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ABSTRACT BOOK

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GENEVA, SWITZERLAND

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ePOSTER ABSTRACT PRESENTATIONS

OP-01Effect of sebelipase alfa on liver parameters over 96 weeks in a diverse population of children and adults with lysosomal acid lipase deficiency

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Background and aims: Lysosomal acid lipase deficiency (LAL-D) is a rare, progressive disease characterized by accumulation of cholesteryl esters and triglycerides in the liver that leads to dyslipidemia, hepatomegaly, and liver cell damage. Sebelipase alfa is a recombinant human lysosomal acid lipase (LAL) indicated for the treatment of LAL-D.
Method: In this multicenter, open-label study, eligible patients >8 months of age were given sebelipase alfa 1 mg/kg by IV infusion every other week for up to 96 weeks. Dose escalation to 3 mg/kg every other week and subsequently to 3 mg/kg weekly was allowed for patients who met protocol-defined criteria; dose reductions for tolerability were permitted to 0.35 mg/kg every other week. Reported here are effects on liver parameters at 96 weeks of sebelipase alfa exposure.
Results: A total of 31 patients were enrolled; median age was 12 years (range, 3-55 years). Descriptive analyses were performed for the overall population; data are summarized as median values. Clinical characteristics at baseline and week 96 are provided in the table. Marked reductions in alanine aminotransferase and aspartate aminotransferase levels were observed. Liver fibrosis improved or did not progress in 7 of 13 patients (54%) with pairwise samples at baseline and week 96 and with a baseline Ishak stage of 0 to 5. Three patients had a ≥1-point reduction in Ishak stage, including 2 patients who had a ≥2-point reduction. One of 3 patients with baseline stage of 6 and a pairwise sample at week 96 improved to stage 2. Sebelipase alfa was generally well tolerated. Most adverse events (AEs) were mild to moderate in severity. Three patients (10%) experienced infusion-associated reactions that were at most mild (n = 2) or moderate (n = 1) in severity. There were no discontinuations due to AEs. Two patients (6%) were positive for anti-drug antibodies on 1 occasion each; neither developed neutralizing antibodies.
Conclusion: Long-term treatment with sebelipase alfa was well tolerated and resulted in sustained improvements in markers of liver injury in this diverse population of patients with LAL-D.

Table:

Characteristic	Baseline (n = 31)	Week 96 (n = 27)	% Change From Baseline (n = 27)
ALT, U/L	63.5	34.0	−44.4
ALT ≤1.5x ULN, n (%)	13/31 (42)	22/27 (81)	
AST, U/L	65.5	42.0	−38.4
AST ≤1.5x ULN, n (%)	16/31 (52)	25/27 (93)	
UK-MELD score	46.5[a]	45.5[b]	−0.6[c, d]
Child-Pugh score	5.0[e]	5.0[b]	0[b]
Fibrosis, n (%)[f]	20/30 (67)	12/17 (71)	
Cirrhosis, n (%)[f]	8/30 (27)	4/17 (24)	
Liver volume, MN	1.4[g]	1.2[a]	−17.6[b, c]
Liver fat content, %	8.1[e]	6.5[a]	−14.9[c, h]
Spleen volume, MN	2.6[g]	2.2[h]	−16.5[c, i]

Results are medians. MN, multiples of normal; ULN, upper limit of normal.
[a]n = 24; [b]n = 22; [c]Patients for whom both a baseline and a week 96 measurement were available; [d]n = 17; [e]n = 28; [f]Fibrosis = Ishak score of 1-4; cirrhosis = Ishak score of 5-6; [g]n = 27; [h]n = 23; [i]n = 21.

OP-02YI Activating the Hormonal Effect of miR-122 Reverses NASH

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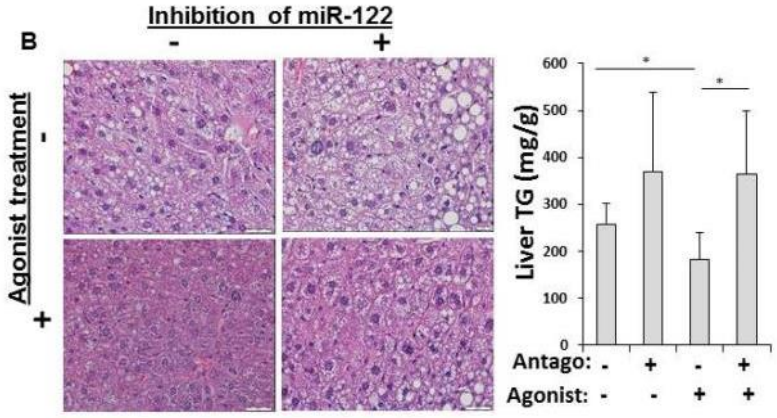
Background and aims: miR-122 is the most abundant liver microRNA consisting of 70% of the total miRNA population. miR-122 regulates metabolic pathways including cholesterol biosynthesis, fatty acid synthesis and oxidation. miR-122 is also found circulating in the blood. In non-alcoholic steatohepatitis (NASH) patients, miR-122 plasma levels are significantly high; while the hepatic levels of miR-122 are reduced. The mechanism by which miR-122 regulates lipid metabolism remained elusive. Moreover, miR-122 regulation by metabolites was not investigated. Currently, there is no medical treatment for NASH. Together with the fact that NASH is extremely common and might lead to hepatocellular carcinoma, effective medical treatments are needed.
Method: We analysed miR-122 promoter activity and validated its target mRNAs using the Luciferase reporter assay. We measured levels of microRNAs by quantitative real-time PCR analysis of RNA extracted from plasma, liver, muscle, and adipose tissues of high-fat-diet (HFD) fed C57BL/6 mice given a ROR-alpha agonist. miR-122 was inhibited using antagomiR-122. Metabolic profiles of mice were determined by histologic analyses of liver tissues.
Results: We found that the following a HFD regime, significantly enhance miR-122 promoter activity and miR-122 secretion from hepatocytes. We further show that miR-122 secreted from the liver, reaches periphery tissues where it represses its target genes. This result indicates that miR-122 acts as a hormone; it is expressed, secreted and functions in a circuitry homeostatic manner.
We identified a putative retinoic-acid-related-orphan-receptor-alpha, (ROR-alpha) site in the miR-122 promoter. We demonstrate that induction of miR-122 promoter activity by FFAs is ROR-alpha - dependent. We validated that two genes which are involved in triglyceride synthesis, are targets of miR-122. Accordingly, inhibition of miR-122 in HFD-fed mice resulted in increased body weight, accumulation of triglyceride (TG) levels in the liver and muscle tissues, and reduced beta-oxidation rates. Treating HFD-fed mice with a synthetic ROR-alpha agonist, increased miR-122 expression and secretion, and led to reduced steatosis and body weight.
Inhibition of miR-122 together with the ROR-alpha activator treatment abolished the beneficial activity of the ROR-alpha agonist on lipid metabolism, indicating that this effect is mediated by miR-122 activity.
Conclusion: We hypothesize that a metabolic circuitry exists wherein FFAs regulate miR-122 expression level via ROR-alpha, and miR-122 in turn, causes a metabolic shift in favor of the beta-oxidation pathway, by inhibiting enzymes involved in the TG synthesis pathway. Our results describe the systemic “hormonal” effect of this miRNA. We suggest that activating miR-122 by a ROR-alpha agonist might serve as a potential therapeutic approach to treat NASH.

Figure:



OP-03YI Human PNPLA3 I148M gene variant impairs secretion of polyunsaturated VLDL-TG and resembles the catalytically inactive PNPLA3 variant in mice

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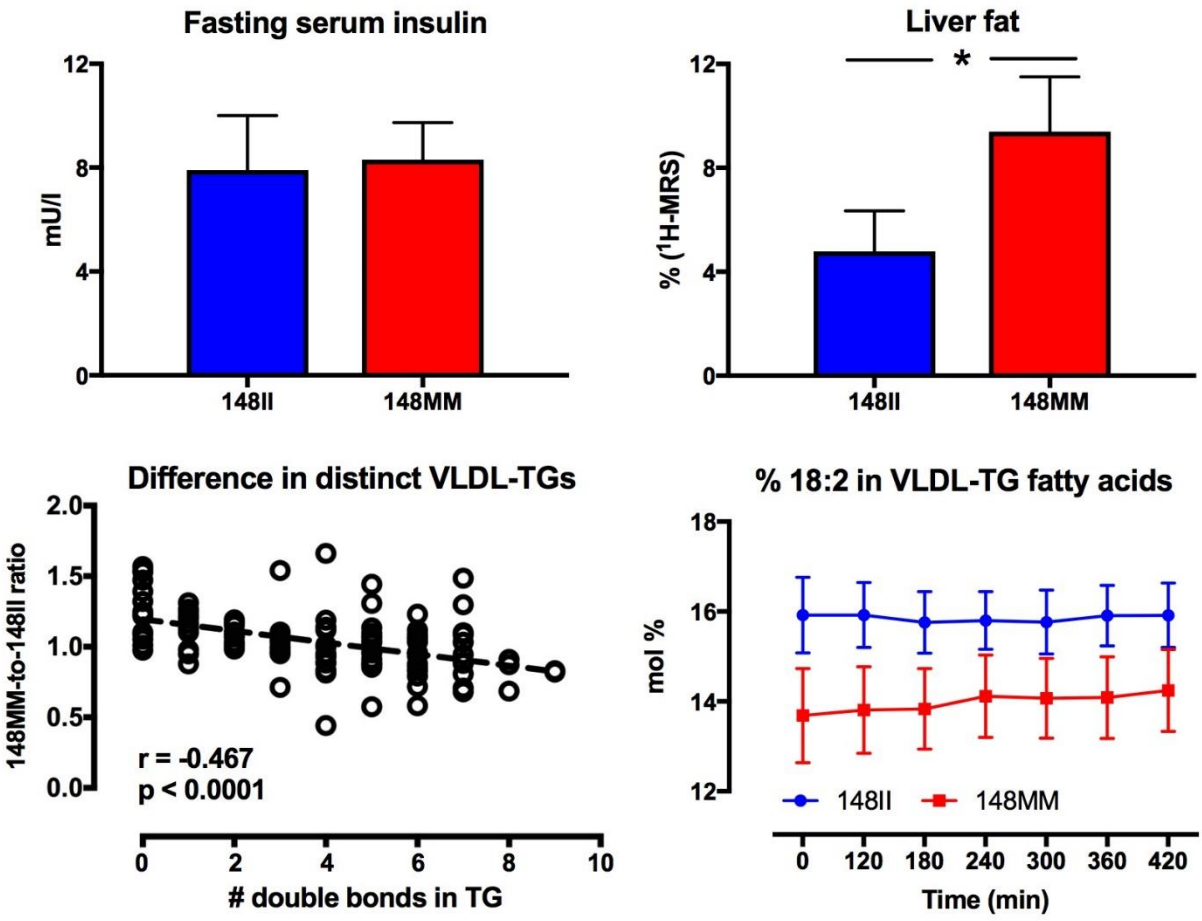
Background and aims: Knock-in mice expressing either the I148M variant or a catalytically inactive variant develop fatty liver disease. Detailed lipidomic profiling of liver lipidome showed opposite effects of the two models on polyunsaturated fatty acids in triglycerides (TGs). Previous human liver lipidomic analysis showed enrichment of polyunsaturated fatty acids in carriers of the I148M gene variant. Mouse and human PNPLA3 are 68% homologous. We determined whether function of the PNPLA3 I148M gene variant resembles either of the two models of fatty liver disease in mice by directly comparing handling of unlabeled and labeled dietary polyunsaturated and saturated fatty acids.
Method: We recruited 12 homozygous carriers (148MM) and 14 non-carriers (148II) of the PNPLA3 I148M gene variant. Liver fat content was measured by proton magnetic resonance spectroscopy (^{1}H-MRS). The subjects ingested a liquid meal containing equal amounts of polyunsaturated and saturated fatty acids labeled with ^{13}C-18:2 and ^{13}C-16:0 fatty acids. Before and for 420 min following the meal, the composition of VLDL-TGs was analyzed by *i*) ultra high performance liquid chromatography (UHPLC) and gas chromatography (GC) combined with mass spectrometry (MS), *ii*) GC for analysis of fatty acid composition of TGs and isotopic enrichments of labeled fatty acids.
Results: The 148MM and 148II groups were similar with respect to age (53.1 ± 2.2 vs. 52.4 ± 1.8 years, respectively, p = NS), BMI (31.8 ± 2.0 vs. 31.8 ± 1.5 kg/m^2, p = NS) and fasting insulin (8.3 ± 1.4 vs. 7.9 ± 2.1 mU/l, p = NS) but differed with respect to liver fat (9.4 ± 2.1 vs. 4.8 ± 1.6 %, p <0.05) (**Figure**). The TGs secreted from the liver in VLDL before and during the meal were depleted of polyunsaturated fatty acids in TGs both when absolute concentrations were measured using UHPLC-MS and relative concentrations using GC-MS (**Figure**). The ratio of ^{13}C-18:2 to ^{13}C-16:0 in VLDL-TG was significantly lower in the 148MM than the 148II group when related to the corresponding ratio in the chylomicron precursor pool (p <0.01).
Conclusion: Function of the human PNPLA3 I148M variant resembles the catalytically inactive PNPLA3 gene variant in mice and is characterized by inability of the liver to normally secrete polyunsaturated fatty acids in VLDL-TGs.
Figure: Fasting serum insulin concentration, liver fat content, difference in distinct VLDL-TG species, and proportion of 18:2 in VLDL-TG fatty acids in the groups. * p <0.05.


Fasting serum insulin
mU/l
12
8
4
0
148II
148MM
Liver fat
% (1H-MRS)
*
12
8
4
0
148II
148MM
Difference in distinct VLDL-TGs
148MM-to-148II ratio
2.0
1.5
1.0
0.5
0.0
r = -0.467
p < 0.0001
0
2
4
6
8
10
double bonds in TG
% 18:2 in VLDL-TG fatty acids
mol %
18
16
14
12
148II
148MM
0
120
180
240
300
360
420
Time (min)

OP-04YI The NLRP3 antagonist IFM-514 decreases fibrosis and inflammation in two mousemodels of non-alcoholic steatohepatitis (NASH)

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Background and aims: Activation of the NOD-like receptor protein 3 (NLRP3) inflammasome contributes to the development of non-alcoholic fatty liver disease and progression to non-alcoholic steatohepatitis (NASH). Therefore, this study tested a novel selective NLRP3 antagonist for mechanistic and therapeutic effects in two mouse models of NASH.
Method: Groups (n = 10) of 12-week-old ApoE-/- mice were fed *ad lib* for 7 weeks with a high-fat cholesterol-rich western diet (WD), and with the methionine/choline deficient (MCD) diet. After 3 weeks of diet-induced injury, mice were injected i.p. with the NLRP3 antagonist IFM-514 (100 mg/kg body weight) or vehicle (0.5% carmellose) every day, 5 days/week for a further 4 weeks. Portal pressure measured invasively via spleen pulpa, and plasma and liver were collected for histological and molecular readouts of fibrosis, inflammation and steatosis.
Results: IFM-514 reduced portal pressure without changes in liver-to-body weight ratio. The readouts for liver fibrosis, sirius red staining, hydroxyproline content, and *acta2*, *col1a1*, *tgfbeta1*, *timp1*, and *ctgf* mRNA expression levels were markedly reduced in both NASH models after IFM-514 treatment. Similarly, hepatic inflammation assessed by HandE staining, and *emr1*, *tnfalpha*, *Il1beta*, and *ccl2* mRNA expression levels, was also attenuated by IFM-514 compared to the vehicle-treated mice. The extent of hepatic steatosis was also reduced as evidenced by oil red O staining, hepatic triglycerides, and *srebp1* and *fas* mRNA expression levels.
Conclusion: IFM-514 reduced fibrosis, inflammation and steatosis in two different mouse models of NASH. These data suggest that blocking NLRP3 might be an attractive therapeutic approach for NASH.

