or obese 36.4% and 63.6% with fatty liver grades from 1 to 3 , pvalue 0.01 . Aortic Intimal Media Thickness mean was as follow in completly normal subjects 0.96_+0.26mm and 1.3_+0.2mm for obese DM while Carotid Intimal Media Thickness was follow0.58_+0.18mm& 0.73_+0.18mm respectively.In obese non DM ,,AIMT was1.24_+0.18mm and in obese DM 1.52_+0.33mm while CIMT was0.86_+0.20mm&1,01_+0.25mm respectively , p value was 0.001.,

Conclusions: CVS complications in form of subclinical atherossclerosis as in AIMT and CIMT of serious health problem in NAFLD patients ,,need the interventions of all ,

THE ASSOCIATION BETWEEN NONALCOHOLIC FATTY LIVER DISEASE AND CARDIOVASCULAR RISK IN DIABETIC PATIENTS

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Background and Aims: NAFDL (non-alcoholic fatty liver disease) can be considered a feature of metabolic syndrome. It is possible that NAFLD patients might have a greater CVD risk.

Methods: We have performed a retrospective observational study in order to see if there is any significant difference between the risks of CVD at the type 2 diabetic patients with NAFDL, comparing with those without NAFDL. 80 type 2 diabetic patients who were hospitalized in the Diabetes Department, Clinical Hospital from Sibiu, during a three months period were studied.

Results: From the whole group, 28.75% were diagnosed with NAFDL. The average age of NAFDL patients was higher than of those without NAFDL (66.91 years, comparing with 60.29, p =0.0015). The risks of CVD at those without NAFDL comparing with those with NAFDL were: for coronary heart disease 21.3% comparing with 33.02% (p=0.0071), for fatal coronary heart disease 15.3% comparing with 26.81% (p=0.0031), for stroke 7.6% comparing with 15.91% (p=0.0001) and for fatal stroke 1.3% comparing with 2.86% (p=0.0004). A linear correlation was found between the Forns index of liver fibrosis and the risk of CVD (r =0.21 for coronary heart disease, r =0.199 for fatal coronary heart disease, r =0.334 for stroke and r =0.325 for fatal stroke). The level of liver cytolysis did not correlate with the risk of CVD, in the NAFDL group.

Conclusions: The diabetic patients with NAFDL have a higher risk of CVD then those without NAFDL. There is a linear correlation between the degree of liver fibrosis and the risk of CVD.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

REAL WORLD EXPERIENCE USING FAST SCORE TO IDENTIFY NASH FIBROSIS

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Background and Aims: FibroScan-AST (FAST) score has demonstrated an effective way to stratify risk for progressive nonalcoholic steatohepatitis (NASH). FAST score is calculated using liver stiffness measurement (LSM), controlled attenuation parameter (CAP), and aspartate aminotransferase (AST). The aim was to assess the capability of FAST score to identify NASH and advanced fibrosis (NASH + $\geq$F2 fibrosis), in a cohort with biopsy-proven NASH.

Methods: Patients with NASH and FibroScan from June 2020 to June 2021 were identified from the electronic medical record. Clinical data was obtained. FAST score $\geq$0.35 cut-off was used to identify NASH + $\geq$F2 fibrosis.

Results: 58/67 patients with FAST score $\geq$0.35 and 46/58 had $\geq$F2 were identified. Rule out cut off of 0.35 performed with sensitivity 95.8%, NPV 77.7% and PPV 79.3%. Specificity 37% was low given sample size. FAST score outperformed FibroScan in classifying NASH+$\geq$F2 fibrosis (79% vs. 67%). Independent logistic regressions showed diabetes (p=0.05) and FAST score (p=0.003) to be associated with correctly identifying NASH+$\geq$F2. Multivariable logistic regression ascertaining the effects of diabetes on the predictability of the FAST score was statistically significant, $\chi2(1) = 5.762$, p=0.016. Age (p=0.097), hypertension (p=0.89), LSM $\geq$ 8kPa (p=0.051) and BMI (p=0.433) were not found to be statistically significant associations. FAST score $\geq$0.35 (OR=20.7,CI:3.11-137) and diabetes (OR=4.8,CI:1.19-19.16) were likely to predict histological indication of NASH, correctly identifying 79% of cases.

Conclusions: These results add to our knowledge of noninvasive tools to identify NASH + $\geq$F2. A FAST score $\geq$0.35 and diabetes can be used to more accurately identify patients with NASH +$\geq$F2.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

DECREASES IN LIVER CT1 ACCURATELY REFLECT HISTOLOGICAL IMPROVEMENT INDUCED BY THERAPIES IN NASH: A MULTI CENTRE POOLED COHORT ANALYSIS

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Background and Aims: Current endpoints in non-alcoholic steatohepatitis (NASH) clinical trials require liver biopsy. MRI-derived biomarkers such as iron-corrected T1 (cT1) are less variable, risky, and costly alternatives. Previous analysis showed that a ~80ms decrease in cT1 corresponds to 2-point decrease in the NAFLD activity score (NAS) and no worsening in fibrosis, indicative of clinically significant histological improvements, as stipulated by the FDA. Here, we validated this observation with data pooled from three interventional NASH studies.

Methods: Study participants underwent MRI and biopsy at baseline and 22-52 weeks following intervention. Participants were characterized as responders (NAS decrease ≥ 2 with no worsening of fibrosis), or non-responders. Median ΔcT1 in the responders was calculated to determine a cut-off for detecting clinically meaningful changes in NASH. Diagnostic accuracy of ΔcT1 to identify responders was quantified using AUROC. Correlations between ΔcT1 and Δhistological markers were tested using spearman's rank.

Results: 193 patients from three NAFLD/NASH-confirmed cohorts were included. Change in cT1 correlated with change in NAS (R_s:0.45;$p<0.0001$) and was significantly higher in histological responders. Using ΔcT1 to identify responders resulted in an AUROC of 0.73[0.65-0.82]. ΔcT1 of 83ms corresponded to a two-point change in the NAS score.

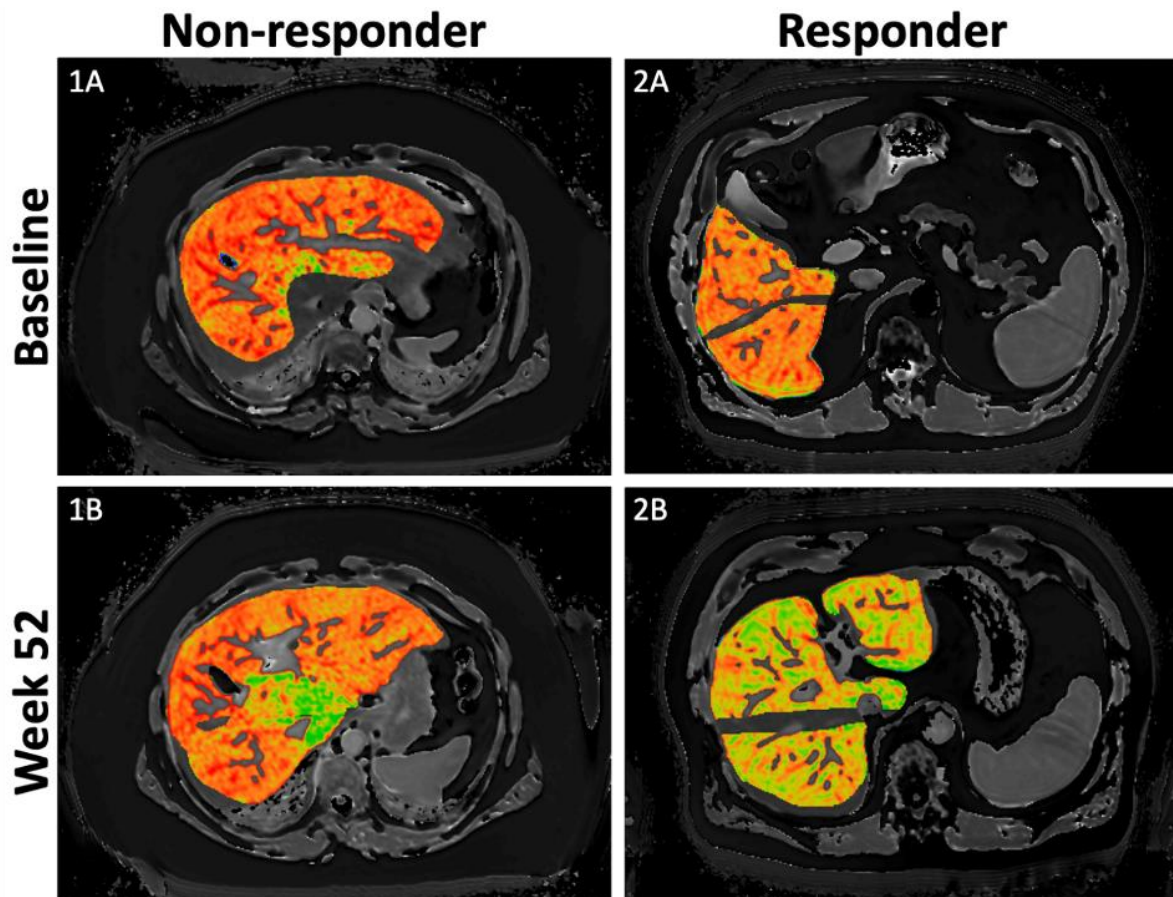**ΔcT1 significantly greater in Responders**
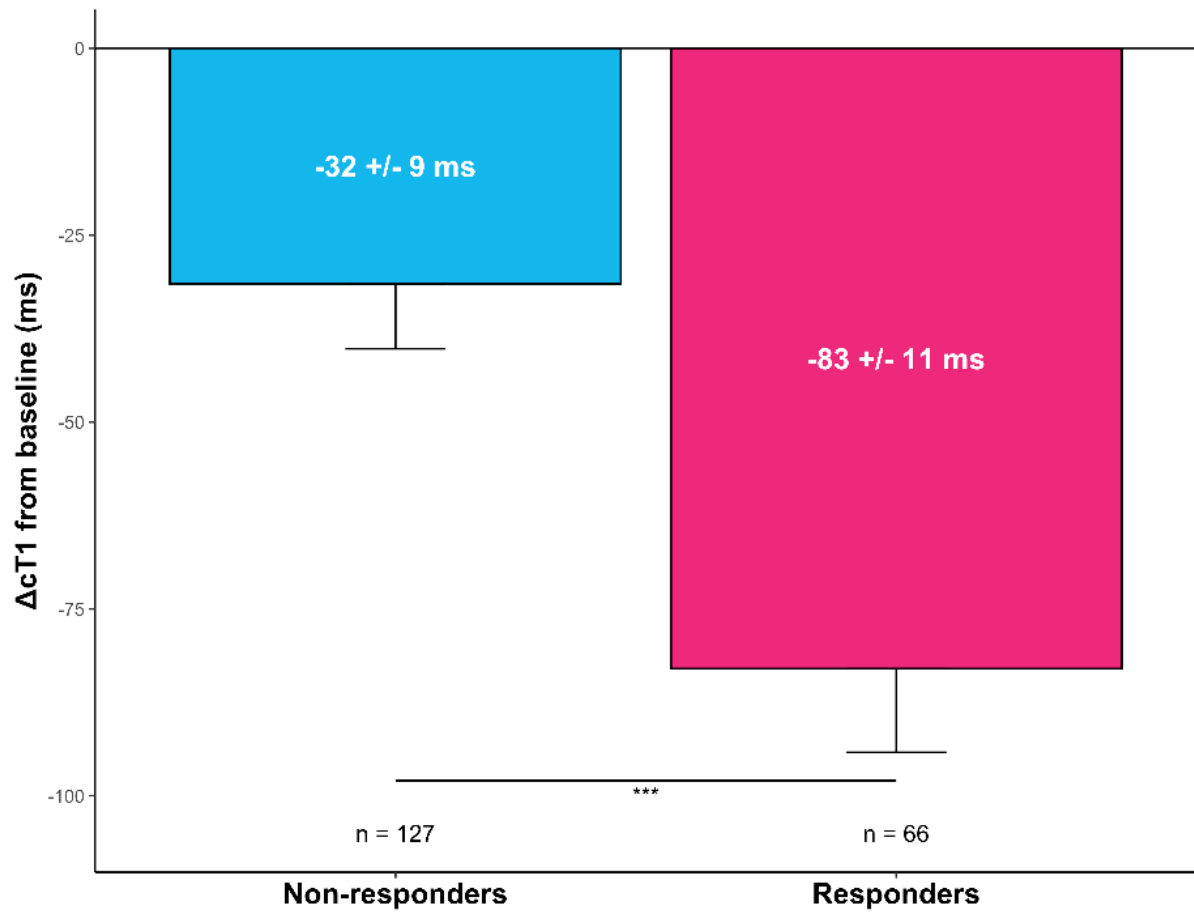
Figure showing the cT1 maps at **A)** baseline and **B)** 52 weeks following intervention in **1)** a non-responder with a ΔcT1 of 35ms and **2)** a responder with ΔcT1 of 82ms.

Conclusions: The previously estimated change in cT1 of ≥ 80ms was validated in a large independent data set with a clinically meaningful change in NASH corresponding to a ΔcT1 value of 83ms in this pooled cohort. These results support the clinical use of cT1 for disease monitoring and its application as a surrogate endpoint for liver biopsy in clinical trials for NASH.

MECHANISM-BASED BIOMARKER PREDICTION FOR LOW-GRADE INFLAMMATION IN LIVER AND ADIPOSE TISSUE

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Background and Aims: Background: Metabolic disorders, such as type 2 diabetes have a large impact on health, especially in industrialized countries. Tissue-specific chronic low-grade inflammation is a key contributor to complications in metabolic disorders. To support therapeutic approaches, it is crucial to gain understanding of the inflammatory dynamics per tissue. To this end, blood-based biomarkers reflecting the tissue-specific inflammatory dynamics would be of great value.

Methods: Methods: An *in silico* approach was used to select candidate biomarkers for tissue-specific inflammation by using a priori mechanistic knowledge from pathway and biomarker databases, and tissue-specific molecules. Verification of is performed in Ldlr-/-.Leiden mice fed a high fat diet (HFD) to metabolically induce tissue specific inflammation in liver and adipose tissue.

Results: Results: The workflow resulted in a list of candidate markers that reflect inflammation in the liver and adipose tissue. First, verification was on murine tissue gene-level by inducing hepatic inflammation and adipose tissue inflammation through a high-fat diet. Second, we evaluated the human translational value by performing a curation step in literature using studies that describe the markers in human studies. This identified hepatic (such as Serum Amyloid A, Haptoglobin, and Interleukin 18 Binding Protein) and adipose (Resistin and MMP-9) inflammatory biomarkers at the highest level of confirmation.

Conclusions: Conclusion: Our approach identified and pre-clinically verified a set of *in silico* predicted biomarkers for metabolically-induced liver and adipose tissue inflammation which can be of great value to study future development of therapeutic/lifestyle interventions to combat metabolic inflammatory complications.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

EVALUATION OF FATTY ACID BINDING PROTEIN 1 IN NAFLD AS SCREENING METHOD :EGYPTIAN PILOT STUDY

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Background and Aims: Although NAFLD is world wide health problem with prevelance variability among countries and in some area it,s prevelance more than 50% of population studies . the cardiac complications with it,s high mortality is reported plus HCC as hepatic complications. Aim of the study to evaluate the level of fatty acid bindnig protein 1 (FABP1), also known as liver FABP as biomarker and screning tool plus US.