P01-01YI Anti-fibrotic properties of OCA and INT-767 in an in vitro model of NASH

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Background and aims: An effective treatment for Non-Alcoholic Steatohepatitis (NASH) is still missing. Fibrosis is the most significant predictor of mortality in NASH. FXR agonists have been proposed as anti-fibrotic treatment. In this study, we assessed the anti-fibrotic effect of the FXR agonist obeticholic acid (OCA, INT-747) and the dual FXR/TGR5 agonist INT-767 in a well-established *in vitro* co-culture model reproducing NASH development.
Method: Human hepatoma (Huh7) and hepatic stellate (HSCs, LX2) cells were simultaneously co-cultured (SCC) at a 5:1 ratio and exposed to 1200 µM of free fatty acids (FFAs) without or with OCA or INT-767. Exposure in absence of FFAs was tested for side effects. Working concentrations were selected based on cell viability curves. Expression of ACTA2, Col1a1, FXR and SHP was evaluatedat 24, 96, and 144h. Extracellular collagen deposition and metalloproteinase 2 and 9 (MMP2-9) activity were evaluated at 96 and 144h (alphaLISA and Innozyme assay kit, respectively) and compared to the FXR agonists tropifexor and GS-9674.
Results: Based on cell viability, 0.1, 1 and 10 µM were selected for both OCA and INT-767. Exposure to FFAs induced HSCs activation (ACTA2 and Col1a1 ($p < 0.05$)) and extracellular collagen deposition ($p < 0.01$ at 144h) vs. CTRL. Compounds co-treatment did not affect ACTA2 and Col1a1 gene expression, while OCA, and to a lesser extent INT-767, significantly reduced FXR and induced SHP expression.
Interestingly, OCA induced a dose-dependent reduction of collagen at both 96h ($p < 0.05$ and 0.01 vs. FFAs, with 1 and 10 µM, respectively) and 144h ($p < 0.01$ with 10 µM). Similarly, INT-767 induced collagen reduction (1 µM at 96h, $p < 0.05$). Tropifexor was more effective at 0.5 and 0.1µM at 144h ($p < 0.05$), while GS-9674 at 96h ($p < 0.01$) but this effect did not persist over time. Neither OCA nor INT-767 altered collagen deposition in the absence of FFAs.
MMP2-9 activity was reduced at 96h in FFA-treaded cells ($p < 0.05$ vs. DMSO), while OCA couneracted this effect ($p < 0.05$). INT-767 also induced a slight increase in MMP2-9 activity. No significant modulation of MMP2-9 activity was induced by tropifexor or GS-9674.
Conclusion: All FXR agonists tested reduce collagen deposition. OCA exerts a more potent and long-lasting effect (-50% vs. FFAs) compared to INT-767 (-30%), tropifexor (-20%) and GS-9674 (-35%). These effects appear to be related to modulation of the FXR pathway, and to increased extracellular matrix degradation as indicated by enhanced MMP2-9 activity.
This work was supported by Intercept Pharmaceuticals.

P01-02YI Deletion of IL-4 Receptor alpha on macrophages attenuates inflammation and fibrosis in murine non-alcoholic steatohepatitis (NASH)

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Background and aims: The role of immune cell populations in NASH-related inflammation and fibrosis remains controversial. However, targeting of specific receptors that switch immune cell phenotypes is attractive. We aimed to study the role IL4 receptor alpha (IL4Ra) which represents a central switch to generate Th2 T cells and M2-type macrophages upon stimulation with interleukin (IL)4 and IL13 in a representative mouse model of NASH.
Method: 8-week-old male Balb/C wild type mice and Balb/C mice with general or macrophage specific deletion of IL4Ra (Balb/C IL4R$^{-/-}$ and LysM^{cre}IL4Rα$^{-/lox}$) mice were fed a choline-deficient, L-amino acid-defined (CDAA) or a choline supplemented (CSAA) diet for an additional 12 weeks. Upon feeding the CDAA diet both KO strains displayed a significantly attenuated weight gain compared to the wild type mice.
Results: HandE stained sections revealed a significant reduction of the NAS score adapted for mice from 6 to 4, and the extent of fibrosis score is reduced to 1 in both IL4Ra $^{-/-}$ strains. Hydroxyproline quantification and Sirius red morphometry confirmed a significant (by 50%) reduction of collagen accumulation in the knockout vs wildtype animals. In wild type mice, steatosis was >66% and macrovesicular, whereas steatosis was reduced to 50%, more concentrated in zone 3 and prominently microvesicular in the IL4Ra knockout mice. Moreover, alpha SMA morphometry indicated a significant reduction of hepatic stellate cell activation, CD68^{+} macrophages, Ki67^{+} hepatocytes, and MPO^{+} neutrophils in both IL4Ra-/- mouse lines were reduced compare to wt mice. RT PCR showed significantly suppressed hepatic transcript levels for col1a1, tgfb1, mmp9 and tnfa, and in FACS analysis a significant reduction of CD 11b^{+}F4/80^{-}Ly6G^{-}Ly6c^{high} inflammatory monocytes-macrophages in the knockout mice vs their wildtype controls.
Conclusion: 1. Ablation of the IL4Ra on monocytes-macrophages and in general comparably suppressed steatosis, inflammation and fibrosis in the CDAA model of NASH; 2. IL4Ra targeted therapies, including inhibition of IL4 and IL13 is a potential therapy of fibrotic NASH.

P01-03 HFD mouse model displays altered copper-related gene expression

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Background and aims: Copper (Cu) is an essential biometal involved in cellular protection from oxidative stress. Interestingly, altered Cu homeostasis has been documented in several chronic diseases, including Non-Alcoholic Fatty Liver Disease (NAFLD) and was indicated as a negative prediction marker of cardiovascular risk. Moreover, NAFLD-cirrhotic patients are characterized by increased Cu serum levels, even more evident in patients affected by NAFLD-related hepatocellular carcinoma. **Aim:** We wonder to evaluate the regulation of Cu homeostasis in liver and heart during NAFLD. Thus, we analyzed Cu-related gene expression in livers and hearts of a mouse model of liver steatosis (HFD mice).
Method: Sixty C57BL/6J mice were divided into six groups and fed with Normal-Fat Diet (NFD) or High Fat Diet (HFD). After 3, 6 and 12 months, livers and hearts were collected. Biochemical parameters and histological features were evaluated. Cu levels were measured by atomic absorption spectrometry. Gene profiling was performed using the Mouse Adipogenesis and Oxidative Stress Qiagen Array kits.
Results: A progressive significant down-modulation of Cu content was highlighted in the livers of HFD mice *vs* control. No differences on Cu levels were detected in the hearts of NFD and HFD mice. Of note, these results were in line with histological characteristics, highlighting an elevated fat deposition in the livers, but not in cardiac tissues of HFD mice. Moreover, Cu amounts were positively correlated with triglycerides levels in HFD mice. Microarray analysis showed a differential pattern of expression of several Cu-related genes in NFD *vs* HFD mice. In particular, the overexpression of *Ppara* and *Rxra* was observed in the liver, whereas *Adig*, *Ccnd1*, *Dio2*, *Klf4*, *Ppargc1a*, *Prdm16*, *Rb1*, *Sfrp1* and *Wnt5a* were overexpressed in the heart of HFD mice. Moreover, looking at the oxidative gene profile, we remarked a differential expression between liver and heart of genes coding for key antioxidant enzyme, such as *Cat* and *Gpx1*, significantly overexpressed in the liver respect to the heart. Interestingly, *Gpx8* was upregulated specifically in the heart of HFD mice. Lastly, a negative correlation between specific Cu-related genes (*Slc31a1, Slc31a2, Sod1, Sod2* and *Ccs*) and Cu intratissutal concentrations in the liver and heart of HFD mice was found.
Conclusion: Overall, our findings point out a tissue specific and Cu-related regulation of adipogenesis and oxidative stress-related gene expression in NAFLD.

P01-04YI Cytokeratin 18 fragment level is a useful biomarker in predicting steatosis and NASH but not fibrosis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease (from minor steatosis to cirrhosis). Differentiating various stages of the disease is important for therapeutic decision making and prognostic outcomes. The gold standard for the diagnosis and staging of NAFLD is liver biopsy. Cytokeratin 18 fragment (CK18-F) levels are a marker of hepatocyte apoptosis. The aim of this study was to evaluate the role of serum CK18-F in predicting steatosis, significant non-alcoholic steatohepatitis (NASH) and fibrosis when compared to the gold standard i.e liver biopsy.
Method: 88 patients with biopsy proven NAFLD were enrolled. Histological findings were classified according to the NAFLD activity score (NAS) proposed by the Non-alcoholic Steatohepatitis Clinical Research Network. In addition to basic serum biochemical profile, serum CK18-F level was measured using M30 Apoptosense ELISA. Patients with diabetes mellitus and significant alcohol consumption were excluded.
Results: Of the 88 patients, 59 (67%) had a NAS score of ≥5 suggesting histopathological NASH and 33% patients had a score of <5. The mean level of CK18-F was significantly (p 0.024) higher (382 ± 138 U/L) when significant steatosis (steatosis grade 2 or 3 on NAS) was present as compared to when there was no/mild steatosis [(320 ± 106.8 U/L) (steatosis grade 0 or 1 on NAS scoring)]. There was a statistically significant difference in the level of CK18-F in the group with NASH (NAS ≥5) when compared to the group with no NASH (NAS<5). Even though the CK18-F level was higher in the group with fibrosis grade 1-2 (381.9 ± 139.22 U/L) as compared to the group with no fibrosis (334.6 ± 117.05 U/L), this was not statistically significant (P 0.087). The AUROC for detection of NASH for CK18-F was 0.82. A value of 304 U/L was 83.1 % sensitive and 82.8% specific for the diagnosis of significant NASH. The AUROC for fibrosis detection using CK18-F was 0.62. A value of 325.5 was 62% sensitive and 61% specificity for diagnosis of fibrosis.
Conclusion: Measurement of serum CK18-F was useful in prediction of significant steatosis and NASH but not fibrosis.

Figure: Correlation between histopathology and CK18-F

	CK-18 fragments (Mean ± SD)	p value
Steatosis 0-1 (n = 36) 2-3 (n = 52)	320.08 ± 106.84 382.99 ± 138.38	**.024**
NAS ≥5 (n = 59) <5 (n = 29)	395.82 ± 135.83 278.79 ± 66.55	**0.0001**
Fibrosis Grade 0 (n = 46) Grade 1-2 (n = 42)	334.16 ± 117.05 381.97 ± 139.22	.087

P01-05 Endothelial disfunction as a mechanism of the hepatic microcirculation disorders and its correction at the non-alcoholic steatohepatitis patients

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Background and aims: the basis for the formation of portal hypertension is an increase of intrahepatic vascular resistance due to not only mechanical components (inflammation, fibrosis), but also a dynamic components, which are initiated in early stage of liver diseases. Our aims was to study endothelial disfunction as feature of intrahepatic hemodynamics disorders at the non-alcoholic steatohepatitis patients (NASH) with initial stage of liver fibrosis.
Method: we investigated 45 NASH patients with liver fibrosis 0-1stage, determined by liver transient elastography (Fibroscan) and liver biopsy (METAVIR). For the estimation of liver microcirculation we used non-invasive method of modificated liver impedansometry- polyhepatography (PHG). Endothelial function was estimated by peripheral arterial tonometry (EndoPAT-2000, Israel). For correction of hepatic blood flow disorders we used L-ornithine-L-aspartate.
Results: at all patients we revealed the disorders of the intrahepatic microcirculation on sinusoidal level (out-flow) with increase of basic resistance and decrease of blood filling of the liver. The endothelial disfunction was identified at the patients: low index of active hyperemia (RHI)-1.44 (normal RHI >1.67). L-ornithine-L-aspartate improved liver microcirculation in all patients after treatment in during control PHG.
Conclusion: at the NASH patients with even initial stages of liver fibrosis we detected hepatic microcirculation disorders in sinusoidal zone. NASH is characterized by signs of endothelial disfunction. L-ornithine-L-aspartate improves liver microcirculation.

P01-06 Non-invasive evaluation of liver fibrosis, steatosis, and non-alcoholic steatohepatitis in biopsy-proven NAFLD patients

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is becoming a major cause of chronic liver disease worldwide. In this broad spectrum disease, the development of a non-invasive method is urgently needed to identify more severe form of disease including non-alcoholic steatosis and advanced fibrosis. In this study, we compared hepatic fibrosis and steatosis using MR imaging and transient elastography (TE) and tried to find non-invasive diagnostic marker for NASH and advanced fibrosis.
Method: This is a multicenter prospective study of patients with biopsy-proven NAFLD. The patients were underwent laboratory test, liver biopsy, MRI and TE 6 months before enrollment. MRI examination included mDIXON, MR spectroscopy (MRS), and MR elastography (MRE). TE measured liver stiffness and controlled attenuation parameter (CAP).
Results: Ninety-four patients with biopsy-proven NAFLD patients were enrolled from October 2016 to March 2018. Mean age and BMI were 51.29 ± 13.38 years and 29.12 ± 5.64 kg/m2, respectively. Female was dominant (58, 61.7%) and other co-morbidities were diabetes (n = 37, 39.4%), hypertension (n = 39, 41.5%) and dyslipidemia (n = 28, 29.8%). For diagnosis of advanced fibrosis (stage 3-4), the AUROC of MRE tended to be superior (0.844; 95% CI, 0.748-0.915) comparing with TE (0.787; 95% CI, 0.683-0.870) (p = 0.272). For diagnosis of severe steatosis (stage 2-3), CAP (0.706; 95% CI, 0.595-0.802) showed lower AUROC compared with mDIXON (0.832; 95% CI, 0.733-0.905; p = 0.027) and MRS (0.842; 95% CI, 0.744-0.913; p = 0.029), respectively. Age, BMI, DM, dyslipidemia, AST, platelet are associated with NASH in univariate. In multivariate analysis AST, PLT, and MRE were significant factor for diagnosis of NASH.
Conclusion: MRI (mDIXON, MRS and MRE) tended to identify more severe steatosis and fibrosis compared to TE in patients with biopsy-proven NAFLD. AST, PLT, and MRE were significant factor for diagnosis of NASH. Non-invasive modalities using AST, platelet, and MRI could be potential tools for diagnosis of NASH.

P01-07YI Genetic susceptibility to increased intestinal permeability is associated with diabetes and progressive liver disease in patients with non-alcoholic fatty liver disease (NAFLD)

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Background and aims: Many studies demonstrate that increased intestinal permeability (IP) plays a key role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM). Considering the great impact of Gut-Liver Axis in NAFLD pathogenesis, genes involved in the regulation of intestinal barrier integrity and modulation of IP are perfect candidates in exploring SNPs that might impact on fatty liver disease severity.
The aim of our study was to assess whether a single nucleotide polymorphisms (SNP) (rs2542151 G→T) of Protein Tyrosine Phosphatase Non-Receptor Type 2 (PTPN2), known to be involved in regulation of IP, is associated with severity of NAFLD (non-alcoholic steatohepatitis -NASH- and/or liver fibrosis) and type 2 diabetes mellitus.
Method: We recruited a prospective consecutive cohort of NAFLD cases and healthy controls among Caucasian patients from two Italian tertiary care centers. PTPN2 genotype was assessed both in patients and controls. Clinical data, anthropometrics and laboratory data were collected for each patient. A subgroup of patients (345) underwent liver biopsy. Unconditional multiple logistic regression models were used to investigate the association between selected SNP (PTPN2 rs2542151 G→T), comorbidities and histological severity of liver disease.
Results: We enrolled 566 cases (males 64, 6%, mean age 45, 3 ± 13, 8 ys) and 377 controls (males 67, 1%, mean age 41, 3 ± 3, 1 ys). PTPN2 genotype distribution was consistent with Hardy-Weinberg equilibrium both in controls' and in patient's cohort and didn't significantly differ between NAFLD patients and controls. Liver biopsy was available for 345 patients (60, 9%); 198 (57.4%) had NASH and 75 (21.7%) had advanced fibrosis (F3). In patients' population, considering a genetic dominant model, the multiple logistic regression analysis showed that PTPN2 rs2542151 G→T is associated with T2DM (OR 1.74 95% CI 1.03-2.93, p = 0.04) independently from age, BMI and sex. At a subgroup analysis of patients who underwent liver biopsy, rs2542151 G→T of PTPN2 was associated with the presence of severe steatosis (OR 2.19 95% CI 1, 35-3.55 p <0, 01), NASH (OR 1.81 95% CI 1, 13-2.90 p <0, 05) and severe fibrosis (OR 2.55 95% CI 1, 50-4.33 p <0, 01) independently from age and sex.
Conclusion: Our study shows that rs2542151 G→T of PTPN2 is associated with the severity of fatty liver disease (steatosis, fibrosis and NASH) histologically assessed. Furthermore, rs2542151 G→T of PTPN2 is associated with the presence of T2DM in patients affected by NAFLD. These results suggest that individual genetic susceptibility to an impaired IP could be a key factor in pathogenesis and disease progression of fatty liver disease and in the onset of type 2 diabetes mellitus in NAFLD patients.

P01-08 Comparison of FS3 with different biomarkers to identify patients with active NASH (NAS≥4) and advandced fibrosis (F≥2)

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Background and aims: Given the upcoming market release of drugs for non-alcoholic steatohepatitis (NASH) patients at risk of progression to cirrhosis or with cirrhosis, Echosens have developed a simple score named FS3 based on three biomarkers (FibroScan liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) and ASAT) to detect patients with active NASH (NAS≥4) and advanced fibrosis (F≥2). The objective of this study is to compare the FS3 performance with non-invasive markers of fibrosis or steatosis or other blood parameters linked to liver damages to detect patients with NASH+NAS≥4+F≥2.

Method: Patients with suspected NASH prospectively underwent FibroScan and liver biopsy (LB) at 7 British centres. LB were read in a blinded manner with consensus by two expert pathologists (VP and PB). NASH was diagnosed using the FLIP algorithm. Diagnostic accuracy to detect patients with NASH+NAS≥4+F≥2 was compared using area under the ROC curve (AUC) for the FS3, Fibroscan LSM and CAP, Fib4, NAFLD fibrosis score (NFS), BARD score, ASAT to platelet ratio index (APRI), ASAT/ALAT ratio (AAR), hepatic steatosis index (HSI), ASAT, ALAT, hyaluronic acid (HA) and alpha-2 macroglobulin (A2M). AUC of the best biomarkers (with the highest AUC) was compared to others using Delong test.

Results: 297 patients were analyzed. Median body mass index (BMI) was 32.3 [inter-quartile range = 7.8] kg/m2, age 54 [19] years. 42% were female. 61% had NASH, 63% had NAS≥4, 57% had F≥2 and 44% had NASH (NAS≥4+F≥2). Comparison of all biomarkers is given in Table 1.

Conclusion: The FS3 which is a simple algorithm based on FibroScan E, CAP and ASAT developed to detect patients with NASH (NAS≥4+F≥2) outperforms the detection of the patient with active NASH (NAS≥4) and advanced fibrosis (F≥2) when compared with other biomarkers of liver damages. The application of FS3 score is very promising for identifying patients eligible for treatment when drugs will be market approved as well as the screening of patients for drug trials.

Table 1: Comparison of all biomarkers to detect patients with NASH+NAS≥4+F≥2

Biomarker	FS3	E	CAP	ASAT	A2M	Fib4	HA	NFS	APRI	BARD	ALAT	AAR	HSI
AUC *[95% CI]*	**0.83** *[0.78-0.87]*	**0.73** *[0.68-0.79]*	**0.71** *[0.65-0.77]*	**0.71** *[0.65-0.77]*	**0.70** *[0.64-0.76]*	**0.69** *[0.63-0.75]*	**0.69** *[0.63-0.75]*	**0.68** *[0.62-0.74]*	**0.68** *[0.62-0.74]*	**0.63** *[0.57-0.69]*	**0.63** *[0.57-0.69]*	**0.58** *[0.51-0.64]*	**0.55** *[0.48-0.61]*
P value Delong test (/FS3)	-	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-3}$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<10^{-6}$	$<10^{-7}$	$<10^{-8}$	$<10^{-10}$	$<10^{-11}$

P01-09 ACCi/DGAT2i combination therapy for the treatment of NASH

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Background and aims: Disordered lipid metabolism and elevated lipogenic flux underlie the pathogenesis of NAFLD/NASH. Acetyl-CoA carboxylase (ACC) and diacylglycerol acyltransferase 2 (DGAT2) are two a key enzymes regulating lipid metabolism. Pfizer is presently developing both an ACC inhibitor (ACCi) and a DGAT2 inhibitor (DGAT2i) for the treatment of NASH with fibrosis. Combination of the two therapies may provide greater patient benefit than can be achieved with any single pharmacological mechanism of action (MoA). Mechanistically, an ACCi/DGAT2i fixed-dose combination has the potential to re-balance the elevated lipogenic tone in NAFLD/NASH by the following mechanisms: 1) inhibiting de novo fatty acid synthesis, 2) promoting fatty acid oxidation, and 3) inhibiting expression of key lipogenic genes. Further, inhibition of DGAT2 may suppress the elevations in circulating TGs observed at high doses of the ACCi, a known consequence of complete ACC inhibition.
Method: Chronic oral administration of ACCi, DGAT2i and co-administration of ACCi and DGAT2i was evaluated in the Western diet induced steatotic rat model.
Results: Administration of ACCi and DGAT2i as monotherpies resulted in dose-dependent suppression in hepatic steatosis. However, relative to DGAT2i monotherapy, administration of ACCi resulted in a greater reduction of hepatic steatosis. Co-administration of ACCi and DGAT2i suppressed hepatic steatosis in a dose-dependent manner and to a greater magnitude than either agent as monotherapy. Administration of ACCi as a monotherapy resulted in an elevation in circulating TGs in the fed and fasted state, relative to vehicle administered Western diet fed animals. Administration of DGAT2i as a monotherapy resulted in dose-dependent decreases in circulating TGs. Co-administration of ACCi and DGAT2i resulted in dose dependent reductions in circulating TGs, completely blocking the ACCi mediated increase in circulating TGs. Relative to vehicle administered Western diet fed rats, administration of ACCi as a monotherapy increased nuclear localization of SREBP-1, indicative of increased SREBP-1c activation. Co-administration of ACCi and DGAT2i blunted the ACCi mediated increase in SREBP-1 nuclear localization and SREBP-1 activation. Additionally, co-administration of ACCi and DGAT2i blunted the ACCi mediated increase in hepatic SREBP-1c and target gene expression.
Conclusion: Thus, an ACCi/DGAT2i combination has the potential to deliver greater efficacy than either monotherapy and to mitigate ACCi mediated increases in circulating TG levels.

P01-10 Lysosomal acid lipase deficit alters the response to therapy of NASH patients

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Background and aims: Lysosomal acid lipase is an enzyme that is active in the intracellular lipid metabolism, especially in hepatocytes, where it interacts with the metabolism of cholesterol esters and triglycerides. When there is a lack of this enzyme (LAL-D), an accumulation of esterified cholesterol and triglycerides takes place in lysosomes which results in a lower cell availability of free cholesterol thus inducing an increase in the synthesis of endogenous cholesterol. This is a very rare condition and it is estimated to have an incidence of 1 case out of 100, 000. If LAL-D is not promptly diagnosed in babies, it can lead to premature death within 6 months. In the following years, the clinical picture can be distinguished into 2 phenotypes: Wolman disease (WD) is diagnosed by the paediatrician, whereas cholesteryl ester storage disease (CESD) manifests itself in adulthood. In both cases, the onset of hepatic steatosis and the alteration of the lipid profile are peculiar aspects of the disease.
The aim of this project was to characterize the prevalence of this condition in the patients with hepatic steatosis who did not responde to conventional treatment for dyslipidaemia and NASH.
Method: The main methods to identify enzyme deficiency of the lysosomal acid lipase are Dried Blood Spots (DBS), a highly-specific non-invasive test to assess enzyme activity of LAL-D, based on collecting a few blood drops and the genetic tests.
Among the patients treated in our unit, those with a clinical presentation compatible with LAL-D were investigated: we selected the patients most suitable for the test out of 210 being treated for NAFLD and poorly responding. The main criteria to perform the test are: BMI<28, ALT>40 mg/dL, LDL>160 mg/dL, HDL<40 mg/dL in males <50 mg/dL in females, possible liver damage, cryptogenic cirrhosis and presence of microvesicular/mixed steatosis. The patients were selected, and DBS test was performed on them.
Results: The results have shown that about 20% of patients have a partial enzyme deficiency, ranging from 30 to 40% of reduction and no total deficit was found. Based on the data that we collected, we have as foreseen outlined a food plan, since a conventional drug treatment cannot be provided for these patients with partial deficiency. The food plan was created to compensate the enzyme deficiency by balancing meals through load and glycemic index and selected low-fat food. Already after 3 months we could asses a decrease in the hepatic enzyme not seen during the precedent therapeutic approaches.
Conclusion: The number of LAL-D is higher in our patients compare to the expected prevalence in the population. In these patients with partial deficit no specific drug is supplied but we achieved a positive result with a nutritional approach.

P01-11Effectsof Pioglitazone and L-ornithine-L-aspartate LongTherapy on NASH course in Patients with Diabetes Mellitus Type 2

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Background and aims: Non-alcoholic steatohepatitis (NASH) occurs during the progression of non-alcoholic fatty liver disease. Diabetes Mellitus type 2 (DM2) has an adverse effect on the course of NASH. The aim of this study was to investigate the effects of pioglitazone and L-ornithine-L-aspartate on lipid metabolism, markers of insulin resistance (IR), fibrosis and histological liver changes in NASH with DM2 patients (ps).
Method: In 44 NASH with DM2 ps [age, 45.6 ± 5.5 yr; body mass index (BMI), 33.9 ± 3.2 kg/m^2; glycated hemoglobin (HbA_1C), (8.92 ± 0.75)%] and 10 healthy volunteers [age, 45.8 ± 4.8 yr; BMI, 25.3 ± 2.4 kg/m2] obesity and IR were determined by BMI, dyslipidemia and HOMA-IR. Circulating insulin, leptin, cytokeratin 18 (CK18 M30, CK18 M65M), fibroblast growth factor 21 (FGF21), connective tissue growth factor (CTGF), and type IV collagen levels were measured by ELISA. Histologic liver changes of the hepatocytes were determined. 15 ps were treated with metformin 1500 mg/day (group I), 15 ps were treated with metformin 1500 mg/day and L-ornithine-L-aspartate 9 g/day (group II), 14 ps were treated with metformin 1500 mg/day and L-ornithine-L-aspartate 9 g/day and pioglitazone 15-30 mg/day for year (group III).
Results: After treatment in ps of group I has increased of HOMA-IR (p = 0.01), circulating triglycerides (p = 0.01), low density lipoprotein cholesterol (LDLC) (p = 0.02), leptin (p = 0.009), CK18 M30 (p = 0.006), CK18 M65M (p = 0.004), FGF21 (p = 0.006), CTGF (p = 0.003), and type IV collagen (p = 0.007); this associated with an increase of the histological parameters: steatosis (p = 0.008), inflammation (p = 0.02), ballooning (p = 0.03), fibrosis (p = 0.009). In groups II and III we observed reductions in circulating triglycerides (p = 0.03 vs. p = 0.008), LDLC (p = 0.02 vs. p = 0.006) and histological parameters of inflammation (p = 0.01 vs. p = 0.003) and ballooning (p = 0.04 vs. p = 0.008). Compared with groups I and II, in the III group HOMA-IR (p = 0.008), circulating leptin (p = 0.01), FGF21 (p = 0.009), CK18 M30 (p = 0.007), CK18 M65M (p = 0.008), CTGF (p = 0.01) and type IV collagen (p = 0.03) decreased, which were associated with a reduction of steatosis (p = 0.003) and fibrosis (p = 0.01).
Conclusion: The pioglitazone and L-ornithine-L-aspartate combination with metformin for year therapy has metabolic, anti-inflammatory and antifibrotic effects and reduces progression of NASH course in NASH with DM2 patients.

P01-12 Development of a novel nomogram to detect significant liver fibrosis in biopsy-proven non-alcoholic fatty liver disease

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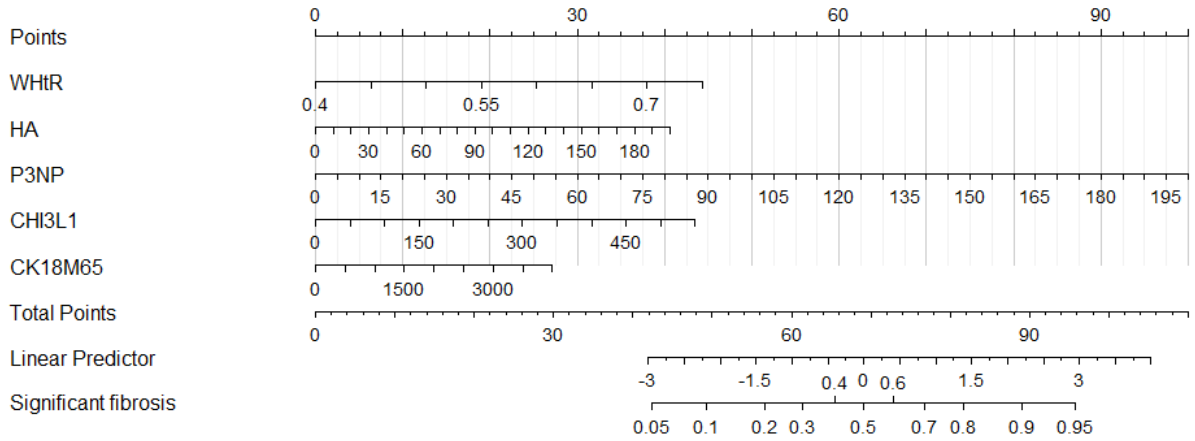
Background and aims: Fibrosis is deemed as a pivotal determinant of the long-term prognosis in non-alcoholic fatty liver disease (NAFLD). In this study, we aimed to develop a novel nomogram-based non-invasive model to accurately predict significant fibrosis in NAFLD patients.

Method: We designed a large prospective cohort study including 232 biopsy-proven NAFLD patients recruited from 2016.12 to 2018.1. Detailed anthropometric and fibrosis-related laboratory parameters, including hyaluronic acid (HA), procollagen-III-peptide (P3NP), chitinase-3-like protein 1 (CHI3L1), CK-18 M65 et al, were collected. The nomogram was compared with NAFLD fibrosis score (NFS), FIB-4 and BARD. Significant fibrosis was defined as ≥F2. Diagnostic accuracy was assessed according to AUROC, sensitivity, specificity, and positive and negative predictive values and likelihood ratios.

Results: Variables included in nomogram were: waist to hip ratio, HA, P3NP, CK-18 M65 and CHI3L1, which were independently associated with significant fibrosis (Figure). The discrimination ability of nomogram (AUROC = 0.850, 95%CI 0.788-0.913) was significantly superior to NFS (AUROC = 0.619, 95%CI 0.502-0.735), FIB-4 (AUROC = 0.643, 95%CI 0.534-0.753) and BARD (AUROC = 0.594, 95%CI 0.481-0.707) for significant fibrosis (all p <0.001).

Conclusion: This novel nomogram was more accurate, calibrated, and achieved higher benefit than NFS, FIB-4 and BARD. It could be useful as non-invasive method to screen fibrosis in the overall population with NAFLD.