Methods: Method: This was a cross sectional study, conducted on 44 Subjects both males and females was DM vesrus non DM obese or non and 44 subjects obese , non obese healthy control who evaluated thorough full history taking, clinical examination, biochemical assessment and abdominal ultrasound,FABP1 was determined using ELISA kits.Data were collected, tabulated, statistically analysed using Statistical Package of Social Science (SPSS) version 22 to obtain **Descriptive data and Analytical data.**

Results: FABP1 was 0.90_+ 0.71ng/ml & 1.4_+1.2 ng/ml in non DM non obese or obese respectively .and in DM non obese FABP1 was0.60_+0.24ng/ml while in DM obese subjects it was 0.85_+0.85 ng/ml and p value =0.01 of statistical significant . NFLD was present with different grades in all groups except in subjects whon non obese 8/22 (36%) and DM non obese 4/22(18%). , p value 0.01

Conclusions: FABP1 is a good biomarker to screening NAFLD especially in obese subjects with diffcult US examination

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

EVALUATING DIAGNOSTIC PATHWAY OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND NONALCOHOLIC STEATOHEPATITIS (NASH) IN AN INTEGRATED U.S. MANAGED CARE HEALTH SYSTEM

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Background and Aims: Liver biopsy, the gold standard for staging NASH/NAFLD, is invasive, painful, costly, prone to variability, and risky. A clear diagnostic pathway using non-invasive alternatives does not exist, resulting in diagnostic odysseys and high costs. This study evaluated the frequency and cost of diagnosis-related procedures in NASH/NAFLD patients in an integrated U.S. managed care health population.

Methods: This descriptive retrospective analysis used Highmark claims between 7/1/16 – 6/30/20 to identify members =>18 years, with =>1 ICD-10 NASH/NAFLD diagnosis code between 7/1/17 – 6/30/18. Index date was the first diagnosis record. Continuous enrollment was required 1 year pre- and 2 years post-index. Mean diagnosis-related medical costs were calculated using allowed amounts across a 1-year post-index period. Procedure utilization was captured annually and reported at 1-year and 2-year post-index.

Results: 28,725 patients met inclusion criteria. 79% were newly diagnosed at index (NDx; N=22,707) and 21% were previously diagnosed with NASH/NAFLD in the 1-year pre-index period (PDx; N=6,018). Mean diagnosis-related costs were substantial and higher for PDx ($1,218 vs $970). Diagnoses attributed to liver fibrosis and cirrhosis were also higher in PDx (20.5% vs 4.7%). In all patients, 1-year post-index procedure utilization was low in liver biopsy and highest in ultrasound (1-2% vs. 28-39%).

Mean Diagnosis-Related Medical Costs in 1-Year Post-Index Period Across Highmark Patients with NASH/NAFLD
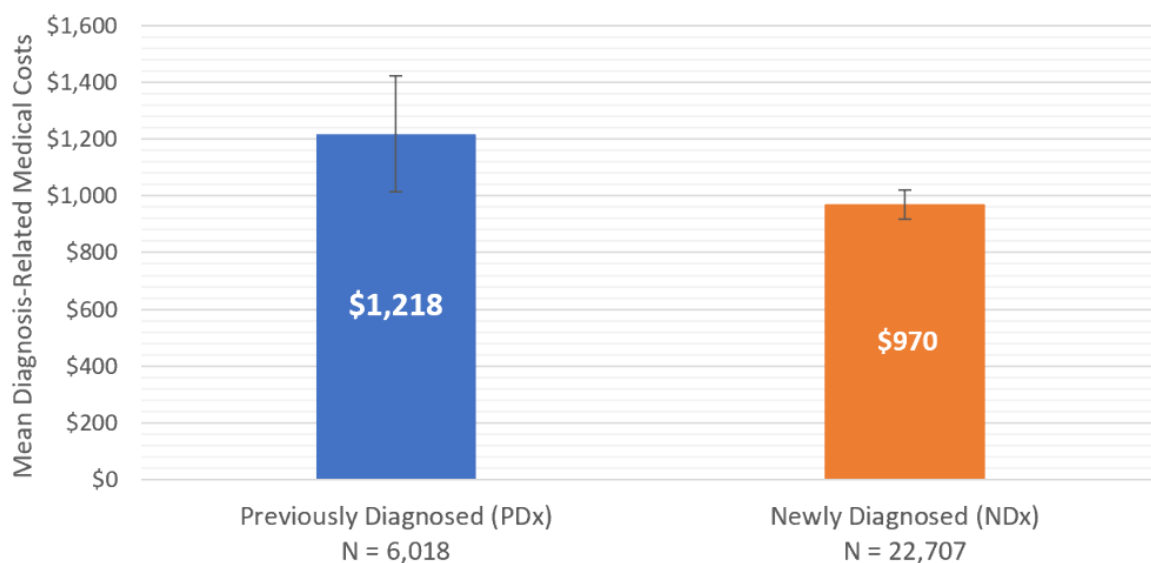
Conclusions: Increased economic burden in previously diagnosed patients could be linked to unclear, invasive, and lengthy diagnostic journeys, leading to uncoordinated care. These results suggest that in real-world settings, where liver biopsies are not used, introduction of accurate, non-invasive diagnostics in a coordinated pathway could lead to cost savings and improved clinical outcomes.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

MARKERS OF SYSTEMIC INFLAMMATION IN A GROUP OF ROMANIAN NON-ALCOHOLIC FATTY LIVER DISEASE PATIENTS

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Background and Aims: This paper evaluates a group of 125 patients with non-alcoholic fatty liver compared with healthy subjects in terms of serological markers of inflammation. Interleukin 6 (IL6), Erithropoetin(EPO), C-reactive protein (CRP) and TNF-alpha are markers of inflammation, involved in processes of metabolic syndrome and nonalcoholic liver disease. We aimed to study if there is any correlation between IL6, TNF, CRP and liver biochemical tests.

Methods: We measured IL6, IL8, TNFα, EPO and PCR in 43 non-alcoholic fatty liver patients and 34 healthy subjects.

Results: In fattty liver patients we found a tight linear correlation between levels of inflammatory markers IL6- TNF, IL6- CRP, TNF-CRP. At the same time we see an acceptable degree of association between TNF levels-waist circumference, CRP-waist circumference, waist circumference - IL6, CRP-GGT, GGT-IL6.There is a significant linear correlation between markers of inflammation and fibrosis Forns index (IL6-Forns - r = 0.47, TNF-Forns - r = 0.32; EPO-Forns - r = 0.25). The highest values of these cytokines were found in patients with moderate obesity (BMI> 30) studies showing that increased expression of TNF and IL 6 is highly significantly correlated with obesity associated insulinresistance.

Conclusions: Close linear correlations between the level of inflammation markers TNF-IL6, IL6, CRP, TNF-CRP confirmed the involvement of these mediators in the pathogenesis of non-alcoholic fatty hepatopathy.The association between levels of TNF-waist , CRP-waist , waist - IL6 confirms the importance of involvement in the promotion of abdominal obesity and metabolic disorders. Increased expression of TNF and IL 6 is highly significantly correlated with obesity associated insulinresistance.

PEDIATRIC METABOLIC INDEX IN THE SCREENING OF FATTY LIVER DISEASE

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Background and Aims: Fatty liver disease is increasingly common in early childhood, however, early diagnosis is limited by lack of non-invasive diagnostic tools that exceed the gold standard of hepatic biopsy. Recent studies have reported that the Pediatric Metabolic Index (PMI) could be a marker of fatty liver disease in children with Prader Willi syndrome, however to date this has not been studied in children without genetic obesity. The objective of this study is to evaluate the correlation between PMI and the presence of non-alcoholic fatty liver disease in school children with and without acquired obesity.

Methods: Two hundred and twenty-three children without evidence of hypothyroidism, and genetic, chronic or metabolic diseases were included . Anthropometry, liver ultrasound as well as lipids serum levels were collected. The PMI was calculated by the formula established for age and gender.

Results: PMI correlated positively with adiposity variables such as BMI, waist and hip circumference, as well as percentage of fat. In addition, PMI showed a strong association with fatty liver disease (r=0.69, p <0.001).

Conclusions: PMI could be a useful tool for the early diagnosis of fatty liver disease in children. however, future studies are necessary in order to establish validated cut-off points.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

FLUORESCENT AGE LEVELS IN ELSA-BRASIL STUDY: A POTENTIAL PLASMATIC BIOMARKER FOR RISK STRATIFICATION OF NAFLD-ASSOCIATED STEATOSIS

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Background and Aims: Liver diseases are associated with the excess formation of advanced glycation end-products (AGEs), which induce tissue inflammation and oxidative damage. However, the trend of oxidative marker levels according to the steatosis grade in NAFLD is unclear. For this purpose, serum AGE levels were compared between participants with NAFLD accordingly to steatosis severity in the baseline ELSA-Brasil population.

Methods: The participants (n=305) were grouped according to the severity of steatosis: mild and moderate/severe pooled, classified by ultrasound hepatic attenuation. The measurement of serum fluorescent AGE concentrations was based on spectrofluorimetric detection. Serum AGE content and clinical and laboratory characteristics of the participants were compared between groups. Logistic regression analysis was used to investigate the relationship between serum AGE levels and steatosis severity.

Results: Higher serum AGE content was present in the moderate/severe group of individuals than in the mild group (p=0.008). In addition, the serum AGE levels were correlated with the steatosis grade in the overall sample (rho=0.146, p=0.010). Logistic regression analysis, after adjusting for confounding variables, showed that subjects with higher serum AGE content had a 4.6-fold increased chance of having moderate or severe steatosis when compared to low levels of serum AGEs.

Conclusions: According to the results of the ROC analyses (AUC=0.83), AGEs could be a potential marker of steatosis severity in patients with NAFLD, strengthening the involvement of AGE in NAFLD pathogenesis. Therefore, plasmatic fluorescent AGE quantification by spectroscopy could be a promising alternative method to monitor progression from mild to severe NAFLD accordingly to steatosis grade.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

THE ACCURACY OF NON-INVASIVE DIAGNOSTICS FOR NAFLD IN PATIENTS WITH TYPE 1 DIABETES

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Background and Aims: The prevalence of NAFLD in type 1 diabetes (T1D) is uncertain, partly due to the lack of cross-validation of diagnostics for steatosis. This study aims to determine the accuracy of non-invasive diagnostics in a T1D cohort.

Methods: Patients underwent ultrasound (ultrasound steatosis score [USS] 0-3), elastography with controlled attenuation parameter (CAP), and magnetic resonance spectroscopy (MRS). 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 was 27.2% (FLI), 29.7% (USS ≥1) and 78.3% (CAP ≥248 dB/m). The AUROC was 0.85 (0.75-0.96) for M-CAP and 0.67 (0.49-0.84) for XL-CAP, while the FLI yielded an AUROC of 0.56 (0.38-0.75). The AUROC for 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 XL-CAP. The USS ≥1 criterion yielded a good sensitivity of 0.91, with a specificity of 0.79. Correlation was strong between the 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, 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.

Conclusions: 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.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

EXENDIN-4 REDUCES STEATOSIS IN HEPG2 CELLS BY DOWNREGULATING FABP1 THROUGH THE ACTIVATION OF THE WNT/B-CATENIN SIGNALING PATHWAY AND DECREASING FOXA1 EXPRESSION

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the leading chronic liver disease worldwide. Agonists of the glucagon-like peptide-1 receptor (GLP-1R), currently approved to treat type 2 diabetes, hold promise to improve steatosis and even steatohepatitis. However, due to their pleiotropic effects, the mechanisms underlying their protective effect on NAFLD remain elusive. We aimed to investigate these mechanisms using an in vitro model of steatosis treated with the GLP-1R agonist Exendin-4 (Ex-4).

Methods: We established steatotic HepG2 cells by incubating HepG2 cells with 400 µM oleic acid (OA) overnight. Further treatment with 200nM Ex-4 for 3 hours significantly reduced the OA-induced lipid accumulation ($p < 0.05$).

Results: Concomitantly, Ex-4 substantially reduced the expression levels of Fatty Acid-Binding Protein 1 (FABP1) and its primary activator, Forkhead box protein A1 (FOXA1). Interestingly, the silencing of β-catenin with siRNA abolished the effect of Ex-4 on these genes, suggesting dependency on the Wnt/β-catenin pathway. Furthermore, after β-catenin silencing, OA treatment significantly increased the expression of nuclear transcription factors SREBP-1 and TCF4, whereas Ex-4 significantly decreased this upregulation.

Conclusions: Our findings suggest that direct activation of GLP-1R by Ex-4 reduces OA-induced steatosis in HepG2 cells by reducing fatty acid uptake via FABP1 downregulation. This effect is achieved by activating the Wnt/b-catenin signaling pathway, which regulates the expression of the transcription factors FOXA1 and TCF4.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: The objective of this study was to assess the possible correlations between the circulating interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) levels and the anthropometric and abdominal fat distribution in patients with non-alcoolic steatohepatitis (NASH) compared with a control group.

Methods: We studied a group of NASH patients hospitalized in o two months period in the Medical Departments, to whom we have analyzed the BMI (body mass index), abdominal waist line, IL-6 and TNF-alpha levels.

Results: We studied 36 patients (16 diagnosed with NASH; 20 were the control group). There was found a correlation between the abdominal waist line and the level of IL6 ($r = 0.4834$) and between the abdominal waist line and the level of TNF alpha ($r = 0.554$), at the patients with NASH. There is a correlation between the BMI and the level of IL6 ($r = 0.3340$) and between the BMI and the TNF alpha level ($r = 0.3904$), at the NASH patients. The glycemia is correlating with IL6 ($r = 0.535$) and TNF alpha level ($r = 0.629$) in these patients. There is an association between the triglycerides level and IL6 and TNF alfa in the NASH group ($r = 0.297$, respectively, $r = 0.237$).

Conclusions: The BMI and the abdominal waist line are correlating with the IL6 and TNF alpha levels in NASH patients. IL 6 and TNF alpha are well correlated with the glicemia and triglycerides levels, at these patient. These findings suggest that the pro-inflammatory cytokines are involved in the pathogenesis of NASH.

Topic: *AS03 Screening & Biomarkers - How Should I Evaluate NASH in My Clinic?*

LOW SCREENING RATES BUT HIGH PREVALENCE OF ADVANCED LIVER FIBROSIS IN PEOPLE WITH DIABETES FROM PRIMARY AND SECONDARY CARE IN THE LIVERPOOL CITY REGION, UK

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Background and Aims: Diabetes is a key driver of MAFLD including liver fibrosis, yet UK guidelines do not advocate widespread screening for this complication in this patient cohort. We aimed to determine prevalence of advanced liver fibrosis and assess current practice in examining fibrosis markers in people with diabetes.

Methods: We extracted HbA_{1c} results ≥48mmol/mol from Liverpool University Hospital's pathology system 31/12/2019-31/07/21. Requests in those <35 years or from inpatient/cancer/dialysis services were excluded leaving primary care and outpatients. We examined the proportion of individuals who had AST, ALT and platelets results requested, allowing calculation of non-invasive fibrosis tests (NIT): the fibrosis-4 (FIB-4) score, AST to platelet ratio index (APRI) and AST:ALT ratio (advanced fibrosis: FIB-4>2.67, or APRI≥1.0, or AST:ALT≥1.0).

Results: Appropriate tests were requested in only 1.3% of people with diabetes (370/27,369). After excluding tests >6 months from the HbA_{1c} result, 338 individuals remained (median age 60 yr, 57.7% male). Hepatic fibrosis was detected by FIB-4 in 18.3% (n=62), APRI in 13.9% (n=47) and AST:ALT in 17.5% (n=59). Where any score was used 28.1% (n=95) of people with diabetes and an NIT result available had evidence of fibrosis. Within this group 28.4% NIT requests originated from primary care, 61.1% from hepatology clinics and 10.5% from outpatient services. Fibroscan data to follow.

Conclusions: Real-world data demonstrates that liver fibrosis is infrequently screened for in people with diabetes despite >25% of patients being at risk. This supports the need for high quality cost-effectiveness analyses into widespread screening for liver fibrosis in people with diabetes.

BONE MORPHOGENETIC PROTEIN 2 IS A NEW MOLECULAR TARGET LINKED TO METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE WITH POTENTIAL VALUE AS NON-INVASIVE SCREENING TOOL.

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Background and Aims: Liver biopsy is the gold standard to establish metabolic dysfunction-associated fatty liver disease (MAFLD) diagnosis but, given its associated risks, the design of accurate non-invasive methods to identify these patients is of upmost importance. Bone morphogenetic protein 2 (BMP2) plays a key role in metabolic homeostasis, however, little is known about its involvement in MAFLD onset and progression. The aim of this study was to elucidate the impact of BMP2 in MAFLD pathophysiology.