Figure:



P01-13 A gut hormone can play a role in NAFLD

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Background and aims: Ghrelin is a gut hormone with various functions including energy metabolism and inflammation inhibition. Nuclear factor-kappa B (NF-κB) participates in the initiation and the progression of inflammation, particularly, the cardiovascular and adipose tissue inflammation. To date the combined role of Ghrelin and NF-κB in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) is a matter of debate. We investigated whether acyl ghrelin level and NF-κB could interplay a role in lipid metabolism and inflammatory injury in NAFLD.
Method: Ninety three adult participates were included in the study, 28 patients had proved non-alcoholic steatohepatitis (NASH), and 40 patients had simple steatosis, as well 25 healthy subjects, matched for age, gender and Body mass index (BMI) to the patients were included in the study as a healthy control group. Full history and clinical examination, abdominal ultrasonography and liver biopsy were done when indicated. Liver function tests, lipid profile, blood sugar, insulin and C- peptide, fasting insulin, and plasma acyl ghrelin concentrations were measured. Nuclear NF-kB microRNA expression was measured by quantitative real time polymerase chain reaction.
Results: Fasting insulin, insulin C-peptide, homeostatic model assessment insulin resistence (HOMA-IR), alanine amino-transferases (ALT) and gamma glutamyl transpeptidase (GGT) were significantly increased and high density lipoprotein cholesterol (HDL-C) was significantly decreased in NAFLD group compared to control group. In addition, a significant increase in ALT, GGT, fasting insulin, insulin C peptide and HOMA-IR were detected in the NASH group compared to group of simple steatosis. The plasma levels of acyl-ghrelin was significantly decreased in NAFLD groups compared to normal control group, the lowest level was detected in NASH group as compared to group of simple steatosis. The expression of NF- κB microRNA was significantly increased in NAFLD groups compared to normal control group and its level was significantly increased in NASH compared to simple steatosis. The NF-kB mRNA was positively correlated with BMI, HOMA-IR, ALT, fasting insulin, insulin C-peptide and liver histopathology and acyl-ghrelin was inversely correlated with BMI, HOMR-IR, ALT, fasting insulin, insulin C peptide and liver histopathology. . Both were significantly correlated with HDL-C.
Conclusion: Acyl ghrelin attenuated NAFLD-induced liver injury through down regulation of NF-κB and they are associated with disease progression. Further large scale studies are recommended to consider ghrelin as promising drug for the prevention and treatment of NAFLD.

P01-14YI Increased liver expression of Vitamin D Receptor is associated with NAFLD, visceral obesity and adipose tissue inflammation

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in the Western countries; mechanisms behind the development and progression of NAFLD and steatohepatitis (NASH) are still debated. Besides, vitamin D is a hormone exerting relevant influence on several metabolic and inflammatory pathways and a direct effect into the liver has been hypothesized and partially proven in both humans and animal models. Recently, knock-out mice for vitamin D receptor (VDR) have been shown to be protected from hepatic fat accumulation. Obesity and chronic adipose tissue (AT) inflammation are major determinants of NAFLD and AT represents the main site of vitamin D accumulation in humans. Whether a relationship exists between liver expression of VDR in NAFLD/NASH and the presence of visceral AT (VAT) inflammation in obesity has not been investigated yet. Therefore, aim of this study was to investigate the hepatic expression of VDR in obese patients with and without NAFLD/NASH in relation to the presence of VAT inflammation and local VDR expression.
Method: Forty consecutive obese individuals candidate to bariatric surgery (M/F: 11/29, mean ± SD age: 43.7 ± 9.6 years) were recruited at Sapienza University of Rome, Italy; intra-operative liver and VAT biopsies were performed for detecting NAFLD/NASH and VAT inflammation. VDR expression was evaluated by immunohistochemistry (IHC) and rt-PCR, VAT inflammation was determined through IHC and the analysis of specific genes' expression using rt-PCR.
Results: Simple steatosis was diagnosed in 50% and NASH in 25% participants; hepatocyte VDR expression linearly correlated with greater intrahepatocyte steatosis ($p = 0.02$), whereas VDR mRNA expression positively associated with NAS steatosis ($p = 0.02$) and inflammation ($p = 0.002$) scores. A significant association was found between greater liver VDR mRNA expression, waist circumference ($p = 0.04$), VAT VDR expression ($p = 0.01$) and VAT inflammation, as indicated by increased macrophage infiltration ($p = 0.008$) and greater VAT expression of inflammatory markers such as IL-8 ($p = 0.01$), MIP1a ($p = 0.04$), MIP2 ($p = 0.002$) and DPP4 ($p = 0.01$).
Conclusion: VDR is overexpressed in hepatocytes in the presence of NAFLD; however, in more severe stages of the disease VDR expression does not show further increases. Therefore, VDR expression in hepatocytes may be likely induced by aberrant hepatic fat accumulation *per se* and specifically associated with VAT accumulation and inflammation.

P02-01YI Effect of Silybin on fibrogenesis in an in vitro model of NASH

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Background and aims: Silybin (milk thistle) is a natural bioactive compound known for its wide hepatoprotective properties. Non-alcoholic Fatty Liver Disease (NAFLD) is a liver disease without an efficient and long-lasting therapy.
The aim of this study is to assess the *in vitro* effect of Silybin in a well-established co-culture model of NASH[1] (hepatocytes and hepatic stellate cells (HSC)) that reproduces the initial phases of NASH development and to compare with the effect on HSC monoculture.
Method: LX2 were seeded alone or together with Huh7 (SCC) at a 5:1 ratio and exposed to 1200 µM of free fatty acid (FFA) (oleic and palmitic acid, in ratio 2:1) alone or in combination with silybin (5 and 7.5µM). Cells exposed to silybin only were tested for possible side effects.Cell proliferation and viability, HSC activation (α-SMA, Col1a1), extracellular collagen deposition, metalloproteinase 2 and 9 (MMP2-9) activity and ROS generation were determined at short (1, 24h) and long (96, 144h) exposure time.
Results: Cell proliferation and viability were not affected by silybin treatment in the SCC. Exposure only to FFA induced a significant induction of LX2 proliferation ($p < 0.001$ vs DMSO), which was counteracted by the presence of silybin ($p < 0.01$, 7.5µM). Exposure to FFA induced the activation of LX2 (α-SMA) and collagen expression (Col1a1, $p < 0.05$) followed by a progressive deposition of extracellular protein ($p < 0.01$ at 144h) in both monoculture and SCC. Interestingly, co-treatment with silybin induced a significant time- and dose dependent reduction of collagen deposition only in the SCC system. Treatments without FFAs did not alter collagen deposition. Mirroring collagen deposition in SCC, MMP2-9 activity was reduced in FFA-treated cells ($p < 0.05$ vs DMSO) while co-treatment with silybin induced a dose dependent increase with the highest activity at 7.5µM ($p < 0.05$ vs FFA). Silybin showed also antioxidant properties by reducing the FFA induction of ROS after 1h of treatment.
Conclusion: In addition to the known protective effect of silybin on hepatocytes, we report that it exerts beneficial effects by reducing HSC proliferation and ROS generation. Interestingly, a net reduction of collagen production, a hallmark of HSC activation, was observed only in SCC, indicating that silybin can modulate the cross-talk between hepatocytes and HSC.

1. Giraudi, P. J. *et al.*T. *Exp. Mol. Pathol.***98,** 85-92 (2015).

P02-02YI Dietary wheat amylase trypsin inhibitors worsen chronic liver disease in pre-clinical models of NASH and liver fibrosis

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Background and aims: Specific nutrient signals from the intestine may be important drivers of obesity and non-alcoholic fatty liver disease (NAFLD) and liver fibrosis. A common dietary component, wheat amylase trypsin inhibitors (ATI), activate intestinal macrophages and dendritic cells via toll like receptor. These cells then migrate and propagate the inflammatory stimulus to the periphery (Zevallos et al, Gastroenterology 2017). We therefore studied how far nutritional ATI would affect murine NAFLD and liver fibrosis.
Method: Male C57Bl/6J mice received a carbohydrate and protein (zein) defined low fat or high fat diet (HFD), with or without 30% of the protein being replaced by wheat gluten (G, naturally containing 0.15g ATI per 10g), or 0.7% of the zein as purified ATI for 8 weeks. Male Mdr2-knockout (Mdr2KO) mice were fed the ATI containing and ATI free diets for 6 weeks. In the NASH model, insulin resistance (IR) was assessed by an intraperitoneal glucose tolerance test.
Results: Mice on the HFD gained significant weight and developed IR. Compared to the HFD alone, mice fed the HFD/G/ATI or the HFD/ATI diets gained significantly more weight and displayed significantly higher serum transaminases and triglycerides, increased liver, epididymal, mesenteric and inguinal fat, and a higher IR. ATI feeding promoted liver and adipose tissue inflammation, M1 macrophage polarization and infiltration, and enhanced the fibrogenic response in the liver. Moreover, there were significantly increased CD68+ macrophages, CD86+ dendritic cells and MHC-II+ cells in the distal small intestine of mice fed HFD/ATI compared to HFD diet alone. In Mdr2KO mice that display features of PSC, liver weight and Sirius red stained collagen area were significantly increased in the ATI fed vs the ATI free group. Moreover, CD68+ and YM-1^{+} staining demonstrated a shift from M1- vs M2-type macrophages, and aSMA morphometry an increased hepatic stellate cell activation. Hepatic transcripts for col1a1, asma and col3a1 were significantly upregulated ATI fed mice vs ATI free mice.
Conclusion: When ingested in quantities comparable to average human consumption, wheat ATI exacerbate the key features of rodent NAFLD and the metabolic syndrome despite their irrelevant caloric value. Moreover, dietary ATI promoted liver fibrosis in the NASH and the Mdr2KO mouse model of PSC.

P02-03 Does oleuropeininduce apoptosisor autophagic processes in HFD mice?

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Background and aims: Several studies showed that the use of antioxidant compounds, such as Oleuropein (Ole), reduces the body weight gain and visceral adiposity in mice fed with high fat diet (HFD), and is able to negatively modulate the autophagy process by activating apoptosis.
Autophagy is a catabolic process involved in the elimination of protein aggregates and damaged organelles. In keeping with, it is well known a strictly association between the regulation of autophagy and hepatic complications related to non-alcoholic fatty liver disease (NAFLD).
Thus, we wonder to understand how Ole regulates autophagy in the presence of unhealthy diet.
We looked at the effects on autophagic processes induced by Ole treatment using a NAFLD mouse model.
Method: Twenty-four C57BL/6 mice were assigned to one of the following dietary group (6 mice for each group, 3 male and 3 female): normal diet (ND); HFD; 3% of Ole-supplemented ND diet and 3% of Ole-supplemented HFD diet. After 16 weeks, we performed Q-RT-PCR analysis of *Becn1*, *Casp3* and *LC3b*.
Results: Our preliminary data showed that Ole, in ND and HFD mice, induces a different modulation of genes codifying for proteins involved in the control of the late autophagic pathway. In particular, we observed, after treatment with Ole, a significant transcriptional increase of both *Casp3* and *LC3b* levels in ND and HFD mice. Interestingly, this effect correlates to gender, with a more pronounced modulation in female respect to male mice.
However, despite these observations, upon treatment with Ole, *Becn1* (beclin) appeared modulated in opposite way in ND *vs* HFD mice. Beclin, in fact, was up-regulated in ND+Ole mice, suggesting an activation of the autophagic process. On the contrary, we observed a reduced expression of *Becn1* in HFD+Ole mice.
Conclusion: Our data highlight that treatment with Ole induces apoptosis in HFD mice. Intriguingly, in ND mice, the use of Ole seems to enhance the autophagic activity. Autophagy is the natural, regulated, destructive mechanism of the cell that disassembles unnecessary or dysfunctional components, and is currently considered essential for preventing diseases such as: cancer, neurodegeneration, cardiomyopathy, diabetes, liver disease, autoimmune diseases and infections. Of note, both observed effects related to Ole are gender-related.
Further investigations on genes and proteins involved in the autophagic and apoptotic processes are currently ongoing. Immunochemistry is under investigation, too.

P02-04YI Lean versus overweight/obese non-alcoholic fatty liver disease-a clinic pathological comparative study

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) refers to the accumulation of fat (mainly triglycerides) in hepatocytes. A fraction of NAFLD patients (especially Asians) do not meet weight criteria of obesity. The aim of this study was to compare the clinicopathological and biochemical profile of lean and overweight/obese NAFLD patients with a special emphasis on insulin resistance.
Method: This prospective study was conducted in biopsy proven NAFLD patients aged 18 to 75 years in a university hospital in India. On the basis of Asia Pacific criteria, the patients were divided into lean (BMI <22.99 kg/m ^2) and overweight/obese (BMI ≥23 kg/m ^2) NAFLD. Histopathological NASH was defined by NAFLD activity score (NAS) ≥5. Patients with other liver diseases and diabetes mellitus were excluded.
Results: Among 88 NAFLD patients, 29 (33%) and 59 (67%) were in the lean and overweight/obese group respectively. The mean age was 33.31 years. Metabolic syndrome was present in 68.2 % patients. In the lean and overweight/obese group, 55.2% and 74.6% patients had metabolic syndrome respectively (p 0.06). In the lean group, the mean weight, height, waist circumference, hip circumference, waist:hip ratio was significantly lower as compared to overweight/obese group. The median triglyceride level (190mg/dl vs 147 mg/dl; p 0.018) and mean VLDL level (50.40mg/dl vs 33.99mg/dl; p <0.0001) was significantly higher in the lean NAFLD group. However, the median levels of fasting serum insulin (5.57µU/ml vs 10.83µU/ml; p 0.009) and HOMA-IR (1.37 vs 2.62; p 0.010) were significantly lower in the lean NAFLD group. NASH was present in 67% patients. 75.9% of the lean patients and 62.7% of overweight/obese patients had NASH (p 0.22). There was no difference in the level of ALT, HDL or total cholesterol in the two groups.
Conclusion: Lean NAFLD patients had lower mean weight, height, waist:hip ratio. Dyslipidemia was more common in the lean NAFLD group whereas insulin resistance was less in lean NAFLD group. Metabolic syndrome and NASH were equally present in both the groups irrespective of BMI.

Figure:
Comparison between lean and overweight/obese NAFLD

Variable	Total (Mean ± SD) (n = 88)	Lean (Mean ± SD) (n = 29)	Overweight/obese (mean ± SD) (n = 59)	P value
Weight (kg)	68.45 ± 7.73	62.76 ± 6.29	71.25 ± 6.81	**<0.0001**
Height (m)	1.67 ± 0.07	1.71 ± 0.06	1.66 ± 0.07	**<0.0001**
BMI (kg/m^2)	24.45 ± 2.81	21.31 ± 1.37	26.00 ± 1.89	**<0.0001**
Waist circumference (cm)	89.35 ± 10.29	83.00 ± 9.85	92.47 ± 9.07	**<0.0001**
Hip circumference (cm)	93.00 ± 8.76	89.14 ± 7.59	94.90 ± 8.74	**0.003**
Waist: hip ratio	0.96 ± 0.05	0.93 ± 0.05	0.97 ± 0.43	**<0.0001**
TG* (mg/dl)	176.0 (105.0-243.25)	190.00 (125.50-292.00)	147.00 (101.00-228.00)	**0.018**
VLDL (mg/dl)	39.40 ± 20.52	50.40 ± 27.90	33.99 ± 12.88	**<0.0001**
Fasting insulin* (µU/ml)	8.09 (4.91-14.46)	5.57 (3.52-8.75)	10.83 (5.16-15.31)	**0.009**
HOMA-IR*	2.02 (1.09-3.62)	1.37 (0.98-2.20)	2.62 (1.22-3.82)	**0.010**

P02-05 Liver microcirculation disorders and its correction by Hepa-Merz at the non-alcoholic steatohepatitis

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Background and aims: The basis of initial component of increased intrahepatic vascular resistance at the chronic liver diseases are endothelial disfunction, activation of hepatic stellate cells, hyperammoniemia. In some only experimental studies in vivo and in vitro was demonstrated effect of hypoammoniemic drugs for liver microcirculation due to decrease of activity of hepatic stellate cells, portal hypertension, increase of endothelial nitric oxide synthase. Aims of our study are to estimate intrahepatic microcirculation and efficacy of hypoammoniemic L-ornithine-L-aspartate (Hepa-Merz) for correction of intrahepatic hemodinamics disorders at the non-alcoholic steatohepatitis (NASH) patients.
Method: We investigated 78 patients with NASH, minimal fibrosis 0-1 stage. Stage of liver fibrosis was estimated by transient elastography (FibroScan). Intrahepatic hemodinamics are determined by polyhepatography-modificated hepatic impedansometry, non-invasive method (PHG). PHG registers a blood flow in projection of zone of hepatic right, left lobes and spleen, integral body impedansography (Fig.1). Our study manifested a high degree of sensitiveness (99%) and specificity (89%) of PHG for definition of localization of hemodynamic disorders in liver (presinusoidal, sinusoidal). For correction of blood flow disorders we used hypoammoniemic drug Hepa-Merz in dosage 5 grams 3 times daily 4 weeks. Efficacy of Hepa-Merz we looked in 2 and 4 weeks via the control PHG.
Results: Analysis of PHG demonstrated, that at all patients with NASH we revealed a liver microcirculation disorders- increased blood resistance, abnormal forms and amplitude of waves (“plateau” wave) in sinusoidal level (out flow zone) (Fig.2). Analysis of efficacy of Hepa-Merz showed, that Hepa-Merz was effective for correction of hepatic microcirculation disorders. In 2 weeks of the treatment we observed normalization or improvement of the wave form, in 4 weeks-wave amplitude (Fig.3).
Conclusion: NASH is characterized by disorders of intrahepatic microcirculation on sinusoidal level even in initial stage of liver fibrosis. Hepa-Merz improved liver microcirculation at the NASH patients.