Methods: To this end, hepatic and circulating levels of BMP2 was quantified in serum and liver biopsy from 115 biopsy-proven MAFLD patients and 75 subjects with histologically normal liver. In addition, the expression of BMP2 was determined in cultured human hepatocytes upon palmitic acid (PA) overload.

Results: We found that BMP2 expression was abnormally increased in livers from MAFLD patients and, interestingly, this was reflected in higher serum BMP2 levels than in subjects with normal liver. Notably, we observed that PA upregulated the expression and secretion of BMP2 by human hepatocytes. An algorithm based on serum BMP2 levels and variables clinically relevant to MAFLD had an accuracy
of 0.886 (95%CI, 0.83–0.94) to discriminate MASH. We used this algorithm to develop SAM (Screening Algorithm for MASH): a SAM < 0.3 implied a low risk and a SAM ≥ 0.6 indicated high risk of MASH diagnosis.

Conclusions: In conclusion, this proof-of-concept study shows that BMP2 is a new molecular target linked to MAFLD and introduces SAM as a simple and efficient algorithm to screen individuals at risk for MASH.

DE NOVO NON-ALCOHOLIC FATTY LIVER DISEASE AFTER LIVER TRANPLANTATION AS DIAGNOSED BY MAGNETIC RESONANCE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the fastest growing cause of liver diseases; arising after liver transplantation (LT) for another indications bears the name de novo NAFLD. We set out to determine incidence of de novo NAFLD and its associations with BMI and fibrosis in patients (pts) after LT at the single transplant centre.

Methods: We organized an observational study of consecutive pts after LT for non-NAFLD causes between January 2015, and december 2020. At baseline, we recorded demographics, etiology of cirrhosis, MELD, Child-Pugh score; at months 6, 12, 24, we recorded BMI, MR spectroscopy (MRS, [≥5%=NAFLD]) and MR Elastography (MRE, [≥2,88kPa=significant fibrosis, ≥3,54kPa=advanced fibrosis]).

Results: We enrolled 164 pts after LT, excluded 37% for pre-defined criteria, and analyzed 104 pts aged 53 years, 38% women, with median MELD 15 points and BMI 25.4. The median BMI at 6, 12 and 24m were 25.5 vs 27.3, (p=0.032) and 26 vs 27.8, (p=0.062). MRS % at 6, 12, and 24m were 4.5 vs 5.1, (p=0.2) and 4.4 vs 7, (p=0.012). Significant fibrosis at months 6, 12, and 24m was found in 27%, 35%, and 46%, respectively (p=0.09), and advanced fibrosis in 4.7%, 1.2%, and 15%, respectively (p=0.003).

Conclusions: Over the 2 years after LT for various non-NAFLD indications, we identified rising BMI, rising incidence of de-novo NAFLD, and rising incidence of significant and advanced fibrosis.

Topic: *AS05 NAFLD Therapeutics*

METABOLIC, BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF LONG-TERM TREATMENT WITH SEMAGLUTIDE IN THE GAN DIET-INDUCED OBESE AND BIOPSY-CONFIRMED MOUSE MODEL OF FIBROSING NASH

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Background and Aims: The glucagon-like-peptide (GLP)-1 analogue semaglutide, currently approved for the treatment of type 2 diabetes and obesity, is in advanced clinical development for non-alcoholic steatohepatitis (NASH). The present study aimed to evaluate the therapeutic effect of long-term semaglutide treatment in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of fibrosing NASH.

Methods: Male C57BL/6J mice were fed the GAN diet high in fat, fructose, and cholesterol for 39 weeks prior to study start. Only animals with liver biopsy-confirmed steatosis (score ≥2) and fibrosis (stage F2-F3) were included and stratified into treatment groups. DIO-NASH mice received (SC, QD) vehicle (n=17) or semaglutide (30 nmol/kg, n=18) for 16 weeks. Pre-to-post liver biopsy histology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and Fibrosis Stage. Additional endpoints included whole-body composition, glucose tolerance, blood/liver biochemistry, and quantitative liver histology.

Results: Compared to vehicle-dosed DIO-NASH mice, semaglutide induced a marked fat-specific weight loss of approx. 20%, improved glucose tolerance, hepatomegaly, and plasma transaminases as well as plasma/liver lipids and inflammation markers. Semaglutide reduced NAS (≥2 point) without improving fibrosis stage. Semaglutide reduced 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).

Conclusions: Semaglutide improved metabolic, biochemical, and histological hallmarks in GAN DIO-NASH mice. Consistent with clinical findings, semaglutide improved NAS without influencing fibrosis stage, further supporting clinical translatability of the model. Concurrent reductions in quantitative markers of fibrosis illustrate the relevance of long-term treatment intervention for promoting fibrosis regression.

MITOCHONDRIAL HYPERACTIVATION DETERMINES A GUT MICROBIOTA PROFILE WITH A TRANSFERABLE PROTECTIVE EFFECT AGAINST METABOLIC ASSOCIATED FATTY LIVER DISEASE

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Background and Aims: Mitochondrial dysfunction is involved in MAFLD multifactorial pathogenesis. Methylation-Controlled J (MCJ) protein absence is linked with a protective effect on this disease. Our aim was to evaluate gut microbiota involvement in the protective effect of MCJ deficiency in MAFLD development.

Methods: Wild-type (WT) and MCJ knock-out (MCJ-KO) mice were fed with control or choline-deficient, L-amino acid-defined, high-fat diet (CDA-HFD) for 6 weeks. A donor mouse from each group was selected regarding MAFLD-related parameters. Fecal microbiota transplantation was performed to germ-free mice (GFm), which were under same feeding conditions for 3 weeks. Liver disease progression, mitochondrial status and gut microbiota composition were analysed.

Results: CDA-HFD induced an inflammatory and fibrotic status compatible with steatohepatitis development. MCJ-KO mice showed a reduced expression of inflammatory markers and decreased liver fibrosis. Similar effects were observed in CDA-HFD-fed GFm and colonized with microbiota from MCJ-KO genotype donors. Moreover, niconinamide-adenine dinucleotide (NAD) intestinal production and its related synthesis enzymes were incremented in MCJ-KO mice, which augmented their fatty acids oxidation potential comparing to WT mice. Besides, metagenomic analysis showed a specific gut microbiota profile associated to MCJ-KO, increasing *Dorea* and *Oscillospira* and decreasing *AF12, Allobaculum* and *Ruminococcus.* This microbial pattern was transferred to GFm colonized with MCJ-KO microbiota, which was associated with an incremented intestinal and hepatic NAD synthesis.

Conclusions: The protective effect of MCJ deficiency in MAFLD involves a mitochondrial hyperactivity mechanism that is transmissible through gut microbiota.

Topic: *AS05 NAFLD Therapeutics*

REAL WORLD PROSPECTIVE ANALYSIS OF SAROGLITAZAR VS VITAMIN E IN NAFLD PATIENTS

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Background and Aims: This study was done to compare the real world efficacy of Saroglitazar 4mg vs Vitamin E 400 IU in reducing AST, ALT, Serum triglycerides and LSM on Fibroscan in NAFLD patients.

Methods: This is a prospective study in which 50 patients each on Saroglitazar 4mg OD & Vitamin E 400 IU OD with USG evidence of fatty liver were enrolled for a period of 1 year. All Patients were followed up for LFT, Serum triglycerides & LSM, at an interval of 6 months and 1 year. Statistical analysis was done using paired sample T-test.

Results: At the end of 1 year, a significant reduction of 21.70% in LSM was seen in patients with Saroglitazar 4 mg **(Table 1)** while only 5.05 % in LSM was seen in patients with Vitamin E. The Liver enzymes AST & ALT have shown significant improvement in patients with Saroglitazar compared to Vitamin E (**Table 2**). **Table 1. Saroglitazar changes in parameters, N=50**

Parameters	Baseline	6 months	1 year	% reduction 1 year	P-Value
ALT(IU/L)	54.76±26.31	34.94±16.99	32.27±12.84	-41.08%	<0.001
AST(IU/L)	46.72±22.47	32.76±14.89	29.20±10.82	-37.50%	<0.001
TG(mg/dL)	194.54±57.24	133.40±56.23	133.06±37.59	-31.60%	<0.001
LSM(kPa)	12.24±10.17		9.58±4.62	-21.70%	<0.05

Table 2. Vitamin E changes in parameters, N=50

Parameters	Baseline	6 months	1year	% reduction 1 year	P-Value
AST(IU/L)	32.88±14.77	26.36±10.67	25.18±8.35	-23.42%	<0.001
ALT(IU/L)	42.64±17.10	36.18±14.06	32.92±11.35	-22.80%	<0.001
TG(mg/dL)	178.50±67.10		170.92±55.05	-4.25%	<0.05
LSM(kPa)	10.29±6.78		9.77±6.54	-5.05%	<0.001

Conclusions: Saroglitazar 4mg OD significantly improved LSM, Serum triglycerides, AST and ALT compared to Vitamin E.

Topic: *AS05 NAFLD Therapeutics*

METABOLIC, BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF SEMAGLUTIDE IN A DIET-INDUCED NON-OBESE AND BIOPSY-CONFIRMED RAT MODEL OF NASH WITH PROGRESSIVE FIBROSIS

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Background and Aims: The glucagon-like-peptide (GLP)-1 analogue semaglutide, currently approved for treatment of type 2 diabetes and obesity, is in advanced clinical development for non-alcoholic steatohepatitis (NASH). The present study evaluated the therapeutic effects of semaglutide in a diet-induced non-obese rat model of NASH with progressive fibrosis.

Methods: Male Sprague-Dawley rats were fed a choline-deficient L-amino acid-defined high-fat diet (CDAA-HFD) for 5 weeks prior to study start. Only animals with liver biopsy-confirmed fibrosis (grade 1-2) were included and stratified into treatment groups. CDAA-HFD rats received (SC) vehicle (n=15) or semaglutide (30 nmol/kg/day, n=15) for 8 weeks. Pre-to-post liver biopsy histology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and Ishak Fibrosis Score. Other terminal endpoints included plasma/liver biomarkers and quantitative liver histology.

Results: Compared to baseline, CDAA-HFD control rats demonstrated ≥3-point worsening of fibrosis score resulting in advanced bridging fibrosis/cirrhosis (grade 4-6) and macroscopic tumor development. Semaglutide induced minor weight loss (5%) with improvements in hepatomegaly and plasma lipids. Interestingly, semaglutide did not exert anti-steatotic action nor improved NAS, albeit semaglutide prevented progression to advanced bridging fibrosis/cirrhosis in 9/15 (60%) of CDAA-HFD rats concurrent with significant reductions in quantitative histological markers of inflammation (galectin-3), fibrogenesis (α-SMA) and fibrosis (PSR, collagen 1a1). Additionally, semaglutide significantly reduced numbers and largest tumor size in CDAA-HFD rats.

Conclusions: Semaglutide promoted weight-independent and non-steatotic improvements in inflammation, fibrosis and tumor burden in CDAA-HFD rats. Collectively, the biopsy-confirmed CDAA-HFD rat model of non-obese NASH is highly applicable for probing therapeutic drug effects focusing on advanced fibrotic progression including tumor development.

Topic: *AS05 NAFLD Therapeutics*

ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTI-FIBROTIC PROPERTIES OF TRITERPENIC ACID AND ACTEOSIDE IN IN VITRO MODELS OF NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: There is no consensus regarding effective pharmacological treatment for NASH. Triterpenic acid (TA) and Acteoside (AC) have been reported to exert hepatoprotective effects in animal models but very little data regarding their molecular mechanisms are available. We assessed the effects of both compounds in our well-established *in vitro* models of steatosis and early-stage NASH.

Methods: Human hepatocytes (HuH7) and hepatic stellate cells (LX2) were exposed to free fatty acid (FFA) alone or in combination with Triterpenic Acid and Acteoside as mono- or co-culture. Steatosis, inflammation, fibrogenesis, generation of reactive oxygen species (ROS), and collagen deposition were determined using standard assays.

Results: Treatment of both compounds alone or with FFA did not alter the intracellular fat content in mono- or co-culture models. Co-treatment with TA+FFA exerted an apparent but not statistically relevant antioxidant effect. Interestingly, AC+FFA showed a significant antioxidant effect at all the concentrations assessed. TA (10nM and 50nM) resulted in a non-significant reduction in IL-6 and IL-8 expressions but a significant downregulation of TNF-alpha. Likewise, AC reduced the expression of all the cytokines, with significant downregulation in TNF-alpha at 0.1µM and 10µM. In the co-culture model, both compounds decreased COL1A1 gene expression and extracellular collagen deposition. The compounds alone did not induce any effects in the extent of steatosis, inflammation, ROS generation, and collagen deposition.

Conclusions: Either Triterpenic Acid or Acteoside reduces the FFA-related inflammation, ROS generation, and collagen deposition. These *in vitro* data suggest that these compounds deserve further investigation for possible use in NASH treatment.

Topic: *AS05 NAFLD Therapeutics*

ORAL DELIVERY OF EXTRACELLULAR RNAS THROUGH BOVINE-MILK DERIVED EXOSOMES TO TARGET THE LIVER AND OTHER TISSUES

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Background and Aims: Extracellular RNAs (exRNAs) are unstable and rapidly degraded, reducing the possibility of successfully exerting a biological function in distant target cells, unless protected. exRNAs can be protected by different tools for therapeutic usage. However, the development of efficient, tissue-specific and nonimmunogenic delivery methods are scarce. Since exRNAs can be naturally transported within exosomes, a type of extracellular vesicle (EVs), that may increases miRNA stability, they could be ideal delivery vehicles for miRNA-based therapy. We aimed to evaluate the use of milk-derived EVs as vehicles for exRNA drug delivery to the liver and other tissues.

Methods: For that, exosomes were isolated from raw bovine milk, combining ultracentrifugation and size exclusion chromatography, loaded with different exRNAs, and test their gastrointestinal (GI) stability in vitro. Transfected exosomes or their control were administered by oral gavage to C57Bl/6J mice and sacrifice at 1 and 3 hours. The miRNAs incorporated into exosomes were analysed in liver and other tissues by qRT-PCR.

Results: Digestive in vitro stability analysis showed high overall degradation of exogenous miRNAs, although EV-protected miRNAs better resisted GI digestion compared to free miRNAs. Orally delivered EV-loaded miRNAs reached host organs, including the liver, with potential to exert a biological activity.

Conclusions: Overall, our data suggest that bovine milk exosomes can be used as nanocarriers of functional exRNAs to target the liver (and other tissues) with potential in RNA-based therapy.

THE EFFECTS OF THE SPIRULINA ON NONALCOHOLIC FATTY LIVER DISEASE CHARACTERISTICS AND ON INTESTINAL MICROBIOTA IN WISTAR RATS

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Background and Aims: Introduction: The main objective of this study was to determine the effect of a diet, enriched in fructose and spirulina for 16 weeks, on the induction of Nonalcoholic fatty liver disease (NAFLD) and its characteristics as well as the modification of the intestinal microbiota (IM) in male Wistar rats.

Methods: Forty Wistar healthy male rats were randomly divided into four groups of 10 rats and received diets of equal quantities (20g/day/rat). The first group received a control diet, the second group received a 40% fructose enriched diet, the third and the fourth groups were assigned the same diet composition as the second group but enriched with spirulina at 5% and 10% respectively.

Results: At week 16, the fourth group had a decrease in steatosis percentage, in triglyceride and TNF-α blood levels ($P < 0.05$). The energy intake/rat/week was also significantly reduced as compared to other groups ($P < 0.05$). At the phylae level, a significant increase in the average percentages of *Firmicutes* and a decrease in *Bacteroidetes* were shown in the second group ($p < 0.05$). At the family level, the 2 groups enriched in spirulina showed a significant increase in the average percentages of *Bacteroidaceae, Lactobacillaceae, Prevotellaceae* and *Fibrobacteraceae* ($p < 0.05$).

Conclusions: The results suggest that spirulina, at 10% concentration, could be considered to increase satiety, reduce steatosis percentage, and ameliorate lipid and inflammatory parameters. The diets enriched in 5% and 10% spirulina showed a significant improvement in the composition of IM through increasing its diversity and bacterial richness.