Figure 1. Normal PHG.

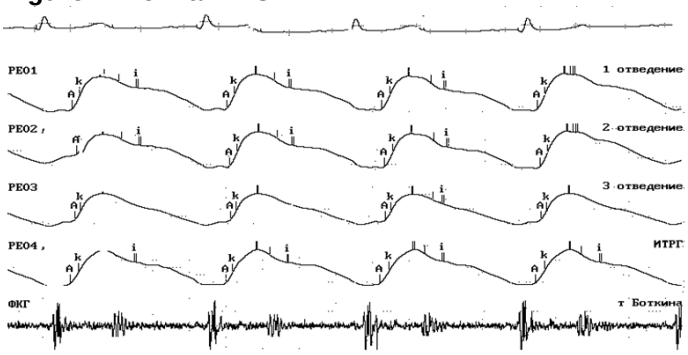


Figure 2. PHG at the NASH patient.

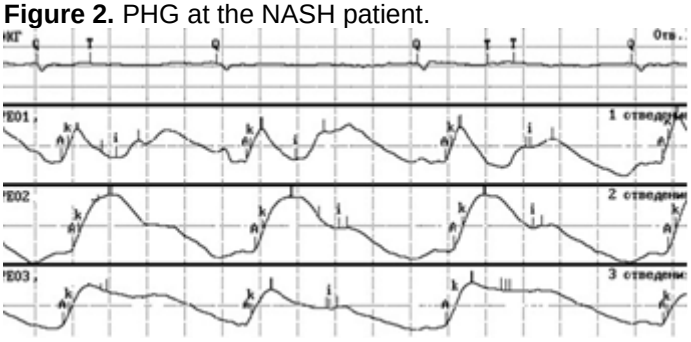
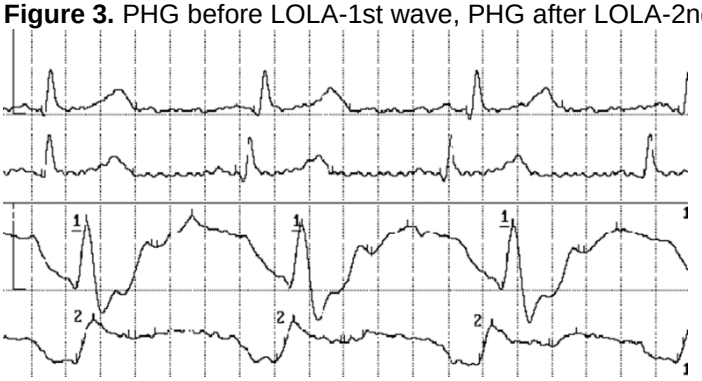


Figure 3. PHG before LOLA-1st wave, PHG after LOLA-2nd wave.



P02-06 The Association between NAFLD and breast cancer: High prevalence and High recurrence

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Background and aims: Breast cancer is most common cancer in women worldwide, and it is a main cause of death in women. The incidence of breast cancer is correlated with metabolic component including diabetes, hypertension, and obesity. Likewise breast cancer, metabolic components are important risk factors for development of non-alcoholic fatty liver disease (NAFLD). In this study, we analyzed the prevalence of NAFLD in patients with breast cancer and the effect of NAFLD on the prognosis of breast cancer.
Method: Total 492 patients with breast cancer who received operation were enrolled from January 2010 to June 2014. Patients who had other chronic liver disease including chronic viral hepatitis B/C, autoimmune hepatitis, and primary biliary cholangitis and significant alcohol abuse (more than 140 g/week in women and 210 g/week in men) were excluded. Hepatic steatosis was evaluated by non-enhanced computed tomography (CT) scan. We measured values of regions of interest (ROI) for 5 times in liver and spleen, respectively. We diagnosed NAFLD when the average ROI of liver is higher than it of spleen 15 or more. 135 healthy controls who took non-enhanced CT scan were also analyzed.
Results: Mean age and BMI were 51.6 ± 11.0 years and 24.7 ± 9.9 kg/m2, respectively. The prevalence of DM and hypertension were 9.5% and 24.6%, respectively. The prevalence of NAFLD in patients with breast cancer was 43.5% (214/492) and it was significantly higher comparing with healthy control (29.6%, 40/135) (p = 0.004). Overall survival did not showed significant difference between NAFLD group and non-NAFLD group (p = 0.958 by log-rank test). However, recurrence rate was significantly higher in patients with NAFLD (10.7%, 23/214) comparing with those without NAFLD (5.4%, 15/278) (p = 0.027). The disease free survival was significantly higher in in patients with NAFLD comparing with those without NAFLD (p = 0.033 by log-rank test). 15.9% of patients (34/214) experienced ALT elevation above 3 times of upper normal range, whereas only 10.4% of patients (29/278) showed elevation of ALT above 3 times of upper normal range (p = 0.049).
Conclusion: The prevalence of NAFLD in patients with breast cancer is significantly high compared to healthy control group. Moreover, breast cancer patients with NALFD showed poor prognosis in aspect of recurrence. Therefore, diagnostic evaluation of NAFLD would be important in patients with breast cancer.

P02-07YI Clinical impact of comorbidities in NAFLD patients referred in a tertiary centre in Italy

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Background and aims: Non- Alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide and is associated to several clinical conditions. Epidemiological studies did not deeply evaluate prevalence of comorbidities associated to NAFLD so far.

The aim of this study is to assess the prevalence of common chronic diseases in a cohort of NAFLD subjects referred to a tertiary center. Moreover, we evaluate the association between comorbidities and liver fibrosis (non-invasively assessed) with the hypothesis that comorbidities could have an impact on severity of liver disease.

Method: From December 2016 and January 2018 we prospectively recruited subjects with ultrasound diagnosis of steatosis (with or without hypertransaminasemia) referred to the Outpatient Liver Unit of Fondazione Policlinico Gemelli in Rome. Patients with chronic liver disease related to viruses (HBV, HCV), alcohol, autoimmune and metabolic disease were excluded. Anthropometric measurements, medical comorbidities, and laboratory tests were obtained during assessment. The Charlson Comorbidity Index (CCI) was used for the stratification of patients based on comorbidity. Multimorbidity was defined as CCI≥2. Liver fibrosis was non-invasively assessed by Fibrosis-4 score (FIB-4).

Results: We enrolled 398 NAFLD patients (males 55, 7%, age 56, 9 ± 13, 8 y). 207 patients (52%) fit the diagnostic criteria for Metabolic syndrome, 115 (28, 9%) have type 2 diabetes mellitus and 207 (52%) have arterial hypertension.

Considering comorbidities included in CCI, diabetes mellitus is the most common one in our cohort (52%) followed by non-metastatic tumours (7.5%). The most common coexistent chronic conditions, excluding those included in CCI, were gastroesophageal reflux (14, 5%), hypothyroidism (10, 8%), cholelithiasis (6%) and sigmoidal diverticulosis (5, 8%).

Multimorbidity (CCI≥2) was present in 63, 3% of patients. Multimorbid patients, compared with non-multimorbid ones, has a higher prevalence of metabolic syndrome (68.3% vs 28.6%), diabetes (43.6 % vs 3.4%) and a higher severity of liver fibrosis at non-invasive evaluation (FIB-4 2.0 ± 3.8 vs 0.9 ± 0.4).

Conclusion: Comorbidities have a high epidemiological and clinical impact in NAFLD patients, especially considering those affected by metabolic syndrome or diabetes. Moreover, multimorbidity is associated with a higher stage of liver fibrosis (non-invasively evaluated). These results suggest that a multidisciplinary evaluation of these patients, especially in those with significative fibrosis, represents the best clinical approach.

P02-08 Algorithm to identify patients with an activity grade >2 in type 2 diabetic patients with non-alcoholic fatty liver disease (NAFLD) Development in a large prospective multicenter UK study

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Background and aims: To address the problem of high screen failure rates NAFLD clinical trials, we aimed to develop a simple algorithm to detect the presence of NASH with an activity (A) from SAF score >2 in patients with diabetes.

Method: FibroScan examination was undertaken within 2 weeks of a clinically indicated liver biopsy (LB) for suspected NAFLD. Recruitment took place (Mar 2014-Jan 2017) at seven UK centres. LB were read in a blinded manner with consensus by two expert pathologists using the (steatosis, activity, fibrosis) SAF score. Activity was the sum of ballooning (graded 0-2) and lobular inflammation (graded 0-2) scores. NASH was diagnosed using the FLIP algorithm. Only those patients with T2DM were considered in this analysis.

An algorithm was developed to identify patients with A>2 with the following objectives:

improve the screen failure rate (SFR = 1-PPV) of at least 30%,

keeping the missed cases rate (MCR = 1-sensitivity (Se)) below 15%.

The following steps were repeated 3 times:

Univariable analysis between the patients with A>2 and the bio-clinical parameters (E, CAP (controlled attenuation parameter), Fib-4, NAFLD fibrosis score, Forn score, BARD score, AST to Platelet Ratio Index (APRI), body mass index (BMI), age, liver enzymes normalized by the upper limits for normal (ULN), fasting glucose, lipid parameters, platelet, albumin, creatinine, urea, phosphatase alkaline, ferritin, hypertension, hypercholesterolemia and gender),

Keep for comparison parameters significantly different in the target patients (at the given step + those initially significantly associated),

Compute cutoffs based on high Se≥0.95 for all parameters in the comparison list,

Select the parameters that has the highest sum Se + specificity (Sp),

Exclude patients with parameters value <cutoff,

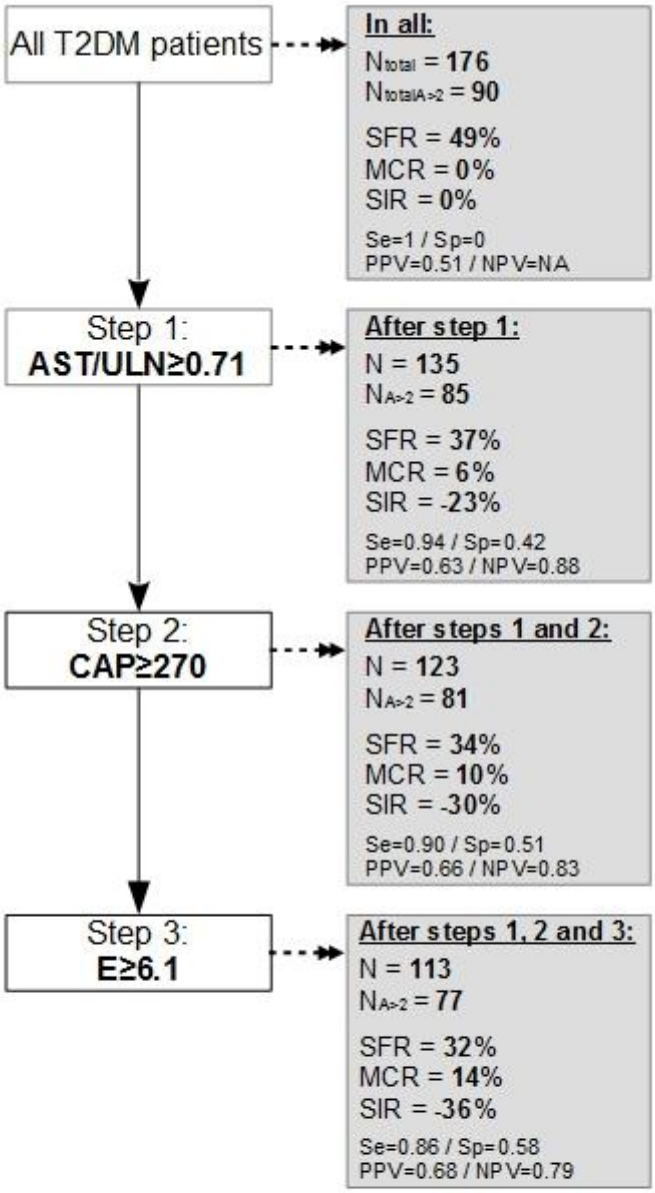
Compute performances: SFR and MCR.

Results: Among the 408 patients who completed the study, 188 were diabetic (46%). 176 were considered in the present analysis due to missing data. 49% were female, with a median age 58 [IQR 14] years and BMI 34.4 [8.7] kg/m². 77% had NASH. 51% had A>2.

The algorithm was devised with the 3 optimally determined parameters: ALT/ULN≥0.71, CAP≥270 dB/m and E≥6.1 kPa. Performance is presented in the figure below.

Conclusion: A simple algorithm based on FibroScan E, CAP and ALT was developed to improve detection patients with A>2 in NAFLD diabetic patients. If applied as a pre-screening tool in clinical trials, it improved the SFR by 35% and reduced the number of patients that would undergo a LB by 35% with missed cases being below 15%.

Figure :



Legend:
SFR: screen failure rate (1-PPV)
MCR: missed cases rate (1-Se)
*SIR: screen improvement rate ((N-Ntotal)/Ntotal*100)*
Se: sensitivity / Sp: specificity
PPV-NPV: positive and negative predictive values
ULN: upper limit of normal

P02-09 DGAT2 inhibition improves end points associated with NASH pre-clinically

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a growing epidemic associated with obesity and metabolic disorders. By 2020, it is projected that NASH will be the leading cause of liver transplantation in the United States. NASH is a multifactorial disease comprised of hepatic steatosis, inflammation, hepatocellular ballooning and fibrosis. The hepatic steatosis results from accumulation of triglycerides and other lipid intermediates. There are two distinct diacylglycerol acyltransferases (DGAT1 and DGAT2) which catalyze the conversion of diacylglycerol to triacylglycerol. DGAT2 activity predominates in the liver during the metabolic fed state and plays a central role in the assembly and secretion of hepatic very-low density lipoprotein (VLDL). Previous studies have demonstrated that reducing the expression of DGAT2 in liver and fat using specific antisense oligonucleotides (ASO) reduced hepatic lipid burden by decreasing diacylglycerol and triacylglycerol; conversely, over-expressing DGAT2 in liver increased hepatic lipid concentrations.
Method: PF-06865571 is a potent and selective oral DGAT2 inhibitor being investigated for the treatment of non-alcoholic steatohepatitis (NASH) with fibrosis.
Results: In primary human, monkey and rat hepatocytes, PF-06865571 dose-dependently inhibited the synthesis of triglycerides (TG). In pharmacodynamics studies, a single oral dose of PF-06865571 in sucrose-fed rats dose-dependently reduced plasma TG levels and in longer duration studies in western-diet fed rats dose-dependently reduced both circulating and hepatic TG. In addition to lowering TG in vivo, administration of PF-06865571 in these rat models suppressed the expression of multiple hepatic genes involved in lipid biosynthesis.
Conclusion: Based on observations from these nonclinical studies it is hypothesized that DGAT2 inhibition with PF-06865571 will impact physiologic processes contributing to NASH including direct inhibition of TG production as well as promoting adaptive responses further decreasing hepatic de novo lipogenesis (DNL).