Topic: *AS05 NAFLD Therapeutics*

MULTICENTER EXTERNAL VALIDATION OF FIB-6; A NOVEL, MACHINE-LEARNING, SIMPLE BEDSIDE SCORE TO RULE OUT SEVERE LIVER FIBROSIS AND CIRRHOSIS IN PATIENTS WITH MAFLD.

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Background and Aims: (NITs) are urgently required to evaluate hepatic fibrosis in MAFLD. We previously developed and validated a non-invasive diagnostic index based on routine laboratory parameters for predicting the stage of hepatic fibrosis in patients with chronic hepatitis C (HCV) called FIB-6 through machine learning with random forests algorithm using retrospective data of 7238 biopsy proven chronic hepatitis C (CHC) patients. Our aim is to validate this novel score in patients with MAFLD.

Methods: Performance of the new score was externally validated in cohorts from one site in Egypt (n=674) and in 5 different countries (n=1798) in Iran, KSA, Greece, Turkey and Oman). Results were compared with METAVIR scoring system and FIB-4, APRI, and AAR.

Results: Using the optimal cutoffs of FIB-6 showed sensitivity = 70.5%, specificity = 62.9%. PPV = 15.0% and NPV = 95.8% for diagnosis of cirrhosis. For diagnosis of severe fibrosis (F3 and F4), the results were 86.5%, 24.0%, 15.1% and 91.9% respectively, while for diagnosis of significant fibrosis (F2, F3 and F4), the results were 87.0%, 16.4%, 24.8% and 80.0%). Regarding ruling out severe fibrosis and cirrhosis, FIB-6 has the highest sensitivity and NPV (97.0% and 94.7%), as compared to FIB-4 (71.6% and 94.7%), APRI (36.4% and 90.7%), and AAR (61.2% and 90.9%).

Diagnostic performance of FIB-6 optimal cutoffs in all cohorts compared to the liver biopsy results

	Cutoff	Sensitivity	Specificity	PPV	NPV
Cirrhosis (F4 vs. F0123)	2.3159	70.5 (64.0-76.2)	62.9 (60.9-64.9)	15.0 (12.9-17.4)	95.8 (94.7-96.7)
Severe fibrosis (F34 vs. F012)	1.8992	86.5 (82.5-89.8)	24.0 (23.3-25.9)	15.1 (13.6-16.8)	91.9 (89.4-93.9)
Significant fibrosis (F234 vs. F01)	1.7720	87.0 (84.1-89.5)	16.4 (14.8-18.1)	24.8 (23.0-26.7)	80.0 (75.7-83.7)

Diagnostic performance of FIB-6 rule-out cutoffs for severe fibrosis (F3) or cirrhosis (F4) in all cohorts compared with that of FIB-4, APRI, and AAR

	Cutoff	Sensitivity	NPV
FIB-6	1.5033	97.0 (94.6-98.4)	94.7 (90.4-97.1)
FIB-4	1.45	71.6 (66.6-76.2)	94.7 (93.6-95.7)
APRI	0.70	36.4 (31.5-41.7)	90.7 (89.5-91.8)
AAR	1.00	61.2 (55.9-66.3)	90.9 (89.3-92.3)

Conclusions: FIB-6 score is an accurate, simple, noninvasive test for ruling out advanced fibrosis and liver cirrhosis in patients with MAFLD better than APRI, FIB-4 and AAR.

Topic: *AS05 NAFLD Therapeutics*

WEIGHT LOSS WITH GLUCAGON-LIKE PEPTIDE 1-RECEPTOR AGONISTS IN A REAL-WORLD SETTING

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Background and Aims: Glucagon-like Peptide-1 Receptor Agonists (GLP-1RA) are powerful compounds that are capable of reducing body weight through multiple mechanisms. This has made them an intriguing therapeutic option for patients with non-alcoholic fatty liver disease (NAFLD) where a weight loss of ≥7% of total body weight has been shown to improve histological disease activity. We aim to characterise the weight loss seen in patients with type 2 diabetes in a specialized outpatient clinic.

Methods: Retrospective data acquisition of patients from our department, who were prescribed a GLP-1-RA (Dulaglutide, Exenatide QW, Liraglutide) between January of 2014 and September of 2020. Inclusion of patients with multiple GLP1-RA was possible, if there was a treatment pause of at least 90 days between therapies.

Results: 589 datasets from different patients were found. For 262 one-year follow-up data was available. Mean age was 60.6 years (SD ±11,5), 44.1% were female. Mean weight loss after initiation of GLP-1RA was 4.9% (SD ±5.9) after 12 months. 34% of patients with treatment persistence achieved weight reduction of ≥7% across all treatment groups. Neither weight trajectory nor glycaemic potency were significantly different between the GLP-1RA (p=0.68 and 0.27, respectively). 38% of patients discontinued GLP1-RA therapy during the observational period.

Conclusions: In a real-world cohort of patients with type 2 diabetes initiation of GLP-1RA treatment led to weight loss of ≥7% in 34%. No significant differences were noted between compounds. Additional pharmacological strategies are needed to prevent NAFLD complications more effectively.

Topic: *AS05 NAFLD Therapeutics*

SIMVASTATIN IMPROVES MICROCIRCULATORY FUNCTION IN NONALCOHOLIC FATTY LIVER DISEASE TROUGH DOWNREGULATION OF OXIDATIVE AND AGE-RAGE STRESS.

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Background and Aims: NAFLD encompasses a broad spectrum of liver disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Vascular dysfunction may play a fundamental role in the progression of NAFLD, as the extent of steatosis is associated with hepatic microcirculatory dysfunction, leading to tissue damage by increased oxidative stress and inflammation. Therefore, strategies targeting vascular homeostasis in NAFLD would be of great clinical importance. We investigated the mechanisms by which simvastatin (SV) exerts its protective effects on hepatic and adipose tissue microcirculation, assessing oxidative stress parameters, including the AGE-RAGE activation.

Methods: NAFLD was established by a high-fat/high-carbohydrate diet (HFHC) for 13 weeks. SV was administered orally between weeks 6 and 13. Leukocyte recruitment was assessed by intravital microscopy, and microcirculation perfusion was assessed by Laser Speckle Contrast Imaging flowmetry.

Results: HFHC exhibited metabolic changes indicative of NASH, whereas treatment with SV protected mice from developing NAFLD. SV prevented microcirculatory dysfunction in HFHC-fed mice, as evidenced by decreased numbers of rolling and adherent leukocytes in the hepatic and fat microcirculation, decreased activation of hepatic stellate cells (HSCs), and improved architecture and density of the hepatic capillary network. Additionally, SV restored hepatic and adipose tissue basal microvascular blood flow. Impairment of the endothelium-dependent vasodilatory response of adipose tissue to acetylcholine was restored by SV. Similarly, treatment with SV decreased lipid peroxidation in liver and adipose tissue and activation of the AGE -RAGE pathway.

Conclusions: Our data suggest that SV improves microcirculatory function in NAFLD trough downregulation of oxidative and AGE-RAGE stress.

Topic: *AS05 NAFLD Therapeutics*

GENE EXPRESSION ANALYSIS REVEALS A POTENT SUPPRESSION OF LIPOGENESIS AS THE MAIN EFFECT OF BERGAMOT FLAVONOIDS ON FATTY LIVER DISEASE.

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Background and Aims: To date, there are no specific drugs approved for non-alcoholic fatty liver disease (NAFLD), but natural products may fill this gap. We have recently showed that Bergamot Polyphenol Fraction (BPF) prevents NAFLD and stimulates autophagy in rat livers. The aim of this work was to investigate the mechanisms of polyphenol-dependent effects.

Methods: We performed RT2-PCR array analysis on 174 energy metabolism and autophagy genes expressed in rat livers exposed for 14 weeks to different diets: standard, cafeteria (CAF) and CAF diet supplemented with 50 mg/kg BPF.

Results: CAF diet caused a strong upregulation of gluconeogenesis pathway (Gck, Pck) and a moderate (around 2-fold) induction of genes regulating lipogenesis (Srebf1, Pparg, Xbp1), lipid and cholesterol transport or lipolysis (Fabp3, Apoa1, Lpl) and inflammation (Il6), but only few autophagy genes were modulated in the CAF group with respect to control animals. While most of these transcripts were significantly downregulated by BPF treatment, we observed a particularly potent effect on lipid synthesis genes, such as Fasn, Acly, Acac and Scd1 that were suppressed far below the mRNA levels of control livers. Such a strong suppression of lipogenesis enzymes was confirmed at the protein level. These effects were accompanied by a moderate transcriptional suppression of pro-inflammatory cytokines (Il6, Tnfa and Il10) and diabetes-related genes and almost no changes in autophagy-related genes.

Conclusions: These data clearly show that chronic BPF supplementation prevents NAFLD and diabetes by modulating energy metabolism and inflammation gene expression programs, with little transcriptional effects on autophagy, but profound transcriptional suppression of de-novo lipogenesis.

Topic: *AS05 NAFLD Therapeutics*

IDENTIFICATION OF A NOVEL BISINDOLYLMALEIMIDE DERIVATIVE WITH POTENT PKCBETA INHIBITION THAN RUBOXISTAURIN, WITH IMPLICATIONS FOR OBESITY

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Background and Aims: Caloric excess and sedentary lifestyle have led to a global epidemic of obesity and fatty liver disease. Various treatment modalities have met with limited success. Hence identification of novel targets that can potentially be employed to prevent obesity remains of great interest. We previously reported that global inactivation of PKCβ in mice has beneficial effects on metabolism and protects from diet-induced adiposity, hepatic steatosis, and insulin-resistance (Hepatology 49:1525-1536, 2009). We also recently reported that hepatocyte-specific PKCβ deficiency recapitulates many of the beneficial effects (Molecular Metabolism 44:e101133, 2021; JCI Insight 6:e149023, 2021). PKCβ selective antagonist could offer a new approach for intervention in the development of obesity and associated fatty liver disease.

Methods: A systemic molecular and pharmacological evaluation of already known and newly synthesized bisindolylmaleimide derivatives was carried out using PKCβ activity assay.

Results: A novel PKCβ inhibitor, named INST3399, was found to inhibit PKCβ activity more potently than ruboxistaurin. INST3399 acts by a mechanism different from ruboxistaurin and offers advantage over ruboxistaurin for in vivo PKCβ inhibition. Targeting of PKCβ outside of the conserved ATP site may be responsible for greater potency. Considering that PKCβ depletion displayed many of the features one would expect in an ideal antiobesity drug, we tested INST3399 for prevention of obesity. As expected, this inhibitor treatment prevented diet-induced obesity and hepatic steatosis.

Conclusions: We propose that this novel PKCβ inhibitor is a promising tool to test therapeutic potential of systemic PKCβ inhibition to treat metabolic conditions with excessive PKCβ activity, including obesity and fatty liver disease.

BIOMARKERS, IMAGING AND SAFETY IN RESMETIROM 52 WEEK NON-CIRRHOTIC NASH PHASE 3 CLINICAL TRIAL, COMPLETED OPEN-LABEL ARM OF MAESTRO-NAFLD-1
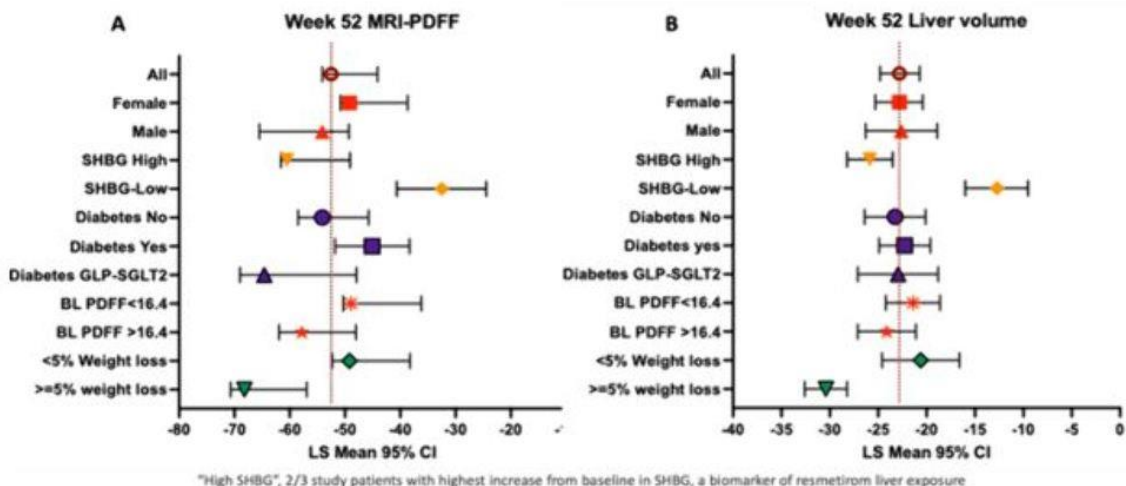
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Background and Aims: Background: MAESTRO-NASH (NCT03900429) and MAESTRO-NAFLD-1 (NCT04197479) are 52-week Phase 3 double-blind placebo controlled clinical trials to study resmetirom, a selective thyroid receptor beta (THR-β) agonist in NASH patients. A goal of MAESTRO-NAFLD-1, a 1200 patient "real life" NASH study is to identify non-invasive markers that correlate with response to resmetirom. The 171 patient 100mg open label arm completed the 52-week study July 2021.

Methods: Methods: Eligibility required at least 3 metabolic risk factors, transient elastography (TE) consistent with ≥F1, and MRI-PDFF ≥8%. The primary and key secondary endpoints of MAESTRO-NAFLD-1 include safety, relative percent reduction of MRI-PDFF (week 16) and LDL-C, Apolipoprotein-B, triglycerides (week 24).

Results: Results: Statistically significant (p<0.0001) reduction of MRI-PDFF -53% (3.3 (SE)) at week 52. Resmetirom reduced liver volume (LV) by -21% (1.0), -23% (1.0) respectively, at weeks 16 and 52 (p<0.0001). LV-corrected mean MRI-PDFF reduction at Week 52 was -61% (2.4). At week 52, MRE (-0.34, p=0.03); CAP (-39 (4.6)) and TE (-1.87, p<0.0001) were reduced relative to baseline. LDL-C (-21% (1.9)), apolipoprotein-B (-22% (1.6)) and triglycerides (-22% (2.6)) were statistically significantly reduced (p<0.0001). No safety flags identified; BP (systolic, diastolic) was reduced by ~2-4 mmHg, bone mineral density (DEXA) was unchanged.

"High SHBG", 2/3 study patients with highest increase from baseline in SHBG, a biomarker of resmetirom liver exposure

Conclusions: Conclusion: Noninvasively identified patients with NASH treated with 100mg per day of resmetirom for up to 52 weeks demonstrated rapid and sustained reduction in 1-hepatic fat and LV; 2-fibrosis (MRE and TE); 3) LDL and atherogenic lipids; 4-liver enzymes and inflammatory biomarkers, supporting the use of non-invasive tests to monitor NASH patient response to resmetirom.

LIVER VOLUME REDUCTION IN RESMETIROM TREATED NON-CIRRHOTIC AND CIRRHOTIC NASH PATIENTS

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Background and Aims: Background: Hepatomegaly may cause symptoms in patients with NASH, possibly driven primarily by high liver fat content. Resmetirom is a liver-directed, highly selective thyroid hormone receptor beta (THR-β) agonist in Phase 3 development for treatment of NASH with significant fibrosis. The purpose of this study was to assess the relationship between liver triglycerides (MRI-PDFF) and liver volume (LV) in placebo and resmetirom-treated patients.

Methods: Methods: Relationship between MRI-PDFF and LV was assessed in MGL-3196-05 (NCT02912260)(n=116), and a NASH-cirrhotic resmetirom-treatment arm of MAESTRO-NAFLD-1 (NCT04197479)(n=105).