P02-10 Impact of western diet and chronic exposure to environmental pollution on the natural history of hepatic steatosis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is nowadays one of the main causes of chronic hepatic damage in developed countries, being linked to metabolic disorders such as obesity, diabetes mellitus (type 2) and hyperlipidaemia. NAFLD prevalence is around 25% in Europe. Hepatic steatosis may evolve overtime into non-alcoholic steatohepatitis (NASH), which is a more serious pathology. When the necro-inflammatory process becomes chronic, it may cause the development of fibrosis, cirrhosis and can eventually bring to hepatocellular carcinoma (HCC). NASH is a complex disease with multifactorial pathogenesis. While its risk and progression factors are not completely identified, bad diet habits and a sedentary lifestyle may have a key role. Also, the lifestyle in Western countries may be a potential issue. Exposition to environmental pollution, in particular to fine particles such as PM10, is another relevant factor.
The aim of this study was to evaluate the role of PM10 and bad diet habits in affecting the pathogenesis of hepatic steatosis and its progression to NASH and hepatocellular carcinoma.
Method: We carried out in vitro studies on murine cell lines: Hepa 1-6, M-HSC, AML-12. In order to evaluate the effect of particulate matter on cell growth, the cells have been stimulated with PM10 at different concentration. In vivo studies have been carried out on C57BL/6N mice, fed with high fat diet (experimental model of NASH) and treated with PM10. The mice went through evaluation of the hepatic morphology and histological investigations to determine the degree of steatosis, inflammation and fibrosis. Furthermore, the degree of oxidative stress following the exposition to PM10 has been evaluated by DMA. In addition, the mice underwent to a syngeneic orthotopic implantation of hepatocellular carcinoma cells.
Results: In in vitro studies, PM10 stimulates proliferation of murine hepatocellular carcinoma cells (Hepa 1-6) and murine hepatic stellate cells (m-HSC), while it reduces hepatocytes growth (AML-12). In the in vivo model, a fat diet associated with PM10 exposure developed hepatic steatosis and NASH: such environment strongly promoted the growth of hepatocellular carcinoma. Immunohistochemistry reveals a greater expression of cellular proliferation indexes and neo-angiogenesis in the hepatic tissue.
Conclusion: The results of the study suggest that exposition to PM10, associated with hyper-lipidic and hyper-glucidic diet, is a significant risk factor for the progression of NAFLD to NASH and hepatocellular carcinoma. The increase of both oxidative stress and proinflammatory cytokines production induced by PM10 and obesity are important physiopathological mechanisms triggering chronic hepatic damage and its evolution.

P02-11 Hypercoagulopathy risk factors in liver cirrhosis patients dueto non-alcoholic steatohepatitis

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Background and aims: Changes of hemostasis with hypo and hypercoagulative symptoms may occur in liver cirrhosis (LC). Characteristics and predictive effects of coagulation profile in LC with non-alcoholic steatohepatitis (NASH) are now debated.
Method: We analyzed 66 patients (ps) with NASH LC (11 females/55 males; age, 45.8 ± 5.7 yr; body mass index (BMI), 33.5 ± 2.8 kg/m^2): 24 ps with Child-Pugh class A cirrhosis (group I), 22 ps with Child-Pugh class B cirrhosis (group II), 20 ps with Child-Pugh class C cirrhosis (group III). The results were compared with those of 20 healthy controls (group IV; 4 females/16 males). The diagnosis of LC was based on *biochemical, ultrasound imaging, and endoscopic findings, suggesting advanced liver disease with portal hypertension,* Desmet's classification of chronic hepatitis in liver biopsy specimens (stage ≥ 3) or liver stiffness assessed via elastography (F-score ≥ 3). Circulating insulin, tumor necrosis factor alpha (TNFα), factor VIII (FVIII), von Willebrand factor (vWF), antithrombin III (AT III), protein C, platelet-endothelial cell adhesion molecule-1, (PECAM-1), P-selectin, plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI) were measured by the immuno-assay method.
Results: Hypercoagulopathy was detected in 41.7% of ps in group I, 72.7%-of ps in II group and 80.0%-of ps in group III; hypocoagulopathy-in 4.54% of ps with II group and 20.0% of ps with III group. In ps with NASH LC a statistically significant increase of FVIII (p = 0.009) and vWF (p = 0.003), PECAM-1 (p = 0.002), P-selectin (p = 0.003), PAI-1 (p = 0.001) and TAFI (p = 0.006) and decrease of AT III (p = 0.006), protein C (p = 0.001) vs. group IV were found. The direct correlations between vWF and Child-Pugh score, low density lipoprotein cholesterol (LDLC), TNFα, HOMA-IR, PAI-1, PECAM-1, P-selectin (r = 0, 5643; r = 0, 6438; r = 0, 7251; r = 0, 5924; r = 0, 7903; r = 0, 6257; r = 0, 6588; accordantly; p <0, 001) and indirect correlations between FVIII and AT III, protein C (r = -0, 7015; r = 0, 8226; accordantly; p <0, 001) were installed.
Conclusion: In NASH LC ps are mostly hypercoagulopathic; risk factors are an increase of Child-Pugh score, dyslipidemia, inflammation, insulin resistance, an increase in procoagulant and endothelial factors, antifibrinolytic activity and a decrease in AT III, protein C.

P02-12 Non-alcoholic steatohepatitis: an independent risk factor for albuminuria in non-diabetic patients

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Background and aims: Increasing evidences suggest that NAFLD in diabetic patients is strongly associated with increased prevalence of chronic kidney disease. However, few data are available on the occurrence of albuminuria in non-diabetic subjects with histologically proven NAFLD. In this study, we aimed to investigate the association between albuminuria and non-alcoholic steatohepatitis (NASH) in non-diabetic patients.

Method: Biopsy-proven NAFLD from the First Affiliated Hospital of Wenzhou Medical University were included. Detailed anthropometric, clinical variables and laboratory parameters were prospectively recorded. NASH was defined as NAS score ≥4, according NASH Clinical Research Network criteria. Albuminuria was defined as urinary albumin-to-creatinine ratio (ACR) ≥30mg/g.

Results: A total of 148 patients were included, of whom 25% had NASH and 75% non-NASH. The prevalence of albuminuria was higher in patients with NASH, compared with non-NASH (24.32% vs. 8.10%, P <0.05). After adjustment of potential confounders, including sex, age, uric acid, hypertension, central obesity and diabetic disease, NASH was associated with increased risk of albuminuria (OR = 7.97). Furthermore, compared with non-NASH in patients without type 2 diabetes, NASH with non-diabetic patients also had higher prevalence of albuminuria. After adjustment of potential confounders, the OR is 13.92 (95%CI: 2.12-91.30, p = 0.006) (table).

Conclusion: This study indicated that NASH was associated with albuminuria in non-diabetic patients, independent of traditional risk factors.

Figure:

Table 1. Association of non-alcoholic steatohepatitis with albuminuria

	unadjusted	P	Model 1	P	Model 2	P
Total patients group (n=148)	3.64 (1.32-10.04)	0.012	3.77 (1.33-10.65)	0.012	7.97 (2.10-30.24)	0.020
Non-diabetic patients group (n=108)	4.28 (1.12-16.36)	0.034	5.41 (1.31-22.44)	0.020	13.92 (2.12-91.30)	0.006

Model 1 is adjusted for age, sex. Model 2 is adjusted for age, sex and comorbidities (hypertension, central obesity, hyperuricemia and type 2 diabetes mellitus). In non-diabetic patients group, Model 2 do not included type 2 diabetes mellitus.

P02-13 The role of endothelial lipase in the diagnosis of cardiovascular risk in patients with non-alcoholic fatty liver disease in hypertension and insulin resistance subjects

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is now recognized as the most common disease in hepatology, and is also often accompanied by hypertension. Insulin resistance (IR) and endothelial dysfunction, in which metabolism endothelial lipase (EL) plays a leading role, are links in a chain and play an important role in the development of cardiovascular risk (CVR). Therefore, our aim was to determine the blood levels of EL for the early detection of CVR in patients with NAFLD in hypertension and IR subjects.
Method: 20 patients with NAFLD and hypertension stage 1 and 2 with and without diabetes mellitus (DM) were followed-up in groups 1 and 2. Groups 3 and 4 consisted of 24 patients with NASH and hypertension stage 1 and 2 with and without DM. Control group 5 consisted of 20 healthy individuals. Patients were selected according to age range and gender equal. The average age was [53 ± 7.5]. The severity of steatosis was determined by the NAFLD index liver fat score.
Results: Blood glucose levels were [7, 35 ± 1, 97] and [6, 39 ± 1, 07] mmol/l in group 2 and 4 respectively and had normal values in groups 1 and 3. Blood insulin level in group 1 was [18, 64 ± 9, 75] μm/ml, group 2-[30, 37 ± 11, 58] μm/ml, group 3-[32, 82 ± 20, 51] μm/ml, group 4-[34, 18 ± 19, 68] μm/ml. HOMA-IR were [4, 338 ± 2, 337], [9, 691 ± 5, 143], [7, 918 ± 5, 652] and [9, 211 ± 4, 577] in groups 1, 2, 3 and 4 respectively. HbA1C levels were [7, 947 ± 1, 555] and [7, 893 ± 0, 41] in group 2 and 4 respectively and [5, 336 ± 0, 581] and [5, 235 ± 0, 438] in groups 1 and 3. The blood level of EL is the lowest in the control group 5-[8, 23 ± 2, 47] ng/ml and a progressive significantly increased in group 1-[11, 299 ± 2, 925] ng/ml, group 2-[11, 714 ± 3, 22] ng/ml and group 3-[11, 84 ± 3, 801] ng/ml. Also significantly (p <0, 05) higher level of EL in group 4 [15, 51 ± 3, 09] ng/ml compared with groups 1, 2, 3 and 5. Spearman correlation analysis showed a significant positive association between the HbA1C and EL levels (r = 0, 386; p <0, 05).
Conclusion: Increased levels of EL is inherent in all patients with NAFLD and hypertension. However, in patients with NASH and hypertension formed an additional association between the level of EL and HbA1C, which allows to consider EL as an additional predictor of CVR.

P02-14YI Prevalence of de novo metabolic syndrome and its risk factors in a liver transplanted population: a prospective study

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Background and aims: *de novo* metabolic syndrome (MS) is an emerging complication in the early period after liver transplantation (LT), resulting in an increased cardiovascular morbidity and mortality. This prospective study aimed to assess the prevalence of MS in the first year after LT and its possible risk factors.
Method: patients who underwent LT from April 2013 to June 2017 with a minimum follow-up of 1 year were prospectively included. Paediatric patients, multiorgan transplantation or re-transplantation and patients who had MS before LT were excluded. For each patient general and metabolic variables were collected at time of LT and at 1-year after LT. Donor variables including, age, gender, body mass index (BMI), diabetes mellitus, graft steatosis were collected to identify predictors to MS. MS was evaluated according to the modified NCEP-ATP III criteria.
Results: fifty-seven liver transplanted patients (77% male, mean age 54.6 ± 8.1 years) were included in the study. The most common indications to LT were HCV and alcohol related cirrhosis (35%), 23% presented HCC and the mean MELD-score at transplantation was 23 ± 8.6. Twenty-five out of fifty-seven patients (44%) developed MS at 1-year after LT. The associated factors to the development of MS were an high BMI pre-LT ($p = 0.03$), a pre-LT history of diabetes mellitus ($p = 0.01$) and the alcoholic liver disease as indication to LT ($p < 0.001$). By multivariate analysis the alcoholic liver disease as indication to LT (OR 7.0, 95% CI [1.9-25.8], $p = 0.003$) was an independent risk factor to de novo MS after LT. No statistically significant associations were observed between the development of post-LT MS and post-LT renal impairment, immunosuppressive regimen nor donor characteristics.
Conclusion: *de novo* MS is a frequent complication after LT, affecting nearly half of patients at 1-year post-LT. Therefore a strict metabolic follow-up is mandatory starting early after LT, particularly in patients transplanted for alcoholic liver disease and with a pre-transplant diagnosis of diabetes.

P03-01YI Cumulative effects of Western diet and alcohol abuse: a novel experimental mouse model of NASH/ASH-derived liver injury

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Background and aims: The life style in the prosperous parts of the world often include the overlapping of the Western diet (WD) (high fat and high sugar diet) (WD) with alcohol intake. Current clinical studies showed a strong causative link between dietary habits and the onset of chronic liver disease. However, the mechanisms by which WD and alcohol together trigger liver damage still remain unclear. In the present work, we aimed to develop an innovative experimental model which resembles the compound effects of WD and alcohol as observed in patient's non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH).
Method: C57BL/6 female mice received 10-20% alcohol in sweet drinking water together with a WD for 10 weeks (*The Cocktail diet*). Mice receiving only WD or alcohol were used as controls. Serum markers of liver damage, blood lipids and glucose, liver histology, hepatic triglyceride content (HTC) and hepatic expression of pro-inflammatory and pro-fibrotic genes were analysed.
Results: The novel *Cocktail diet* mimicked the effects of compound effect of WD and alcohol consumption, and lead to obesity, significant hepatomegaly and glucose intolerance. The *Cocktail diet* treatment resulted in cholesterolemia, accompanied by high HTC, hepatic macrosteatosis and ballooning degeneration of hepatocytes. Significant liver damage was characterized by elevated serum transaminases (eg: ALT, AST) and LDH, as well as positive TUNEL staining. Notable, mice treated with the Cocktail diet exhibited significant hepatic inflammation and intrahepatic accumulation of $CD11b^+$ and $F4/80^+$-positive immune cells accompanied by remarkable perisinusoidal fibrogenic alterations.
Conclusion: The novel *Cocktail diet* is an excellent innovative model for the characterization of patients with signs of NASH/ASH. Obviously, the compound effects of WD and alcohol synergistically enhanced obesity, glucose intolerance, liver damage, and triggered prominent steatohepatitis and fibrosis.

P03-02 Multiplex real-time PCR detection of variants in the PNPLA3 and TM6SF2 genes associated with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of hepatic fat in the absence of excess alcohol consumption, HCV infection or endocrine disorders. NAFLD is assumed to be the leading cause of liver damage in developed countries, and its broad clinical spectrum ranges from steatosis, steatohepatitis, fibrosis and ultimately cirrhosis and hepatocellular carcinoma. Aside from insulin resistance, obesity, physical inactivity and type 2 diabetes mellitus, a strong genetic component was identified as risk factor for NAFLD. The two variants PNPLA3 p.I148M and TM6SF2 p.E167K impair the export of very low-density lipoprotein (VLDL) from the liver and are the two major determinants of inter-individual differences in liver steatosis and susceptibility to progressive non-alcoholic steatohepatitis.
Method: We developed a multiplex TaqMan-based real-time PCR assay (NAFLD mxp RealFast Assay) for the simultaneous detection of the PNPLA3 p.I148M (rs738409) and TM6SF2 p.E167K (rs58542926) variants.
Results: The NAFLD mxp RealFast Assay was tested on a series of DNA samples derived from peripheral blood, buccal swabs or dried blood. PNPLA3 and TM6SF2 genotypes were determined by Sanger sequencing in all samples. In comparison to this reference method, the NAFLD mxp RealFast Assay achieved 100% concordance. It performed equally well on various common real-time thermocyclers, and when applied in a direct-to-PCR approach on unpurified templates combined with ultrarapid PCR, complete genotyping was possible in less than one hour.
Conclusion: The NAFLD mxp RealFast Assay is a quick and convenient PCR test, which can be used to assist in the individual risk prediction of NAFLD patients.

P03-03YI Genetic assessment of the role of bile acids in children affected by non-alcoholic fatty liver disease

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Background and aims: Bile acids (BA) are involved in the regulation of glucose and lipid metabolism, and their potential role in the pathophysiology of non-alcoholic fatty liver disease (NAFLD) is still under investigation.The prevalence of NAFLD has dramatically increased in children, alongside the rise of the obesity rate and diabetes. The rapid progression and the severity of the disease in some children remains undetermined. It is well-known that genetic and environmental factors influence the development of NAFLD, although the specific role of genetic variation within the pathogenesis and the progression of the disease is still unclear. We hypothesized that genetic variants in the BA metabolic pathway may be present in children with NAFLD.
Method: Children attending a paediatric liver unit with biopsy-proven diagnosis of NAFLD and liver fibrosis (stage ≥2), in whom all other known causes of liver disease had been out-ruled, were enrolled in this study. Data and blood samples were collected from King's Paediatric Liver Biobank. Liver biopsies were scored according to Brunt/Kleiner criteria. A next generation sequencing custom panel of 135 genes was designed, based on literature reviews: 22 of these genes are involved in the BA transport and metabolism. The Agilent SureSelect QXT kit was used for the generation of the library and the preparation of the samples.
Results: DNA of 50 children (male = 32; female = 18), with a median age of 13.8 years (range: 6 to 18) and a median BMI z-score of 2.00 (range: 0.16 to 2.80), was sequenced. Within the 682 variants identified across the cohort, a total of 68 missense variants were found in the BA genes. 8 rare variants with a very low minor allele frequency (MAF) in the general population were in *CYP8B1* (rs563690413; MAF: 0.000023 and rs372472190; MAF: 0.000142), *FGFR4* (rs569265847; MAF: 0.000071), *ABCA1* (rs138056193; MAF: 0.000061), *ABCC2* (rs186620377; MAF: 0.000061), *NR1H4* (rs747025458; MAF: 0.000011), *ABCC4* (rs150633056; MAF: 0.000004) and *HNF4A* (rs768495780; MAF: 0.000008). The evaluation of the effect of the missense variants by *in silico* software (Polyphen-2; SIFT) showed a presumable deleterious effect for the variant rs186620377 in *ABCC2* and rs138056193 and rs372472190 in *ABCA1*.
Conclusion: Several variants in the bile acid metabolism pathway may be of relevance in susceptibility to early onset severe NAFLD. Further validation will allow assessment of possible functional relevance in NAFLD and for future therapies.