Results: Results: LV at baseline was elevated in non-cirrhotic and cirrhotic patients with NASH, relative to literature values for healthy controls. Reduction in LV correlated with reduction in PDFF in placebo (r^2=0.25, p=0.001) and resmetirom (r^2=0.38, p<0.0001) treated patients at 12 and 36 weeks. LV reduction was greater in resmetirom -18.6% (1.1), -20.5% (1.2) compared to placebo -0.4% (1.5), 0.1% (1.9) treated at 12 and 36 weeks, respectively (p<0.0001). In cirrhotic NASH patients treated with resmetirom, LV reduction was much greater than expected based on the small reduction in PDFF. Resmetirom-treated patients who had NASH resolution and/or fibrosis reduction on biopsy at week 36 all had an MRI-PDFF reduction ≥30% and/or LV reduction of ≥15% at week 12.
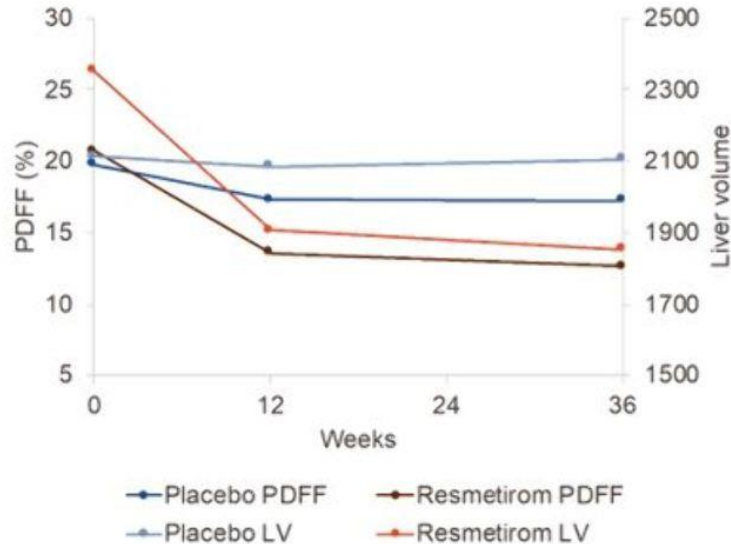
Figure 1, Liver volume (LV) and MRI-PDFF (PDFF) Time course in MGL-3196-05 Phase 2 NASH Study

Conclusions: Conclusion: Reduction in LV in resmetirom-treated patients may be explained in part by reduction in liver triglycerides, but also likely driven by other changes related to its mechanism of action. LV reduction may be associated with histopathologic improvement of NASH and will be further assessed in the Phase 3 MAESTRO-NASH study.

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN 6 IS INVOLVED IN LIPID METABOLISM IN LIVER AND ADIPOSE TISSUE

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Background and Aims: PCSK6 is a protease strongly enriched in human liver however its function in liver has not been fully explored. Here, we aim to investigate the role of PCSK6 in lipid metabolism, and particularly in the context of NAFLD.

Methods: We used biobanks to investigate the expression of PCSK6 in healthy and diseased tissues. In addition, we used Pcsk6$^{-/-}$ mice as well as liver specific knockout mice to investigate the effect of PCSK6 ablation.

Results: Genetic analyses of the PCSK6 locus identified a variant rs7181043 that was significantly associated with PCSK6 mRNA expression in healthy human adipose tissue, liver and in atherosclerotic plaques. The same variant was associated specifically with plaque fat content and atherosclerotic patient's plasma LDL levels. In addition, PCSK6 mRNA expression in plaques was positively correlated with total plasma cholesterol and LDL levels in atherosclerotic patients. Further analyses using public scRNAseq data of healthy human livers, revealed that PCSK6 is expressed in hepatocytes and stellate cells. Microarray comparison of the livers from Pcsk6$^{-/-}$ mice and wild-type controls showed that VLDL particle assembly was one of the upregulated processes. *In vivo* studies showed that Pcsk6$^{-/-}$ mice have higher plasma cholesterol and LPL levels at baseline compared to controls, and lower levels of LDLR in their liver. These findings were further confirmed in our liver specific knockouts. In addition, preliminary studies show that liver specific knockout mice develop increased liver steatosis and fibrosis on a modified western diet.

Conclusions: Our data suggests that PCSK6 is involved in cholesterol and metabolic control.

Topic: *AS05 NAFLD Therapeutics*

THE EFFECT OF TELMISARTAN ON NASH IN NUTRITIONAL MOUSE MODEL

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Background and Aims: The aim of this project was to investigate the effect of telmisartan on NASH in a nutritional mouse model. We focused mainly on changes in mitochondrial respiration of the liver.

Methods: NASH was induced in male C57Bl/6J mice fed *ad libitum* standard control diet (PicoLab RD 20) and tap water or western diet (WD, AIN-76A) and glucose-fructose syrup for 30 weeks. After NASH induction, mice were fed the above diets for an additional 6 weeks with telmisartan (oral gavage, 5 mg/kg b.w./ day). Plasma liver enzymes, protein and mRNA expressions, and histological changes of liver tissue (hematoxylin-eosin, Sirius red) were evaluated. Mitochondrial respiration of liver tissue homogenates was assessed by high-resolution respirometry (OROBOROS Oxygraph 2k).

Results: Induction of NASH was confirmed by liver histology (steatosis, lobular inflammation, fibrosis and NAS score), hepatic triglyceride content and increased plasma ALT and AST activities. Total capacity of respiration (OXPHOS capacity and ETS capacity) and succinate-activated respiration were reduced in liver homogenate of WD-fed mice. Telmisartan reduced absolute and relative liver weight ($p<0.001$), visceral adipose tissue weight ($p<0.001$) and hepatic cholesterol content ($p<0.001$) in WD fed mice. ALT and AST activities were significantly lowered by telmisartn ($p<0.001$), and steatosis grade and NAS score were also reduced in telmisartan-treated animals ($p<0.05$). Moreover, telmisartan reversed decreased total respiration in OXPHOS and ETS states, and succinate-dependent respiration ($p<0.05$).

Conclusions: Telmisartan appears to be effective in reversing the progression of NASH, at least in a mouse nutritional model of the disease.

Topic: *AS05 NAFLD Therapeutics*

METABOLIC, BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF COMBINATIONAL TREATMENT WITH SEMAGLUTIDE AND LANIFIBRANOR IN THE GAN DIET-INDUCED OBESE AND BIOPSY-CONFIRMED MOUSE MODEL OF NASH

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Background and Aims: Semaglutide (SEMA), a glucagon-like-peptide (GLP)-1 receptor agonist, and lanifibranor (LANI), a pan-peroxisome proliferator-activated receptor (PPAR) agonist, have both showed promising therapeutic efficacy in recent phase 2 clinical trials for NASH. The present study aimed to evaluate the effects of SEMA and LANI combination treatment using low doses, as compared to maximal dose monotherapy in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH with hepatic fibrosis.

Methods: Male C57BL/6J mice were fed the GAN diet for 34 weeks prior to study start. Only animals with liver biopsy-confirmed NAFLD Activity Score (NAS) (score ≥5) and fibrosis (stage ≥F1) were included and stratified into treatment groups. DIO-NASH mice received vehicle, SEMA (30 nmol/kg, SC), LANI (30 mg/kg, PO) or SEMA (10 nmol/kg, SC) + LANI (10 mg/kg, PO) for 8 weeks (n=13-14 per group). Pre-to-post liver biopsy histology was performed for within-subject evaluation of NAS and fibrosis stage. Other terminal endpoints included blood/liver biochemistry, quantitative liver histology and transcriptome profile.

Results: SEMA+LANI promoted greater weight loss (-32%) compared to individual monotherapies (-26%; -24%). SEMA and SEMA+LANI reduced hepatomegaly and SEMA+LANI reduced plasma lipids, transaminases, NAS (≥2-point improvement) and histological markers of steatosis and inflammation to a similar extent as SEMA and LANI alone. Furthermore, hepatic gene expression signatures for SEMA+LANI demonstrated improved lipid handling, lowered pro-inflammatory activity and reduced fibrogenesis as observed for monotherapy.

Conclusions: Combined low-dose SEMA and LANI treatment demonstrated similar therapeutic effects on metabolic, biochemical, histological and transcriptomic profile as compared to high-dose monotherapy in biopsy-confirmed GAN DIO-NASH mice.

Topic: *AS05 NAFLD Therapeutics*

SEMAGLUTIDE IMPROVES CARDIOMETABOLIC PARAMETERS IN DIET-INDUCED OBESE NASH HAMSTERS

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Background and Aims: Cardiovascular disease is the leading cause of deaths in nonalcoholic steatohepatitis (NASH) patients. Mouse models, while widely used for drug development, do not fully replicate human NASH nor integrate the associated cardiac dysfunction, i.e. heart failure with preserved ejection fraction (HFpEF). To overcome these limitations, we established a nutritional hamster model developing obesity, NASH and HFpEF. Here we evaluated the effects of the GLP-1 receptor agonist semaglutide (SEMA).

Methods: Hamsters were fed with a free choice diet, which presents hamsters with a choice between control chow (CC) or high fat/cholesterol (HFC) diet, and normal water (NW) or 10% fructose water (FW). After 20 weeks of diet, obese hamsters were treated s.c. QD for 5 weeks with vehicle or SEMA.

Results: Compared with vehicle, SEMA induced a lower HFC/FW and higher CC/NW intake, leading to a 17% body weight loss ($p<0.01$) and a 48% lower visceral fat mass ($p<0.001$). SEMA significantly reduced fasting glycemia, hyperinsulinemia and HOMA-IR index (-77%, $p<0.0001$). SEMA decreased plasma total cholesterol levels (-24%, $p<0.001$) and hypertriglyceridemia (-50%, $p<0.001$). Although SEMA did not improve NAFLD activity scoring and fibrosis score significantly, significant improvement in liver steatosis was observed with lower liver weight (-28%, $p<0.0001$ vs. vehicle) and liver triglycerides levels (-25%, $p<0.01$). SEMA showed substantial benefits on HFpEF with significantly improved E/A, E'/A' and E/E' ratios measured by echocardiography.

Conclusions: SEMA improves cardiometabolic parameters in the obese hamster. This preclinical model will be useful for validating novel drugs or combination therapies for the treatment of NASH and associated HFpEF.

Topic: *AS05 NAFLD Therapeutics*

INDOLE-3-CARBINOL DERIVATIVE 3,3'-DIINDOLYLMETHANE PROTECTS AML12 CELLS AGAINST TGFB1-INDUCED LIVER INJURY BY REGULATING APOPTOSIS AND NRF2/HO-1 PATHWAY

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Background and Aims: 3,3'-Diindolylmethane (DIM), a metabolic compound of Indole-3-carbinol extracted from cruciferous vegetables that exhibits anti-inflammatory and anti-tumor properties. In the past, DIM has also been shown to have anti-fibrotic and anti-inflammatory properties moreover, its protective effects on liver injury has not been clearly elucidated. In this study, we postulated the effects protective effects and molecular mechanisms of DIM on TGFβ1-induced liver injury in mouse hepatocytes.

Methods: Mouse hepatocytes (AML12) were treated with DIM (10,20,40 and 60 μM) with or without TGFβ1 in time and dose dependent manner. The cytotoxicity of DIM was measured by MTT assay. Epithelial-mesenchymal transition (EMT) and apoptotic related proteins, Oxidative stress and inflammatory marker were detected by western blot. TGFβ1 induced reactive oxygen species (ROS) levels was determined by dihydroethidium (DHE) staining. The loss in mitochondrial dysfunction of AML12 cell was observed with tetramethylrhodamine ethyl ester.

Results: DIM treatment significantly reduced TGFβ1-induced cytotoxicity of AML12 cell in dose and time dependent order, restored the mitochondrial membrane potential, reduced ROS levels, inhibits apoptosis and inflammation.

Conclusions: This results concluded that DIM possess a beneficial role in the prevention of hepatocyte injury by attenuating fibrosis, oxidative stress, apoptosis and inflammatory cytokines production through the regulation of Nrf2/HO-1 pathway.

CHOLESTEROL: A REGULATOR OF APOPTOSIS AND FIBROSIS IN HEPATIC STELLATE CELLS, AT THE COURSE OF FATTY LIVER DISEASE PROGRESSION

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Background and Aims: A key target in liver fibrogenesis is the Hepatic Stellate Cells (HSC) at the occurrence of liver injury, HSCs become activated and change to fibroblast-like phenotype cells, which are eventually responsible for producing the ECM (extracellular matrix) and making the liver tissue fibrotic. Our study aimed to investigate NAFLD's disease trajectory following exposure to dietary cholesterol and bile acids.

Methods: Mice (N = 40) were assigned to 4 groups and were fed with one of the following diets: Control (standard AIN-93G diet), CHOL group (standard AIN-93G diet + cholesterol 1 %), CA group (standard AIN-93G diet + cholic acid 0.5 %), CHOL+ CA group (standard AIN-93G diet + cholesterol 1 % + cholic acid 0.5 %, atherogenic diet).

Results: cholic acid supplementation caused a reduction in mice weight gain (CA and CHOL+CA). However, cholesterol addition to cholic acid promoted severe hepatomegaly, elevated total serum cholesterol, FC content in the liver, and significantly decreased serum triglycerides. Cholic acid supplementation (CA) caused significant liver damage, while cholesterol addition (CHOL+CA) attenuated liver damage parameters and fibrosis. Lower HSC presence, and altered ECM modulator expression were observed (MMP-2 and TIMP-2) in the CHOL+CA group. Furthermore, apoptotic HSC were detected in groups supplemented with cholesterol (CHOL and CHOL+CA). In vitro HSC-T6 viability and not AML12 hepatocytes was affected in a dose and time-dependent manner by cholesterol treatments, but not by cholic acid.

Conclusions: In the current study, we demonstrated that cholesterol ameliorated advanced fibrosis induced by bile acids by induction of HSC apoptosis.

Topic: *AS05 NAFLD Therapeutics*

BUTYRATE PROTECTS AGAINST DIET-INDUCED LIVER FIBROSIS AND SUPPRESSES NON-CANONICAL TGF-B SIGNALING IN HUMAN HEPATIC STELLATE CELLS

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Background and Aims: In obesity-associated non-alcoholic steatohepatitis (NASH), persistent hepatocellular damage and inflammation are key drivers of fibrosis, which is the main determinant of NASH-associated mortality. The short-chain fatty acid butyrate can exert metabolic improvements and anti-inflammatory activities in NASH. However, its effects on NASH-associated liver fibrosis remain unclear.

Methods: Putative antifibrotic effects of butyrate were studied in Ldlr-/-.Leiden mice fed an obesogenic diet (HFD) containing 2.5% (w/w) butyrate for 38 weeks and compared to a HFD-control group. Antifibrotic mechanisms of butyrate were further investigated in TGF-β-stimulated primary human hepatic stellate cells (HSC).

Results: HFD-fed mice developed obesity, insulin resistance, increased plasma leptin levels, adipose tissue inflammation, gut permeability, dysbiosis and NASH-associated fibrosis. Butyrate corrected hyperinsulinemia, lowered plasma leptin levels and attenuated adipose tissue inflammation, without affecting gut permeability or microbiota composition. Butyrate lowered plasma ALT and CK-18M30 levels and attenuated hepatic steatosis and inflammation. Butyrate inhibited fibrosis development as demonstrated by decreased hepatic collagen content and Sirius-red-positive area. In TGF-β-stimulated HSC, butyrate dose-dependently reduced collagen deposition and decreased procollagen1α1 and PAI1 protein expression. Transcriptomic analysis and subsequent pathway and upstream regulator analysis revealed deactivation of specific non-canonical TGF-β signaling pathways Rho-like GTPases and PI3K/AKT and other important pro-fibrotic regulators (e.g. YAP/TAZ, MYC) by butyrate, providing a potential rationale for its antifibrotic effects.