P03-04 An open label randomized controlled trial of Vitamin D vs Pentoxifylline in non-diabetic patients of Non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) encompasses a histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Pharmacologic therapy is important when lifestyle measures fail. The aim of this study was to compare the response to two different treatments i.e. Vitamin E and Pentoxifylline (PTX) in non-diabetic patients of NAFLD in terms of clinical and biochemical outcomes.
Method: In this open label study, in addition to lifestyle modification advice, non-diabetic patients with biopsy proven NAFLD were randomized to receive either Vitamin E (800 mg in 2 divided doses) or PTX (1200 mg in 3 divided doses).
Results: A total of 48 patients (26 in Vitamin E group and 22 in the PTX group) with a mean follow-up of 166.8 days completed the study. At follow-up, FBS was less in Vitamin E group (94.69 ± 6.72 mg/dl vs 100.45 ± 9.82 mg/dl; p 0.020). The level of VLDL was lower (p 0.015) in the PTX group (33.15 ± 7.51mg/dl). There was no difference in any other parameter between the two groups. In the Vitamin E group, there was a decrease in systolic and diastolic blood pressure (p <0.0001), weight (67.96 ± 8.2 kg vs 67.00 ± 7.28 kg; p 0.003), BMI (from 24.14 ± 2.93 kg/m2 to 23.75 ± 2.66 kg/m2; p 0.008), waist and hip circumference. There was a decrease in ALT to 55.38 ± 29.90 at follow-up from 67.54 ± 34.50 U/L at baseline (p 0.016). Median triglyceride level decreased from 251.5 mg/dl to 194.5 mg/dl (p 0.002) and HDL increased from 38.08 at baseline to 41.15 at follow-up (p 0.0001). FBS decreased from 97.88mg/dl at baseline to 94.69 mg/dl at follow-up (p 0.018). There was a decrease in the CK18-F level from 348.95 U/L to 319.79 U/L (p 0.0001). In the PTX group, there was a decrease in weight (70.77 ± 7.32 kg to 70.00 ± 6.91 kg; p 0.009) and waist circumference (92.14 ± 11.88 cm to 91.27 ± 11.91cm; p 0.007). ALT decreased from 83.55 ± 38.96 U/L to 64.51 ± 26.54 U/L (p 0.008) and HDL increased from 37.64 mg/dl to 40.5 mg/dl (p 0.0001). There was also a decrease in CK18-F from 344.01 U/L to 303.78 U/L (p 0.022).
Conclusion: Vitamin E therapy was effective in reducing weight, BMI, systolic blood pressure and ALT, improving dyslipidemia as well as blood sugars and CK18-F. PTX therapy improved weight, ALT, HDL and CK18-F. Overall, even though both the treatments decreased ALT and CK18-F, Vitamin E appears to offer distinct advantage over PTX in terms of improvement in dyslipidemia and blood sugars.

Figure:
Comparison of treatment outcomes between both the groups

Parameters	Vitamin E (n = 26) Mean ± SD	PTX (n = 22) Mean ± SD	p value
HDL (mg/dl)	41.15 ± 5.31	40.50 ± 6.45	0.70
LDL (mg/dl)	117.96 ± 29.69	112.18 ± 27.27	0.49
VLDL (mg/dl)	44.29 ± 19.42	33.15 ± 7.50	**0.015**
FBS (mg/dl)	94.69 ± 6.72	100.45 ± 9.821	**0.020**
Fasting Insulin (µU/ml)	5.18 (2.12-10.03)	5.43 (2.93-5.44)	0.85
HOMA IR	1.24 (0.45-1.23)	1.28 (0.74-1.80)	0.98
CK18 fragments (U/L)	319.79 ± 149.85	303.78 ± 74.12	0.65

P03-05 An integrated primary and secondary care algorithm for evaluating non-alcoholic fatty liver disease significantly increases community screening for hepatitis B and C infection

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Background and aims: The World Health Organisation aims to eliminate Hepatitis C as a major public health threat by 2030. However, rates of testing for Hepatitis B and C are low in primary care, where non-alcoholic fatty liver disease (NAFLD) is much more prevalent. As chronic viral hepatitis may present with abnormal ALT or fatty liver on imaging, we hypothesised this population may be a suitable target for increased screening. In 2014, we introduced an integrated pathway for the initial management of suspected NAFLD in primary care including hepatitis B and C testing at the point of entry. We examined the impact of the NAFLD strategy on screening for viral hepatitis in an unselected catchment population of over 650, 000.
Method: Across 3 primary care Clinical Commissioning Groups (CCGs), adults with either a raised ALT or fatty liver on imaging were screened for alcohol misuse and tested for Hepatitis B and C before calculating a NAFLD Fibrosis Score (NFS). Using NFS triage, indeterminate or high fibrosis risk patients were assessed in a secondary care nurse-led NAFLD clinic including elastography (Fibroscan) while low risk patients remained in primary care. All received structured advice on diet and physical activity. The Virology laboratory database was interrogated for all tests for HBsAg, HBcAb and anti-HCV originating from primary care locations between 1st November 2011 and 31st October 2016. As a "control" for trends in blood borne virus testing, HIV serology from the same locations and time period were analysed. Data was scrutinized for number of tests pre and post implementation, new diagnoses and the number of those attributable to the NAFLD pathway.
Results: In the baseline period from November 2011 to April 2014, there were a mean of 173 HBsAg and 139 anti-HCV tests per month from primary care. Following NAFLD pathway introduction, there was a highly significant increase in mean monthly testing to 295 HBsAg and 250 anti-HCV tests per month between May 2014 and October 2016 ($p < 0.001$ for both). HIV testing remained stable with a mean of 68 tests per month prior to and 86 per month following introduction of the pathway ($p = 0.20$, NS). In addition to increased primary care testing rates, the NAFLD algorithm led to new diagnoses of chronic viral hepatitis and we will present further data on the yield of testing for Hepatitis B and C in a community NAFLD population.
Conclusion: We demonstrated a significant increase in testing for chronic viral hepatitis in primary care following the implementation of a NAFLD risk assessment pathway. The number of tests per month and number of requests related to NAFLD were sustained for the past 2 years, suggesting successful integration into routine primary care practice. A NAFLD pathway represents a potential method to improve detection of chronic viral hepatitis and we suggest that a similar approach could have additional impact in areas of higher prevalence of hepatitis B and C.

P03-06 Who cares about NASH? Patients with NAFLD care!

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Background and aims: Fatty liver was described over 150 years ago in obese individuals with an unhealthy diet and "indolent" habits, and could be reversed by improving the diet and doing more physical exercise. Fatty liver was associated with type II diabetes mellitus (T2DM) in the early 20th century, and with liver fibrosis or cirrhosis in the mid-20th century. Non-alcoholic fatty liver disease (NAFLD) is now the most common chronic liver disorder, with risk of non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma. Lifestyle recommendations for treatment of NAFLD have remained relatively unchanged. There are now several clinical trials for treatments of NASH but case ascertainment remains difficult. Our study aim was to describe the referral pattern of patients referred for NASH clinical trials and the related diagnostic findings.
Method: We reviewed a tertiary hospital NAFLD research database to describe phenotypic characteristics of patients referred for NASH clinical trials. We also sought to determine the referral source of the patients and the diagnostic yield of NASH over an 18-month period.
Results: 422 patients were identified. Most referrals (396) were initiated by patients with fatty liver responding to a radio advertisement, 8 from hepatologists, 13 by general practitioners, 1 from a non-hepatology physician and 4 from hepatobiliary surgeons. 353 patients were excluded from further assessment due to the presence or severity of comorbidities or unconfirmed steatosis. 69 patients (59% female) fulfilled criteria for physical assessment, transient elastography using Fibroscan®, and blood tests. Amongst these 66% had abdominal obesity by BMI criteria and 93% using waist circumference criteria, 50% raised alanine aminotransferase (ALT), 26% aspartate aminotransferase (AST), 63% gamma glutamyl transpeptidase (GGT) levels, 30% T2DM, 54% arterial hypertension, 44% hypertriglyceridaemia, 47% low levels of high density lipoprotein cholesterol (HDL-C) and 75% metabolic syndrome. Non-invasive pre-screening for fibrosis found 31% Fibroscan® liver stiffness measurement (LSM) >8.5kPa, 13% AST/ALT ratio >1, 16% FIB-4 >2.67, 13% NAFLD fibrosis score >0.676. Liver biopsy was performed on 28 patients (41%), of whom 15 (53%) had NASH, including 2 (7%) with cirrhosis. Amongst biopsied patients, NASH was more common in females (67%). 12 patients (17%) were enrolled in NASH clinical trials. No single clinical or non-invasive variable was independently predictive of NASH, however 77% of those with NASH had LSM >8.5 kPa.
Conclusion: Community-based patients with NAFLD are concerned about NASH and are willing to participate in NASH treatment trials. LSM >8.5 kPa was the most useful pre-screening test for NASH. Education of the medical community, plus empowering NAFLD patients to initiate assessment of NAFLD severity is critical to early diagnosis and treatment of NASH.

P03-07 Multi-parametric MRI as a composite biomarker for NASH and NASH with fibrosis

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Background and aims: Effective management of the fatty liver disease epidemic requires accurate and scalable approaches to stratify patients to identify those with steatohepatitis (NASH) and fibrosis, who are at greater risk of poor clinical outcomes. Liver*MultiScan*™ is a multiparametric MRI-based method measuring iron-corrected T1 (cT1) and proton density fat fraction (PDFF). PDFF accurately measures steatosis, while cT1 correlates with the key histological features of NASH: ballooning, inflammation and fibrosis, and predicts liver-related outcomes. Here, we evaluate the utility of these MRI metrics, in combination with fasting glucose, a marker of the metabolic syndrome, for the stratification of NASH patients.
Method: 77 individuals from two UK outpatient liver clinics with biopsy-confirmed NAFLD were included for this analysis. Mean age was 50.4 years [18-74]; 39% (30) female; 68% (52) had a BMI ≥30kg/m^2. All participants underwent multiparametric MRI (Liver*MultiScan* protocol; acquisition <10 mins) from which cT1 and PDFF were calculated, alongside measurement of fasting glucose. The ability of these biomarkers to identify individuals with NASH (NAFLD Activity Score (NAS)≥4) and high risk NASH (NAS≥4 and Brunt fibrosis Stage ≥2) was evaluated using AUROC analysis, with logistic regression used to combine biomarkers.
Results: PDFF and cT1 both performed well for identifying NASH with AUROCs of 0.85 (0.77-0.94) and 0.81 (0.70-0.91), respectively. When used in combination, the performance improved further (AUROC = 0.88, 0.81-0.96). For identifying high-risk NASH, cT1 outperformed PDFF, with AUROCs of 0.78 (0.68-0.89) and 0.67 (0.55-0.79), respectively. Combining cT1 with fasting glucose improved the identification of high-risk NASH further with an AUROC of 0.89 (0.81-0.96).
Conclusion: PDFF and cT1 are both effective biomarkers for identifying NASH; however, cT1 outperforms PDFF for identifying NASH patients with significant fibrosis. This performance is further enhanced when cT1 is used in composite with fasting glucose, highlighting the diagnostic utility of multiparametric MRI when used in conjunction with serum markers of metabolic disease.

Figure:

	AUROC	Se.	Sp.	PPV	NPV
cT1	0.78 (0.68-0.89)	0.89	0.62	0.66	0.87
PDFF	0.67 (0.55-0.79)	0.77	0.52	0.57	0.73
Glucose	0.77 (0.66-0.88)	0.57	0.89	0.8	0.71
cT1 + Glu.	0.89 (0.81-0.96)	0.89	0.76	0.76	0.89
cT1 +PDFF + Glu.	0.88 (0.8-0.95)	0.83	0.81	0.78	0.85

Table 1: A table showing the breakdown of AUROC analysis for high-risk NASH (NAS ≥4 and Fibrosis ≥2) using cT1 (optimal cut-off = 853ms), PDFF (optimal cut-off = 9.6%) and cT1 + fasting blood glucose (Glu.).

P03-08YI RIPK3-MLKL mediated necroptosis contributes to ischaemia induced cell death in steatotic hepatocytes

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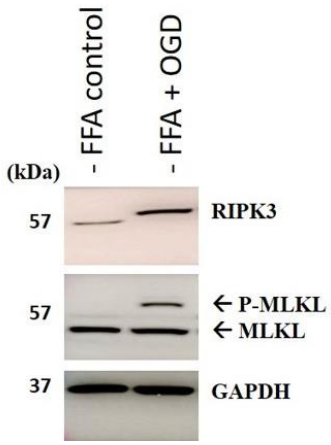
Background and aims: Steatosis in donor livers poses an increased risk of early graft dysfunction due to their vulnerability to ischaemia-reperfusion (I/R) insult. Necroptosis is associated with I/R injury and receptor-interacting protein kinase 3 (RIPK3) orchestrates necroptosis via phosphorylation of mixed lineage kinase domain like pseudokinase (MLKL). However, necroptosis and RIPK3-MLKL dependent cell death has not been fully studied in steatotic liver undergoing I/R injury. Here, we investigated the involvement of RIPK3 and MLKL in cell death during ischaemic insult in our *in vitro* model of hepatic steatosis and I/R injury.

Method: Alpha mouse liver 12 (AML-12) cells were subjected to hepatic steatosis by culturing in media containing 2mM free-fatty acid (FFA; 24 hours) and incubation in hypoxic conditions (1% O_2, 5% CO_2, and 94% N_2) for a further 12 hours in glucose-free media to create oxygen-glucose deprivation conditions (OGD) as seen in ischaemia. Western blot analysis was used for protein expression and real-time polymerase chain reaction (RT-PCR) for mRNA quantification. Cellular necrosis and inhibition of RIPK3 with GSK'872 was monitored by flow cytometry (annexin V/propidium iodide double staining).

Results: We found that OGD conditions induced cell death via RIP-MLKL dependent necroptosis. RIPK3 was increased in FFA+OGD treated cells compared to controls ($p \leq 0.01$). The absence of cleaved-CASPASE3 and presence of phosphorylated MLKL in FFA+OGD treated cells strongly indicated that ischaemic injury in these steatotic cells contributed to RIPK3-MLKL dependent necroptotic cell death. Cell viability assays showed a reduction in viable cells after OGD ($p < 0.01$) and flow cytometry demonstrated the necrotic form of cell death after OGD ($p < 0.01$). Pre-treatment of FFA+OGD cells with GSK'872 deceased RIPK3 expression by 57.4% and downregulated MLKL expression by 44.2% compared to FFA+OGD alone. Flow cytometry results also showed a decrease in necrotic cells in GSK'872 treated cells (22% necrotic) compared to FFA+OGD (45% necrotic). The decrease in inhibitor of nuclear factor kappa-B kinase subunit alpha (*Ikappak-alpha*) and nuclear factor kappa-light-chain-enhancer of activated B cells (*Nf- kappab)* mRNA expression by 89.5% and 70.2% respectively indicated that cells failed to undergo DNA repair after OGD treatment.

Conclusion: Our findings suggest that RIPK3-MLKL dependent necroptosis resulted in ischaemic injury in our *in vitro* model. Further investigation of the inducers of necroptosis will help understand the mechanisms of RIPK3-MLKL medicated necroptosis. In conclusion, targeting RIPK3 may be a promising therapeutic approach to inhibit necroptosis during I/R injury in fatty liver, with the ultimate goal of increasing the donor pool for liver transplantation.

Figure: OGD treatment upregulated necroptosis-associated RIPK3 and MLKL expression.