Conclusions: In conclusion, butyrate protects against obesity development, insulin resistance-associated NASH and liver fibrosis. These antifibrotic effects are at least in part attributable to a direct effect of butyrate on collagen production in hepatic stellate cells, involving inhibition of non-canonical TGF-β signaling pathways.
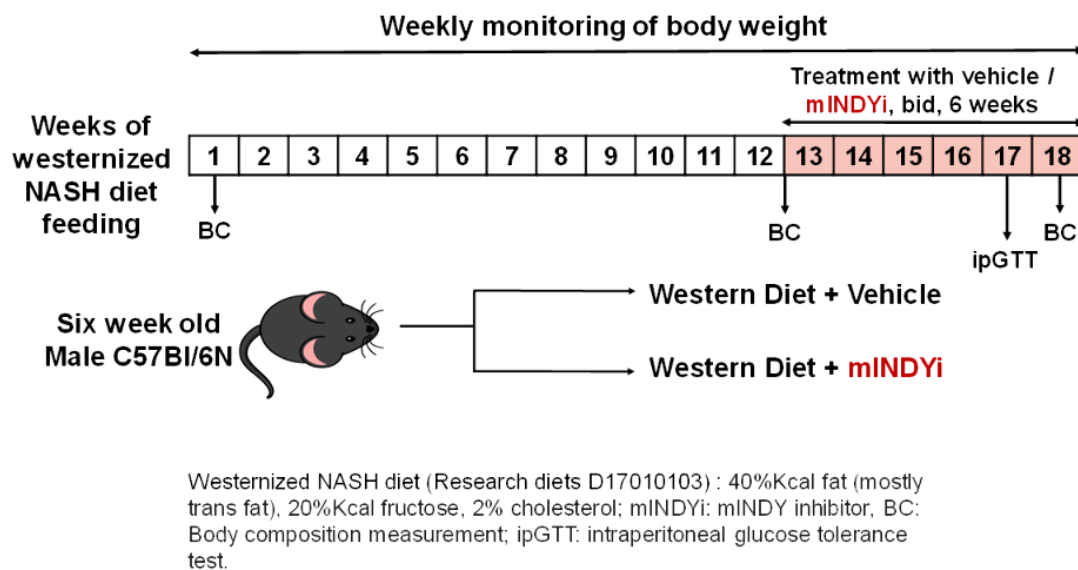
PHARMACOLOGICAL INHIBITION OF MAMMALIAN INDY AMELIORATES HEPATIC INSULIN RESISTANCE, STEATOSIS AND INFLAMMATION IN A DIET-INDUCED NASH MOUSE MODEL

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Background and Aims: Recent data proposed a role of the citrate transporter INDY (Acronym: *I'm Not Dead Yet*) in the development of obesity, insulin resistance and NAFLD suggesting its potential as a therapeutic target for metabolic-related disorders. Our study aims to determine if mammalian INDY (mINDY) inhibitors can be introduced as a new therapeutic option for NAFLD/NASH.

Methods:

Westernized NASH diet (Research diets D17010103) : 40%Kcal fat (mostly trans fat), 20%Kcal fructose, 2% cholesterol; mINDYi: mINDY inhibitor, BC: Body composition measurement; ipGTT: intraperitoneal glucose tolerance test.

Six-week-old C57Bl/6N mice were fed a western diet (WD) for 18 weeks to induce NASH. After 12 weeks of feeding, mice received mINDY inhibitor (PF-06649298, mINDYi; 100 mg/kg) or vehicle bid until end of week 18. During the treatment period, body weight and composition were monitored. ipGTT and FACS analysis of liver inflammatory cells were performed.

Results: mINDYi-treated mice exhibited lower body weight, fat mass and higher lean mass compared to vehicle group. Results from ipGTT revealed improved glucose tolerance and insulin sensitivity. The latter was further confirmed through increased insulin-stimulated Akt phosphorylation in liver, gonadal WAT and BAT. Treatment with mINDYi attenuated WD-induced hepatic injury, steatosis and inflammation as shown histologically and biochemically by reduction in plasma ALT and AST, decrease in hepatic triglyceride accumulation and reduced hepatic lymphoid and myeloid immune cells. Interestingly, these effects were associated with a significant rise in plasma and hepatic Fgf21 together with increased hepatic downstream LKB1 and AMPK phosphorylation.

Conclusions: Our study shows for the first time that mINDYi attenuates diet-induced steatohepatitis; offering a promising novel treatment strategy for NAFLD/NASH. Further investigations are currently ongoing to elucidate the mechanisms underlying mINDY inhibition and whether it is dependent on Fgf21 signaling.

NICOTINAMIDE N-METHYLTRANSFERASE INHIBITION IMPROVES OBESITY-RELATED LIVER DYSFUNCTIONS

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Background and Aims: The multifactorial nature of obesity and its related metabolic dysfunctions pose great challenges while offering diverse targets for developing effective therapeutics. Nicotinamide N-methyltransferase (NNMT), an enzyme highly expressed in white adipose tissue, is a recently discovered drug target that has been preclinically validated and clinically evaluated for the management of obesity, Type 2 diabetes, and liver disease. Located at the nexus of the nicotinamide adenine dinucleotide salvage and methionine metabolic pathways, NNMT serves as a critical regulator of cellular energy metabolism and epigenetic alterations linked to adiposity and insulin sensitivity.

Methods: Industry-standard protocols were used in all studies.

Results: NNMT inhibition by selective small molecules limits body weight and white adipose tissue mass gain, improves glucose tolerance and hyperinsulinemia, increases cellular energy expenditure, and reduces hepatic steatosis and inflammation in diet-induced obese mice. Additionally, obese animals transitioned from a high-fat diet to a normal (lean) diet and supplemented with NNMT inhibitor (NNMTi) treatment show highly accelerated body weight and fat loss, increased whole-body lean mass, reduced liver and epididymal white adipose tissue weights, decreased liver adiposity, and improved liver pathologies (e.g., steatosis) relative to obese animals transferred to lean diet alone. Importantly, lean diet combined with NNMTi treatment normalized body composition and liver adiposity parameters to levels observed in age-matched lean control mice.

Conclusions: Taken together, NNMTi treatment's modulation of body weight, adiposity, liver physiology, and the adipose tissue metabolome strongly support it as a promising therapeutic for obesity and obesity-driven comorbidities.

ISOLEUCINE AND VALINE CORRECT HEPATIC LIPID PROCESSING, REDUCE LIVER STEATOSIS AND SUPPRESS INFLAMMATION IN OBESE MICE WITH MANIFEST NASH

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is associated with disturbed liver lipid handling and excessive accumulation of hepatic fat. Branched-chain amino acids (BCAAs) may beneficially modulate hepatic lipid handling, however it remains unclear whether individual BCAAs can attenuate already established NASH.

Methods: After 26 weeks of pretreatment with an obesogenic diet (FFD), mice were treated with individual BCAA, valine or isoleucine (3% of FFD), for another 12 weeks and were then compared to FFD controls.

Results: FFD controls developed pronounced obesity, dyslipidemia, insulin resistance with increased liver damage markers ALT and CK-18m30 and pronounced NASH pathology after 38 weeks. Valine and isoleucine did not affect obesity and dyslipidemia, but significantly reduced hyperinsulinemia. Valine and isoleucine significantly reduced ALT, CK-18m30, and total liver steatosis. Microvesicular steatosis was strongly decreased, by 61% (valine) and 71% (isoleucine). Functional transcriptome analysis demonstrated an upregulation of BCAA metabolism genes (e.g. BCAT, PP1MK, BCKDHA), deactivation of lipid synthesis regulators (SREBF1, AGT, IGF1) and activation of regulators involved in lipid oxidation (AMPK, ACOX1, EHHADH) with increased mitochondrial biogenesis (PPARGC1α, CLUH). This correction of critical metabolic pathways by valine and isoleucine was associated with decreased lobular inflammation, and a significant suppression of FFD-stimulated inflammatory pathways controlled by IL-1b and TNFα.

Conclusions: In conclusion, FFD feeding disturbed lipid metabolism in liver and induced NASH. Supplementation of FFD with either valine or isoleucine corrected lipid metabolism and reduced liver steatosis. These beneficial metabolic effects were accompanied by a pronounced reduction of liver inflammation which was substantiated by suppression of critical inflammatory pathways.

THE EFFICACY OF DULAGLUTIDE 30-WEEK TREATMENT OF PATIENTS WITH NAFLD AND T2DM IN REAL CLINICAL PRACTICE.

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Background and Aims: Dulaglutide has been approved as an agent for the treatment of pts with T2DM. It was found that aGLP-1 may prevent the progression of NAFLD. To assess the effect of dulaglutide on carbohydrate and hepatic metabolism in this pts.

Methods: Open prospective cohort study of real clinical practice. 92 pts with T2DM and NAFLD, BMI ≥ 28 kg/m2, receiving basic therapy with daily dose of metformin ≥ 1,500 mg as monotherapy or as part of a combination therapy were condacted to the study with treatment of dulaglutide subcutaneus injections 1,5 mg weekly for 30,6 ±9,7 weeks. Mean age 52,4±11,1 years, 23,9% men. All patients undergo physical examination with weekly weight, waist and hip circumference measurement, blood sampling tests (HbA1c, FPG, ALT, AST, GGT, lipids, hematology) , ultrasound and Fibroscan before and after 30 weeks of treatment. Statistical analysis was done using by Statistica Version 12 and Student's t-test for related groups. Results are presented as mean values calculated using Microsoft Excel.

Results: Mean weight loss was -5 kg in 30 weeks. A statistically significant decrease in weight, BMI, waist circumference, ALT levels was detected in the whole group, despite the fact that the goal of more than 5% of the initial weight loss have reached only 56,0% of patients. There was no statistical difference in FPG, AST, GGT, lipids and HbA1c. A downward trend in TE was also noted.

Conclusions: In real clinical practice Dulaglutide may have a positive effect on weight loss, metabolic and hepatic parameters in patients with NAFLD and T2DM.

HIGH DOSE THIAMINE THERAPY PREVENTS THE DEVELOPMENT OF OVERNUTRITION INDUCED FATTY LIVER IN SHEEP

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Background and Aims: Non-alcoholic fatty liver (NAFLD) is a rising global epidemic affecting ~25% of the global population. However, there is yet no approved drug therapeutic agent against it. Thiamine (vitamin B1) as a precursor for thiamine pyrophosphate (TPP) – a cofactor required in the catalytic decarboxylation of alpha-keto acids and transketolase reactions – plays a crucial role in energy metabolism. Employing our recently developed sheep large animal model of overnutrition-induced fatty liver, we tested whether a high thiamine treatment dose boosts carbohydrate and fat combustion to prevent hepatic fat accumulation.

Methods: Thirty-six lambs of ~2 months were randomly assigned to three treatment groups of (n=12 each) of: high-calorie diet (HC), HC with subcutaneous thiamine treatment of 300 mg/animal five times weekly (THC), and low-calorie diet (LC) monitored for an experimental duration of 135 days, followed by postmortem liver analyses.

Results: Thiamine treatment prevented excessive hepatic fat accumulation compared with the untreated HC group (4.8 vs. 8.1%; $P<0.0001$). Whereas thiamine treatment reduced hyperglycemia (72.0 vs. 74.6 mgdL^{-1}; P=0.02), it increased liver glycogen content (2.1 vs. 0.99%; $P<0.005$). Thiamine increased the activity of alpha-ketoglutarate dehydrogenase (P=0.01). Thiamine treatment downregulated Perilipin 2 gene expression (P= 0.002) but upregulated microsomal triglyceride transfer protein (*MTTP)* gene expression (P=0.001). Further experiments revealed that thiamine had no effect on the weight of sheep but increased serum creatinine levels ($P<0.05$).

Conclusions: Thiamine revealed a strong hepatic antisteatotic effect and hence a potentially affordable and safe therapy in the management of the multifaceted fatty liver-related disorders.

INDOLE-3-PROPIONIC ACID AMELIORATES HEPATIC FIBROSIS BY ATTENUATING HEPATIC STELLATE CELL ACTIVATION

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Background and Aims: Indole-3-propionic acid (IPA) is a gut-derived tryptophan metabolite. In the literature, there is some evidence of the beneficial effect of IPA as an anti-non-alcoholic steatohepatitis (NASH) microbial metabolite and a hepatoprotective compound in rodent and cell models. Also, IPA level is decreased in individuals with obesity and type 2 diabetes (T2D).Here, we investigated the association of IPA with human liver histology and transcriptomics, and the potential of IPA to reduce features of hepatic stellate cell (HSC) activation.

Methods: A total of 233 subjects (72% women, age 48.3±9.3 years; BMI 43.1±5.4 kg/m^2) undergoing bariatric surgery with detailed liver histology were included. Circulating IPA levels were measured using liquid chromatography-mass spectrometry (LC-MS) and the liver transcriptomics with total RNA-sequencing. Human hepatic stellate cell model (LX-2) activated with transforming growth factor beta (TGF-β) 1 was used to study the hepatoprotective effect of IPA.

Results: Circulating IPA level was lower in individuals with the liver fibrosis compared to those without fibrosis (p=0.039 for all participants; p=0.013 for 153 individuals without T2D). Accordingly, circulating IPA level associated with the expression of 278 liver transcripts (p<0.01) that were enriched for the genes regulating HSC activation and hepatic fibrosis signaling. Moreover, IPA was sufficient to decrease cell adhesion, cell migration, and the mRNA gene expression of classical markers of HSCs activation (all p<0.05).

Conclusions: In conclusion, the association of circulating IPA with the liver fibrosis and the ability of IPA to reduce activation of HSC suggests that IPA may have a hepatoprotective effect and therapeutic potential.

AN MTORC1-PLIN3 PATHWAY IS ESSENTIAL TO ACTIVATE LIPOPHAGY AND PROTECTS AGAINST HEPATOSTEATOSIS

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Background and Aims: NAFLD is the most common hepatic pathology in western countries and no treatment is currently available. NAFLD is characterized by the aberrant hepatocellular accumulation of fatty acids in the form of lipid droplets (LDs). Recently, it was shown that liver LD degradation occurs through a process termed lipophagy, a form of autophagy. However, the molecular mechanisms governing liver lipophagy are elusive. Here, we aimed to ascertain the key molecular players that regulate hepatic lipophagy and their importance in NAFLD.

Methods: We analyzed the formation and degradation of LD in vitro (fibroblasts and primary mouse hepatocytes), in vivo and ex vivo (mouse and human liver slices) and focused on the role of the autophagy master regulator mTORC1 and the LD coating protein perilipin (Plin) 3 in these processes.

Results: We show that the autophagy machinery is recruited to the LD on hepatic overload of oleic acid in all experimental settings. This led to activation of lipophagy, a process that was abolished by Plin3 knockdown using RNA interference. Furthermore, Plin3 directly interacted with the autophagy proteins focal adhesion interaction protein 200 KDa and autophagy-related 16L, suggesting that Plin3 functions as a docking protein or is involved in autophagosome formation to activate lipophagy. Finally, we show that mTORC1 phosphorylated Plin3 to promote LD degradation.

Conclusions: These results reveal that mTORC1 regulates liver lipophagy through a mechanism dependent on Plin3 phosphorylation. We propose that stimulating this pathway can enhance lipophagy in hepatocytes to help protect the liver from lipid-mediated toxicity, thus offering a therapeutic strategy in NAFLD.

ALTERATIONS IN N-LINKED GLYCOSYLATION ARE ASSOCIATED WITH HISTOPATHOLOGICAL CHANGES IN NALFD AND NASH IN MOUSE AND MAN

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Background and Aims: N-linked glycosylation is one of the most common post-translational modifications. Changes in the N-glycan profile of a glycoprotein can occur in response to environmental and genetic factors and can correlate with disease stages. N-linked glycosylation changes in serum have been reported in Non-alcoholic steatohepatitis (NASH) patients, but the disease stage at which these modifications start, and their tissue origin is poorly understood. Here, we hypothesized that these N-glycan modifications may be associated with histopathological liver damage induced during the progression of non-alcoholic fatty liver disease (NAFLD).

Methods: Human and mouse tissue samples were used in this study. We induced NAFLD and NASH in a murine model through ad libitum feeding with either a high-fat diet (HFD) or a Western diet (WD), respectively. Mouse models developed inflammation, steatosis, and fibrosis, consistent with NAFLD/NASH phenotypes. To validate animal studies, liver biopsy specimens from 51 NAFLD/NASH patients representing the full range of NASH fibrosis stages (NASH CRN) were analyzed.

Results: Overall, N-glycan analysis in liver tissues from NAFLD/NASH mouse models and human NASH biopsies had an increased expression of mannose and complex/fucosylated N-glycan structures compared to control mouse livers and patients with a low fibrosis score and/or level of steatosis. As hypothesized, spatial glycan alterations were localized specifically to fibrotic and fatty areas. In addition, the expression of fucosylated N-glycan structures correlated with the degree of fibrosis in human biopsies.

Conclusions: Overall, we elucidate a promising translational approach by using N-glycan modifications to stratify the stages of NAFLD/NASH and be used as strategies for biomarker development.

Topic: *AS06 Other*

DEVELOPMENT OF A DISEASE-MIMICKING MODEL FOR NON-ALCOHOLIC STEATOHEPATITIS AND FIBROSIS IN A TRIPLE CELL-TYPE, SPHEROID-BASED LIVER-ON-CHIP PLATFORM WITH MICROFLUIDICS

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Background and Aims: Despite ongoing efforts there is currently no effective therapeutic treatment available for non-alcoholic steatohepatitis (NASH). Several drug candidates have failed clinical trials due to lack of efficacy, underlining the need for predictive preclinical models. To this end we developed a disease-mimicking *in vitro* model which closely resembles the pathophysiology of liver fibrosis induced by lifestyle factors.

Methods: Primary human hepatocytes, Kupffer cells, and stellate cells were cultured in a matrix-free environment. Fatty acids, carbohydrates, inflammatory and immunomodulatory factors were used at physiological concentrations to faithfully recapitulate disease development and progression of NAFLD-NASH. Development of steatosis was imaged using confocal microscopy and plate reader assays. Transcription and protein analyses confirmed expression of different collagen isoforms upon full disease induction.

Results: Induction of NASH resulted in hepatocyte steatosis, expression of inflammatory cytokines, expression of secreted protein markers for fibrosis, and deposition of collagen in the cell-matrix fraction of the liver spheroids. Microfluidic flow conditions resulted in a more homogenous distribution and size of lipid droplets as compared to static culture conditions where droplet size was more variable. Collagen deposition was not affected by single drug treatment but combination treatment consisting of drugs with different modes of action reduced collagen deposition compared to vehicle-treated NASH controls.

Conclusions: We present a disease-mimicking cell model for NASH and fibrosis that results in collagen production under static and flow conditions. The model is responsive to pharmacological interventions. We will further investigate the effect of microfluidic flow and experimental drugs currently in clinical trials as single or combination treatment.

Topic: *AS06 Other*

LIVER STEATOSIS IN PATIENTS TREATED WITH METHOTREXATE FOR RHEUMATOID ARTHRITIS IS RELATED WITH BODY MASS INDEX.

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Background and Aims: Methotrexate (MTX) is the principal treatment for rheumatoid arthritis (RA). Long term use has been associated with liver steatosis (LS) and liver fibrosis (LF) (1,2). The aim of our study is to determine if LS in patients treated with MTX for AR is associated with MTX cumulative dose (MTX-CD), metabolic syndrome (MtS), body mass index (BMI) and LF.

Methods: A single centre prospective study of patients receiving MTX for AR was performed from february 2019 to february 2020. Transient elastography (fibroscan, echosens) was used for fibrosis determination (LF>7 KpA). CAP for liver steatosis (CAP>248 dB/m). Demographic variables, laboratory data, MTX-CD (>4000 mg), MtS criteria, BMI (>25), TE and CAP scores were collected from all patients.

Results: Sixty patients were included. Forty-four were women (73,33%) and mean age was 61,58-yr-old (SD 11,63). When we compared MTX-TCD≤4000 mg (26 patients) -No LS 12, LS 14- with >4000 mg (34-patients) -No LS 21, LS 13- no statistical differences were seen (p=0.228). We compared CAP scores with MtS, BMI and LF: The were no significative differences with the presence or not of MtS or LF. CAP/MtS: 51 no MtS (85%), 9 MtS (15%), p=0.156. CAP-Fibrosis: 54 No LF (90%); 6 LF (10%), p=0.261. LS determined by CAP was significatively associated with BMI>25. CAP/BMI: 22 BMI≤25 (36.67%); 38 BMI>25 (63.33%), p=0.001.

Conclusions: Liver steatosis in patients with AR treated with MTX is not associated with MTX-CD, LF or MtS. BMI is significatively related to LS in these patients.

IMPAIRED AUTOPHAGY AND ENHANCED INFLAMMASOME ACTIVATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS CONTRIBUTE TOWARDS PROGRESSION OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Circulating Peripheral Blood Mononuclear Cells (PBMCs) can be used as a surrogate monitor for tissues that are difficult to biopsy or a sensitive monitor to evaluate the physiological state of an organism as they can migrate through various tissues of the body. Considering the prognostic value of PBMCs, we have focussed their role in the progression of NAFLD, where we studied the interplay of autophagy with inflammasome activation in PBMCs.

Methods: Blood was collected from 32 patients with LSM (>7kpa), USG with confirmed fatty liver were enrolled in the study along with healthy volunteers. Major anthropometric, biochemical, dietary parameters were recorded. Important molecular markers of Oxidative stress, Inflammation, Inflammasome activation and autophagic flux in PBMCs were analysed by Western Blot, Flow Cytometry, SEM, ICC and Statistical analysis being undertaken in SPSS 19.0.

Results: Increase in BMI, W/H ratio, SBP, DBP, SGPT, SGOT, γ-GGT, TG and decreased HDL in NAFLD patients ($p<0.05$) were recorded compared to controls. Elevated systemic inflammation was observed in NAFLD, due to higher hsCRP and proinflammatory cytokines ($p<0.05$) in Serum. Nutritional intake of SFA and ω-6 PUFAs in diets were higher in cases. Formation of ROS and NLRP3 Inflammasomes markers were upregulated in PBMCs of NAFLD subjects. The number of LC3B foci, a marker of autophagosomes, was lower in cases than in controls, while the expression of p62/SQSTM1, a protein degraded by autophagy, was increased.

Conclusions: The current research provides evidence of impaired autophagy and intracellular ROS being linked to Inflammasome activation and subsequent pyroptosis of PBMCs in NAFLD.

Topic: *AS06 Other*

THE RISE IN A NON-ALCOHOLIC FATTY LIVER DISEASE AS THE ETIOLOGY OF CIRRHOSIS IN SLOVAKIA

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Background and Aims: Cirrhosis is No1 cause of death in young Slovaks. Worldwide, NAFLD is the fastest growing etiology of cirrhosis; however, region-specific trends are unknown. To investigate the dynamics, we set out to compare two cirrhosis datasets created 10 years apart for the delta in a prevalence of NAFLD.

Methods: Dataset No1 was obtained during our retrospective study of 2009-2011, on consecutive patients(pts) admitted to our liver unit with cirrhosis; the second consisted of a year 2019 extraction from the cirrhosis registryRH7 [founded in 2014, admission characteristics of pts hospitalized with cirrhosis have been recorded]. We included pts with NAFLD cirrhosis and analyzed etiology, sex, age, Model for end stage liver disease(MELD), body mass index(BMI), Mid-arm muscle area (MAMA/mm^2), C-reactive protein (CRP[mg/L]).

Results: Following the criteria, we enrolled 40pts with NAFLD cirrhosis from dataset of 2009-2011 and 63pts from RH7 of 2019. As the main etiology of cirrhosis in years 2009-11 and 2019, NAFLD was found in 2.8%vs12% of pts,respectively, (p=0.086). The median age in a two cohorts was 55.6 and 56.3,(p=0.72), respectively; females predominated p=0.022. MELD was significantly higher in the 2019: 12.61vs15.67,(p=0.018). According to MAMA and CRP, pts were more sarcopenic and inflamed in 2019 as compared to 2009-11: 56.4vs24.65,(p <0.001),and 11.70vs29.4,(p=0.023).

Conclusions: Comparing a two similar cohorts created 10 years apart, we found a numerically increased prevalence of NAFLD as the main etiology of cirrhosis in hospitalized pts.Statistical signifficance was not achiever, most probably due to a small number of pts. At admission, pts from 2019 were more decompensated and inflamed.

Topic: *AS06 Other*

DIFFERENCES IN HEPATIC TRICARBOXYLIC ACID CYCLE-DRIVEN MITOCHONDRIAL RESPIRATION IN MODELS OF DIABETES MELLITUS AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Obesity and type 2 diabetes associate with non-alcoholic fatty liver disease and its progression to steatohepatitis (NASH). Mouse models fully reflecting the human metabolic alterations of combined NASH and type 2 diabetes are still missing. In obese individuals with and without diabetes, hepatic mitochondrial capacity decreased upon NASH development. We examined hepatic tricarboxylic acid cycle (TCA)-driven mitochondrial respiration and underlying mechanisms in respective mouse models.

Methods: Two-days old C57BL/6j mice received streptozotocin (STZ) or vehicle (VCL) and then high-fat diet (HFD) or continued regular chow diet (RCD) from week 4 to 16, yielding 4 models (n=5-9/group): obesity [VCL+HFD]; insulin-deficient diabetes, DM [STZ+RCD]; diabetes+NAFLD, NASH [STZ+HFD] and control [VCL+RCD]. TCA-linked mitochondrial respiration was assessed by high resolution respirometry and whole-body insulin sensitivity by hyperinsulinemic-euglycemic clamp tests. ELISA and qPCR were used to measure reactive oxygen species (ROS) and lipid metabolism-related genes.

Results: Whole-body insulin sensitivity was 50% lower in obesity, DM and NASH than in control (all $p<0.0001$). Maximum TCA-driven respiration was 108% higher only in DM ($p<0.0001$ vs control). Mitochondrial density and respiratory control ratio were not different between the groups. Although systemic concentrations of ROS were higher in both DM and NASH, hepatic ROS were not different between the groups. Of note, mRNA level of hepatic lipid uptake- and lipolysis-related genes *Cd36* and *Lpl* were increased in DM only (both $p<0.01$ vs control).

Conclusions: Short duration of insulinopenic diabetes associates with higher hepatic TCA-supported respiration which result from mitochondrial adaptation to excessive lipolysis and lipid uptake.

Topic: *AS06 Other*

THE DNA DAMAGE RESPONSE IS INVOLVED IN THE METABOLIC DYSREGULATION OF NAFLD

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Background and Aims: A specific feature of NAFLD is the imbalance between processes that regulate the liver lipid input and output. A chronic lipid-rich environment alters the oxidative capacity of mitochondria promoting the generation of species implicated in the DNA damage response (DDR), process linked to NAFLD; however, little is known about the effect that DDR might have in the lipid metabolic dysregulation. Thus, the aims were: to evaluate if DDR could be involved in the metabolic-associated changes involved in NAFLD progression and to elucidate if lipid accumulation might activate DDR.

Methods: A cohort of obese patients with liver biopsies was used. The mitochondrial-complexes activity, pH2AX (marker of DNA damage) liver levels, serum FGF21 and liver and serum lipid concentration were determined. A mice cohort and mouse primary hepatocytes were also used.

Results: Liver pH2AX levels were increased in obese NASH patients and correlated with metabolic features of worse prognosis. Mitochondrial-activities were also increased in NASH patients, and were higher in those with increased pH2AX-activation; however, serum ketone bodies, an indirect marker of fatty-acid oxidation, remained unchanged when compared to no-NASH patients. In the animal cohort, pH2AX levels also correlated, as in patients, with insulin resistance and liver diglycerides. When hepatocytes were were triggered in vitro with UV or H_2O_2 (DDR-inducers) were not able to catabolize the increased intracellular-lipid levels when facing a lipid-rich environment. The results also showed that treatment with fatty-acids alone was enough to activate DDR.

Conclusions: DDR activation triggers metabolic changes related with mitochondrial dysfunction and facilitate lipid accumulation, both related to disease progression.

ALTERATIONS IN THE PHENOTYPE OF LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECS) IN MURINE MODEL OF CHRONIC HEART FAILURE

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Background and Aims: Liver dysfunction often co-exists with heart failure (HF) worsening the prognosis of the underlying disease. In this study, a unique murine model of chronic HF (Tgαq*44 mice) was used to characterize the alterations of LSECs phenotype and function that could contribute to HF progression.

Methods: *In vivo* and *in vitro* studies were conducted at early (4-month-old), transition (8-month-old) and late (12-month-old) stage of HF development in Tgαq*44 mice as compared with age-matched FVB mice. Assessment of portal flow velocity and liver pathology was performed using Doppler, plasma biochemistry, histology and TEM imaging, respectively. The primary LSECs' phenotype was characterized in terms of the number of fenestrations (AFM technique), eicosanoids biosynthesis (UPLC-MS/MS) and bioenergetics (Seahorse XF Analyzer).

Results: Even at the early stage of HF, livers of Tgαq*44 mice displayed disrupted portal flow and sinusoid dilatation associated with mild elevation of ALT and TG that normalized at the later stages. LSEC phenotype in the early and late phases of HF was featured by the defenestration, increased biosynthesis of vasoprotective EETs and decreased secretion of vasodilator prostanoids with preserved LSEC bioenergetics.

Conclusions: HF development induces clear-cut alterations in LSEC phenotype, that could be attributed to disrupted portal blood flow and did not result in severe impairment of liver function and histology. HF-induced LSEC defenestration was associated with shifted eicosanoid biosynthesis from COX to CYP-450-dependent pathway. Altogether, data suggest that altered LSEC phenotype precedes liver parenchyma dysfunctions associated with the development of end-stage HF. Accordingly, LSEC-target therapy might mitigate the worsening of liver-heart cross-talk in HF.

ONE MIR TO RULE THEM ALL - REGULATION OF THYROID HORMONE METABOLISM IN NASH BY MIR-34A-5P

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) affects a quarter of the global population but to date no efficient therapy is available. The more severe stage of NAFLD, non-alcoholic steatohepatitis (NASH), is defined by inflammation in addition to accumulation of fatty acids in the liver and has been associated with impaired thyroid hormone signalling. Therefore, this projects aims to find epigenetic regulators of the thyroid hormone (TH) metabolism that are dysregulated in NASH.

Methods: Expression of miRNA-34a-5p and genes involved in TH metabolism were analysed by qPCR in liver of subjects and mice suffering various grades of NAFLD. Additionally, HepG2 cells were transfected with a miR-34a-5p mimic and inhibitor and genes related to TH metabolism were analysed.

Results: miRNA-34a-5p was increased in livers of subjects and mice with NAFLD and negatively correlated with genes of the TH metabolism. Overexpression of miR-34a-5p in cell culture reduced expression of almost all TH metabolism related genes.

Conclusions: miR-34a-5p might be a critical regulator of hepatic TH metabolism and involved in the development of NASH.

Topic: *AS06 Other*

HEPATIC MACROPHAGE-DERIVED OSTEOPONTIN PROTECTS FROM DIET-INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Osteopontin (OPN) is an immunomodulatory protein involved in chronic liver disease. The expression of OPN in macrophages (MFs) from healthy liver is relatively low; however, it markedly increases in NAFLD. Our aim was to elucidate the role of MF-derived OPN in NAFLD.

Methods: *OPN* expression was analyzed in publically available datasets. *Opn* knock-in and knock-out mice in myeloid cells and Kupffer cells were generated. Mice were fed a NAFLD-inducing or an isocaloric control diet for 6 months.

Results: scRNA-seq showed increased *OPN* expression in MFs in human and mice with NAFLD. Unsupervised clustering identified a MF population with high *Opn* expression. Gene set enrichment analysis of the *Opn*[High] MF transcriptomics revealed low enrichment in pro-inflammatory pathways but high enrichment in lipid metabolism pathways. Both genders of *Opn*[KI-Mye] mice were almost fully protected from NAFLD. *Opn*[KI-Lv.MF] recapitulated the protective phenotype of *Opn*[KI-Mye] mice. Livers from *Opn*[KI-Mye] mice showed higher concentration of urea cycle metabolites along with increased serum urea and decreased ammonia, indicating increased arginase activity. Gene expression analysis revealed that mitochondrial arginase (*Arg2*) but not cytosolic arginase (*Arg1*), was up-regulated mainly in hepatocytes from *Opn*[KI-Mye] mice. ARG2 stimulates mitochondrial oxidative metabolism. Indeed, livers from *Opn*[KI-Mye] mice had increased NAD^+/NADH ratio, up-regulation of *Cpt1a*, *Pparα* and products from mitochondrial β-oxidation. Moreover, *Opn*[KI-Mye] had reduced liver triglycerides containing saturated long-chain fatty acids, which are predominantly oxidized in mitochondria.

Conclusions: Hepatic MF-derived OPN protects from diet-induced NAFLD in mice. The ARG2-mediated increase in mitochondria respiration and β-oxidation could be responsible for the protective effect.

Topic: *AS06 Other*

PREVALENCE AND CLINICAL CHARACTERISTICS OF METABOILC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE IN POPULATION WITH NORMAL BODY MASS INDEX (LEAN MAFLD).

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Background and Aims: Among MAFLD patients, 4.1% have lean MAFLD. The aim was to report prevalence of lean MAFLD according to different cut-off points of CAP (dB/m) and metabolic characteristics associated.

Methods: Retrospective study with data for a check-up unit from 2019-2020, the medical evaluation includes transient elastography for evaluation of fibrosis and steatosis. Prevalence of steatosis was defined with two different cut-off points, (>232 dB/m, and >263 dB/m). Lean MAFLD was defined with: BMI <24.9 and at least >2 metabolic alterations: high blood pressure, hypertension diagnosis, glucose alterations, high triglycerides, low HDL, treatment for dyslipidemia, abdominal circumference and CPR. Patients with other liver diseases, hepatotoxic and high alcohol consumption according to gender were excluded.

Results: Among 3863 patients evaluated, 45.4% presented steatosis with >263 dB/m cut-off point, and 65.9% with >232 cut-off point. Prevalence of lean MAFLD was 5.7% (n=100) with >263 dB/m cut-off point, and 5.3% with >232 cut-off point, most of patients were male (54%) with median age of 46 [IQR 40-56] years, BMI of 23.7 [22.8-24.4] kg/m^2, CAP 293 [271-314] dB/m and liver stiffness 4.0 [3.5-4.7] kPa. 40% of lean MAFLD patients have grade 1 of steatosis (>263dB), 17% grade 2 (>283 dB) and 43% grade 3 (>296 dB). BMI showed significant difference according to degree of steatosis, being greater in patients with grade 3.

Conclusions: Prevalence of lean MAFLD in this population is high, most patients have grade 3 steatosis and higher BMI. Prevalence not change using different cut-off points of CAP.

RELIABILITY FACTORS FOR THE MEASUREMENT OF HEPATIC STEATOSIS BY MEANS OF A CONTROLLED ATTENUATION PARAMETER BY TRANSIENT ELASTOGRAPHY

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Background and Aims: The controlled attenuation parameter (CAP) allow the indirect measurement of liver fat by transient elastography. Current recommendations for quality CAP studies are to obtain valid measurements with IQR <30 or <40, although there is little evidence to support this. We aimed to evaluate the reliability factors of CAP quality by transient elastography.

Methods: Retrospective, observational design of transient elastography studies from 2015 to 2019 using Fibroscan 502 Touch M and XL probes according to manufacturer's recommendations. Univariate and multivariate logistic regression analysis was performed to identify the reliability factors on CAP.

Results: 1153 studies were analyzed, 52.6% male, median age of 54 years [44-63] and BMI 27.4 kg/m2 [24.1-29.7]. Fatty liver screening represented the 48.8% (n = 558), with 66% reporting some grade of liver steatosis. The incorrect probe was used in 26.2% (n = 302). Reliability factors for IQR <40 were the XL probe (OR 0.34; CI 95% 0.26-0.45), age <54 years (OR 0.71 CI95% 0.55-0.92) IQR kPa <30 (OR 0.48 CI 95% 0.28-0.82) and for an IQR <30 the use of the XL probe (OR 0.31 CI95% 0.23-0.42) and IQR kPa <30 (OR 0.35 CI 95% 0.17-0.71). Evaluating only screening studies (n = 558), the XL probe maintained an independent association for both IQR <40 and <30.

Conclusions: The main reliability measure for CAP IQR <40 and <30 is the use of XL probe regardless of the study indication, BMI and degree of fibrosis.

GENE-DIET INTERACTIONS IN MAFLD HIGHLIGHT THE NEED FOR PERSONALIZED NUTRITION

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Background and Aims: Metabolic (dysfunction) associated fatty liver disease (MAFLD) constitutes the liver manifestation of metabolic syndrome. Management of MAFLD is a great challenge for healthcare professionals, since the inter-individual predisposition for disease onset and progression depends both on genetic and dietary or other lifestyle factors. However, studies on gene-diet interactions in MAFLD are scarce.

Methods: Previously published dietary patterns[1] derived from a sample of 134 MAFLD cases and 217 controls were assessed for their interaction with 5 known predisposing genetic variants for the disease and its related metabolic traits: PNPLA3-rs738409, TM6SF2-rs58542926, GCKR-rs780094, GCKR-rs1260326 and MBOAT7-rs641738. Multivariable regression models were applied and a Bonferroni-corrected p-value was used ($\alpha=0.01$). Statistical analysis was implemented with Plink 1.9.

Results: Adherence to a "Prudent" dietary pattern has been negatively associated with triglycerides (TGs) levels. However, carrying at least one copy of the hypotriglyceridemic A allele of rs58542926 increased TGs levels of individuals who adhered better to the dietary pattern, after adjusting for the main confounding factors ($\beta_{interaction}=20.17$ mg/dL, $p_{interaction}=0.007015$). Interaction remained nominally significant after further adjustment for other lifestyle factors. Similarly, a significant positive interaction effect of the "Prudent" dietary pattern and the rs58542926 A-carriers was found on fasting insulin levels ($p_{interaction}= 0.000273$). No more significant gene-diet interactions were found.

Conclusions: Genetic protection of TM6SF2-rs58542926 carriers against hypertriglyceridemia and hyperinsulinemia was reversed when adherence to a dietary pattern rich in healthy fatty acids and dietary fibres was increased, highlighting the necessity of providing with personalised nutritional therapy. [1]IP Kalafati et al. Nutrition 2019;61:105-110. doi: 10.1016/j.nut.2018.10.032.

GLYCOPROTEIN NON-METASTATIC MELANOMA B (GPNMB) MODULATES HEPATIC STEATOGENESIS AND LIVER CANCER

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Background and Aims: Lipid accumulation predisposes to NAFLD, which may progress to cirrhosis and HCC. Hepatocyte derived GPNMB was reported as regulator of fat metabolism in adipose tissue, however, its role in the pathogenesis of NAFLD is not clear.

Methods: We analyzed expression of *GPNMB* in human NAFLD and HCC liver samples. STAM mice with progressive NAFLD were analyzed with IHC, immunofluorescence and molecular profiling. A steatosis model was set up by oleic acid treatment of hepatocytes and AML12 cells. GPNMB levels were modulated by overexpression, knockdown or treatment with recombinant GPNMB protein. Lipid metabolism associated gene expression was assessed by qPCR. Functional assays on cell proliferation and cell death were performed in HUH7 liver cancer cells by MTT and caspase-3 assays, PCR, immunoblot and time-lapse imaging.

Results: mRNA expression data consistently identify GPNMB upregulated in liver of NASH and HCC patients and STAM mice. IHC localizes GPNMB to hepatocytes and cancer cells. Oleic acid treatment induces GPNMB expression in hepatocytes. Lipid accumulation increases upon knockdown and decreases upon overexpression of Gpnmb as evident from triglyceride levels and bodipy staining. Treatment with rGPNMB protein protects hepatocytes from oleic acid mediated lipid accumulation. Gpnmb depletion increases lipogenic and decreases lipolytic gene expression. Complementary results are evident upon GPNMB overexpression. In HUH7 liver cancer cells, GPNMB inhibits proliferation and enhances cell death as measured by live cell imaging and caspase assays. Mechanistically, GPNMB supports cancer cell death by interfering with AKT phosphorylation dependent survival signals.

Conclusions: GPNMB is consistently upregulated in NAFLD, toning down lipogenesis and suppressing tumorigenesis.

Topic: *AS06 Other*

ASSOCIATION OF LEVEL OF PHYSICAL ACTIVITY, DIETARY PATTERN AND LIPID PROFILE WITH NON ALCOHOLIC FATTY LIVER DISEASE - A CASE CONTROL STUDY.

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Background and Aims: Spectrum of liver pathology that develops in the absence of alcohol abuse or any other predisposing medical condition is being recognised as a major health issue, ranging from simple steatosis to florid cirrhosis and hepatocellular carcinoma.

Methods: The study was conducted with 33 persons diagnosed with NAFLD and 31 controls in the department of physiology.

Results: The level of physical activity as measured by MET score in the NAFLD group was 948.80±628.4 (Mean± SD) and 4475.77±3202.3 (Mean ±SD) in the control group with a p value of < 0.001. 83.9% in the control group were having high level of physical activity and only 12.1% had high level of physical activity in the NAFLD group. Low level of physical activity was reported by 15 out of 33 participants in the NAFLD group as compared to none in the control group. The calorie consumption in the NAFLD group was 2576.48±364.41 (Mean ± SD) and 2305.19±323.35 in the control group with a p value of 0.003. Both BMI and the waist hip ratio were found to be significantly greater in the NAFLD group compared to controls. SGPT levels was more in the NAFLD group (70.94±73.2) as compared to controls (37.71±32.5) with a p value of 0.001(OR=5.75 CI=1.948 – 16.968).

Conclusions: Physical activity has a strong inverse relationship with Non-Alcoholic Fatty Liver Disease but excess calorie intake and obesity has vice versa relationship.

Topic: *AS06 Other*

A MOLECULAR DISCRIMINATION FOR THE METABOLIC SYNDROME BY URINE AND SERUM NMR-METABOLOMICS

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Background and Aims: Metabolic syndrome (MetS) is a complex health condition that that leads to an increased risk of developing cardiovascular disease (CVD), stroke and vascular dysfunction. This syndrome originates by a combination of different risk factors: altered glucose metabolism, obesity, elevated levels of triglycerides, low HDL cholesterol and hypertension. MetS constitutes a major health problem worldwide but the impact of each of the risk factors and the role of aging and non-alcoholic fatty liver disease (NAFLD) as possible factors that enhance Mets, is not jet well understood.

Methods: We have used NMR-based metabolomics on a large cohort of urine and serum samples to investigate the molecular signature of MetS. The cohort of samples was designed to populate all possible intermediate conditions from subjects without any risk factor up to individuals with MetS.

Results: Urine analysis shows that NMR metabolomics is sensitive to MetS, with all the contributing risk factors represented by at least one metabolite. Thanks to the obtained results we were able to built a metabolic model of MetS that can discriminate between individuals with and without the syndrome with statistical significance.

Conclusions: These results adds an unprecedented diagnostic molecular dimension to the set of risk factors that currently describe MetS. While aging and NAFLD do not directly interfere with the metabolic discrimination of the syndrome. Ongoing serum analysis are evidencing the different distribution of 115 lipoprotein related parameters: the main VLDL, IDL, LDL and HDL classes and related subclasses. These results will be crucial to understand other aspect of the disease.

Topic: *AS06 Other*

NON-ALCOHOLIC FATTY LIVER DISEASE: IDENTICAL ETIOLOGIC FACTORS IN PATIENTS WITH TYPE 1 AND TYPE 2 DIABETES.
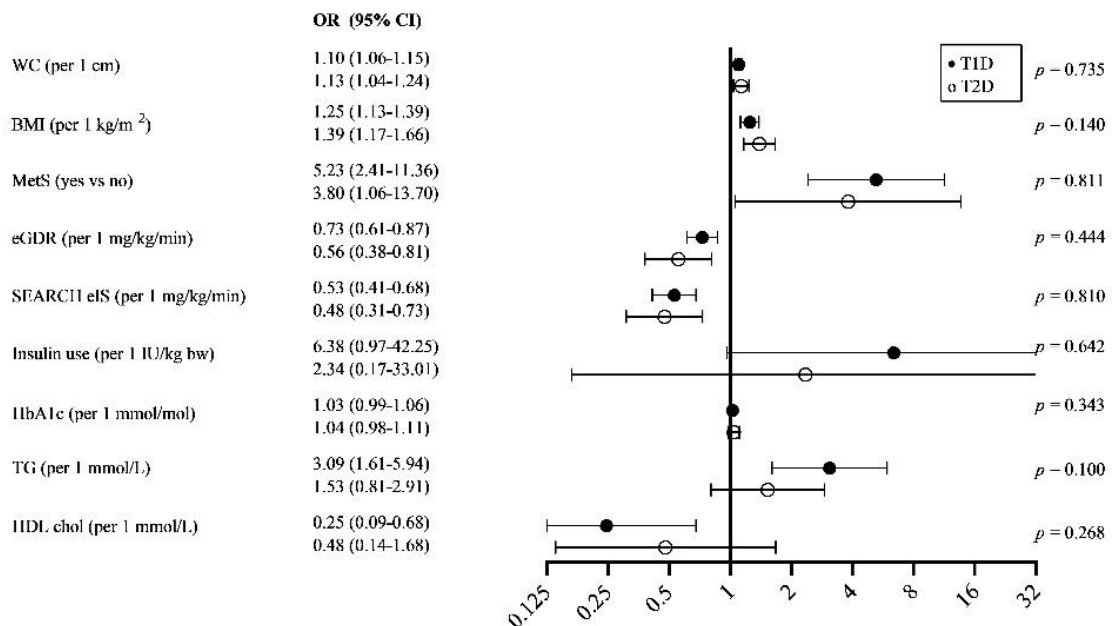
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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. We aimed to compare NAFLD prevalence, distribution and its etiologic determinants in patients with type 1 diabetes (T1D) and type 2 diabetes (T2D).

Methods: In this cross-sectional study, NAFLD status was evaluated by transient elastography in adult outpatients with T1D and T2D. NAFLD was defined as hepatic steatosis with or without fibrosis. Associations between insulin resistance related factors and NAFLD and advanced fibrosis (≥F3) were explored in T1D and T2D separately, using multivariate logistic regression models. Interaction analysis was performed to compare the associations in patients with T1D and T2D.

Results: One hundred and fifty patients with T1D (mean age 47 years, male 55%, mean diabetes duration 25 years, median BMI 25 kg/m^2) and 100 patients with T2D (median age 67 years, male 56%, median diabetes duration 17 years, mean BMI 30 kg/m^2) were included. NAFLD prevalence was 34% in patients with T1D and 84% in patients with T2D. Advanced fibrosis prevalence was 2.7% in patients with T1D and 22% in patients with T2D. In both patients with T1D and T2D, waist circumference, BMI and metabolic syndrome were positively associated, and estimated insulin sensitivity was negatively associated with the presence of NAFLD, adjusted for age, sex and diabetes duration. The *p*-value of the interaction term was not significant for any of the variables.

	OR (95% CI)	
WC (per 1 cm)	1.10 (1.06-1.15)	
	1.13 (1.04-1.24)	$p = 0.735$
BMI (per 1 kg/m^2)	1.25 (1.13-1.39)	
	1.39 (1.17-1.66)	$p = 0.140$
MetS (yes vs no)	5.23 (2.41-11.36)	
	3.80 (1.06-13.70)	$p = 0.811$
eGDR (per 1 mg/kg/min)	0.73 (0.61-0.87)	
	0.56 (0.38-0.81)	$p = 0.444$
SEARCH eIS (per 1 mg/kg/min)	0.53 (0.41-0.68)	
	0.48 (0.31-0.73)	$p = 0.810$
Insulin use (per 1 IU/kg bw)	6.38 (0.97-42.25)	
	2.34 (0.17-33.01)	$p = 0.642$
HbA1c (per 1 mmol/mol)	1.03 (0.99-1.06)	
	1.04 (0.98-1.11)	$p = 0.343$
TG (per 1 mmol/L)	3.09 (1.61-5.94)	
	1.53 (0.81-2.91)	$p = 0.100$
HDL chol (per 1 mmol/L)	0.25 (0.09-0.68)	
	0.48 (0.14-1.68)	$p = 0.268$

Conclusions: Despite differences in population characteristics and pathophysiology between T1D and T2D, insulin resistance related factors are similarly associated with NAFLD in both groups.