P03-09YI Energy metabolism in obesity reveals that NASH requires targeting AMPK/mTOR-driven pathways

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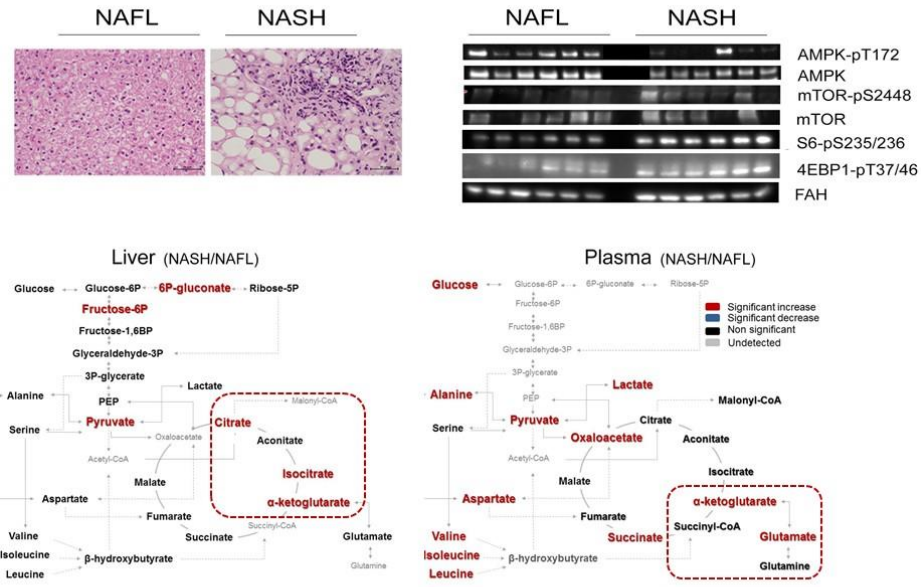
Background and aims: Obesity is usually accompanied by a set of comorbidities, among which the non-alcoholic fatty liver disease (NAFLD) is commonly present. Non-alcoholic steatohepatitis (NASH) is characterized by progressive liver injury, inflammation, and fibrosis related to obesity. However, the mechanisms that govern the transition from hepatic steatosis, which is relatively benign, to NASH remain poorly defined. Multiple pathogenic drivers have been implicated in NASH initiation and progression, including mitochondrial dysfunction, autophagy and acute alterations into metabolism. Exploring to identify potential therapeutic approaches, the aims of our study were to better understand energy metabolic changes in NAFLD progression to find a non-invasive metabolite biomarker.
Method: Patients undergoing bariatric surgery following procedures involving laparoscopic sleeve gastrectomy were recruited as a model of obesity-induced NAFLD in an observational study. Samples were obtained immediately before bariatric surgery. Relevant data and histological features of the liver were obtained prospectively. Several Western Blots were performed to evaluate the state of the AMPK/mTOR pathway and targeted metabolomics assays of energy metabolism intermediaries (GC-EI-QTOF-MS) for liver and plasma samples were carried out.
Results: We observed an important mTORC1 activation in patients with NASH. Hepatic steatosis and chronic low-grade inflammation by the AKT/mTORC1 complex inhibits autophagy and promotes an imbalance of pAMPK/AMPK ratio. Liver metabolic analysis showed that NASH were promoted the accumulation of glycolytic intermediates proximal to glucose-6-phosphate and were increased levels of the citric acid cycle (CAC) intermediates citrate/isocitrate and α-ketoglutarate. When we evaluated plasma, NASH patients led to augmented levels of glycolytic intermediates (glucose and pyruvic acid), that is, those intermediates involved in glucose transport and phosphorylation. Plasma concentration of oxaloacetate, α- ketoglutarate and succinate were increased in NASH patients. Curiously, we observed an important activation of glutaminolysis and high accumulation of aspartate, alanine and branched-chain amino acids (BCAAs).
Conclusion: We demonstrated that α-Ketoglutarate is a key metabolite of energy homeostasis that modulates hepatocyte health in NASH patients through mammalian TORC1 (mTORC1). Thus, hepatic modulation of α-Ketoglutarate may be as a crucial checkpoint for steatosis-to-NASH progression by mTORC1 inhibition pathway to counter liver injury.


NAFL
NASH
NAFL
NASH
AMPK-pT172
AMPK
mTOR-pS2448
mTOR
S6-pS235/236
4EBP1-pT37/46
FAH
Liver (NASH/NAFL)
Plasma (NASH/NAFL)
Significant increase
Significant decrease
Non significant
Undetected
Glucose
Glucose-6P
6P-gluconate
Ribose-5P
Fructose-6P
Fructose-1,6BP
Glyceraldehyde-3P
3P-glycerate
Lactate
Alanine
PEP
Malonyl-CoA
Pyruvate
Citrate
Serine
Oxaloacetate
Aconitate
Acetyl-CoA
Isocitrate
Malate
α-ketoglutarate
Aspartate
Fumarate
Succinyl-CoA
Valine
Succinate
Glutamate
Isoleucine
β-hydroxybutyrate
Glutamine
Leucine

P03-10YI Exercise improves HFD-mediated metabolic syndrome, dysbiosis and associated gut-liveraxis and biliar acid deregulation in an in vivo model of early obesity and NAFLD

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Background and aims: Childhood obesity have reached epidemic levels representing one of the most serious public health concerns associated with metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) and gut microbiota alterations. There is few clinical experience for pediatric NAFLD patients, and since no perfect NAFLD *in vivo* model has been developed, its pathogenesis remain unclear and the therapeutic options are very scarce with respect to safety, effectiveness, and patient compliance. Physical exercise is known to improve obesity and NAFLD progression, modulating the gut microbial balance. Therefore, we aimed to evaluate the benefits of exercise on gut microbiota and metabolic status modulation in an *in vivo* model of early obesity and NAFLD.

Method: 21 days old male Wistar rats fed with control (C) or high fat diet (HFD) were subjected to an interval aerobic training protocol. Metagenomic and metabolomic analyses were performed in fecal samples. The relative expression of genes related to lipids and biliar acids metabolism as well as gut-liver axis alterations were measured.

Results: Exercise decreased HFD-induced body weight gain, metabolic syndrome, liver disfunction, and intrahepatic lipid accumulation as a result of its lipogenic metabolism modulatory capacity. Exercise training also reduced the subsequent lipotoxicity and improved the inflammatory response, downregulating the NF-κB transcriptional activity induced by HFD. Some of this effects seems to be mediated by its capacity to preserve intestinal barrier functionality, which in turn prevents gut-liver axis deregulation and improves the bile acids enterohepatic circulation and homeostasis. Besides, exercise effectivelly conteracted HFD-induce dysbiosis, increasing the *Firmicutes/Bacteroidetes* ratio *vs* sedentary rats. Moreover, a specific microbiome with a functionaly-associated metabolomic profile, was found in relation to diet, age, and exercise.

Conclusion: We provide scientific evidences highlithing the benefits of physical exercise protocols to modulate the intestinal microbiota in the management of childhood obesity and NAFLD development, via its anti-inflammatory, lipids and biliar acids metabolism modulatory and prebiotic capacities. Suported by LE063U16 (JCyL and FEDER), BFU2017-87960-R, GRS1428/A/16 and CIBERehd funded by ISC III.

P03-11YI The Zucker Diabetic Sprague Dawley rat: a novel model for Type 2 diabetes-related non-alcoholic steatohepatitis

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Background and aims: Non-alcoholic steatohepatitis (NASH) is highly prevalent in Type 2 diabetes mellitus (T2DM). NASH progresses into cirrhosis and hepatocellular carcinoma and is known to worsen the prognosis and mortality in T2DM. Our understanding of the mechanisms underlying NASH development in T2DM is hindered by the absence of a good experimental model that can physiologically develop clear T2DM and NASH. This study sought to investigate the suitability of the Zucker Diabetic Sprague Dawley (ZDSD) rat for studying T2DM-related NASH.
Method: Eight, fifteen-week old ZDSD rats were fed a diabetogenic diet for 5 weeks. Six, age-matched, Sprague Dawley rats were used as controls and fed normal rodent chow. We monitored body mass, fasting glucose, fasting triglycerides (TGs) and glucose handling. We also measured circulating levels of the liver function enzymes; alanine transaminase and alkaline phosphatase and scored livers for NASH
Results: ZDSD rats developed frank T2DM and exhibited impaired glucose handling, chronic hyperglycaemia (fasting glucose 19.45 ± 5, 36 vs 4.07 ± 0.58 in controls), deranged lipid metabolism (fasting TGs 2.73 ± 1.03 vs 1.22 ± 1.29 in controls) and impaired liver function (ALP 366.38 ± 72.51 vs 178.50 ± 20.19 in controls; and ALT 114.88 ± 35.58 vs 84.00 ± 50.84 in controls). Histopathological analyses of ZDSD rat livers showed the presence of >66% steatosis, 33-66% inflammation, 33-66% hypertrophy and advanced fibrosis.
Conclusion: The cooccurrence of both T2DM and advanced NASH in the ZDSD rat validates our hypothesis of its potential as a model for studying the pathogenesis of T2DM-related NASH.

P03-12YI Ultrasound-based quantitative assessment of hepatic fat content: the Seato-score system

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Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD) is reaching epidemic proportion and is associated to many other pathological conditions. For these reasons a non-invasive, cheap and easy-to-use system for quantifying the hepatic fat content is needed. We developed a new system for quantitative evaluating liver fat content post-processing ultrasound (US) images.

Method: We enrolled 105 subjects for which Magnetic Resonance Spectroscopy (MRS) measurements and Controlled Attenuation Parameter (CAP) values were obtained. For each subject, US images were acquired and employed for assessing hepatic-renal ratio (HR), hepatic-portal vein ratio (HPV), attenuation-rate (AR), diaphragm visualization (DV) and portal-vein-wall visualization (PVWvis). *Steato-score* was obtained combining these five parameters using multivariate regression analysis using MRS data as ground-truth. The diagnostic performances of the *Steato-score* were evaluated dividing the population on the MRS base using previously specified cut-off values correspondent to biopsy steatosis classes S0, S1, S2 and S3. In particular, 3 classifications were performed: S0vsS1S2S3 using a MRS cut-off of 3.12%; S0S1vsS2S3 using a MRS cut-off of 8.77%; S0S1S2vsS3 using a MRS cut-off of 13.69%. The same analysis was repeated using the correspondent CAP cut-off values: 233.5 dB/m, 268.5 dB/m and 301.2 dB/m.

Results: Steato-score was dependent on HR, AR, DV and PVWvis and was significantly correlated with both MRS and CAP values. As concerns the comparison with MRS, area under the ROC curve (AUROC) was equal to 0.90, with 81% sensitivity, 88% specificity for the comparison S0vsS1S2S3, was equal to 0.98, with 100% sensitivity and 88.8% specificity for the comparison S0S1vsS2S3 and equal to 0.97, with 91% sensitivity and 94% specificity for the comparison S0S1S2vsS3. The same analysis performed for the CAP assessments provided lower values: AUROC:0.74, sensitivity:60%, specificity:84% for S0vsS1S2S3; AUROC:0.82, sensitivity:79%, specificity:79% for S0S1vsS2S3; AUROC:0.87, sensitivity:94%, specificity:73% for S0S1S2vsS3.

Conclusion: This proposed system could be a valid alternative for a non-invasive, simple and inexpensive assessment of intrahepatic fat.

P03-13YI Changes in liver fatty acid delta-9, delta-6 and delta-5 desaturase activities in two animal models of hepatic steatosis

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Background and aims: Changes in liver fatty acid desaturase activities were proposed as major factors involved in the development and progression of liver steatosis. The aim of this study was to investigate the modifications in liver fatty acid delta-9, delta-6 and delta-5 desaturase (D9D, D6D and D5D, respectively) in two rat models of NAFLD such as the methionine and choline deficient (MCD) diet model and Obese Zucker rats.

Method: Eight-week-old male Wistar rats fed for 3-week with MCD diet and relative control diet were used as nutritional model of NAFLD. Twelve-week-old Obese and Lean male Zucker rats were used as genetic model of NAFLD. Serum levels of hepatic enzymes (AST, ALT, Alkaline Phosphatase) were quantified. Hepatic TBARS and ROS were evaluated as index of oxidative stress. Liver fatty acid profiling was performed by Gas Chromatography-Mass Spectrometry analysis (GC-MS). The estimated desaturase activities were calculated using ratios of 16:1n−7/16:0 (D9-16D), 18:1n−9/18:0 (D9-18D), 18:3n−6/18:2n−6 (D6D) and 20:4n−6/20:3n−6 (D5D). The anti-inflammatory fatty acid index (AIFAI) was also quantified.

Results: Liver D9-18D activity increased both in MCD and Obese Zucker rats but it was considerably upregulated in MCD rats. No changes in D9-16D occurred in MCD group while an increase was found in Obese Zucker rats. Four and two fold decrease in hepatic D-5D activity occurred in MCD and Obese Zucker rats, respectively. A comparable trend occurred for Delta-6D in MCD rats that correlated with oxidative stress assessed by TBARS and ROS ($r = 0.74$, $P < 0.01$ and $r = 0.87$, $P < 0.001$, respectively). Conversely, no D-6D was detectable in Obese Zucker rats. Liver docosahexaenoic acid (DHA; 22:6n-3) and AIFAI were lower in MCD when compared with Obese Zucker rats. No significant difference in serum AST, ALT and Alkaline Phosphatase were found comparing the two NAFLD animal models.

Conclusion: Extensive changes in D9-18D, D6D and D5D occurred in MCD rats when compared with Obese Zucker rats. These events were associated with marked oxidative stress and decrease in AIFAI and DHA, this last recently found to protect against the progression to steatohepatitis that spontaneously occurs only in MCD rats. These results may contribute to the understanding of NAFLD progression and provide the basis for the identification of potential therapeutic targets able to counteract this common disorder.

P03-14YI The deletion of perforin protects from non-alcoholic steatohepatitis (NASH) development

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Background and aims: In industrial countries non-alcoholic steatohepatitis (NASH) is the third most common reason for liver transplantation and the fastest growing medical problem. The protective effect of the perforin-granzyme system in CCL4 induced fibrosis is well known. Further it was shown before that the cytotoxic molecule perforin is involved in mediating ConA-induced hepatitis. The importance of the mediator of NKT- and CD8 T cell cytotoxicity perforin in NASH development remains unclear.
Method: Therefore, we investigated WT and perforin$^{-/-}$ mice in the methionine-choline deficient (MCD) and two different high fat diet (HFD) models of murine steatohepatitis.
Results: MCD (4 weeks) and HFD (24 weeks) fed perforin$^{-/-}$ animals showed less invasive development and progression of steatosis. Perforin$^{-/-}$ mice showed a dampened manifestation of typical characteristics of the metabolic syndrome, namely a decreased liver:body weight ratio, an improved insulin resistance and decreased levels of cholesterol and serum triglycerides in both mouse steatohepatitis models. Attenuated disease progression was further reflected by less histomorphological changes with maintenance of an intact liver architecture and less fatty liver degeneration by decreased intrahepatic triglyceride accumulation. Ameliorated pathogenesis of diet-induced steatohepatitis was further displayed by lower levels of serum transaminases and proinflammatory cytokines in perforin$^{-/-}$ mice compared to WT. Consistent with the less invasive phenotype, perforin$^{-/-}$ animals exhibit less hepatic infiltration of pro-inflammatory immune cells such as CD11b^{+}Ly6G^{+} neutrophils and monocyte derived inflammatory macrophages.
All these changes finally lead to less collagen accumulation in livers of MCD and HFD treated perforin$^{-/-}$ animals compared to WT controls.
Conclusion: The deletion of perforin in mice resulted in a protection against diet-induced steatohepatitis in two mouse models of steatohepatitis induced by dietary treatment. Therefore, the disruption of the cytotoxic perforin-granzyme system could provide a potential target for anti-NASH intervention.

P04-01 Sheep as a large animal model for fatty liver research

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Background and aims: Fatty Liver (FL) develops when the hepatic synthesis and uptake of lipids exceed their rate of breakdown and export out of the liver. High-producing ruminants are susceptible to enhanced hepatic fat accumulation, which can lead to severe and often lethal metabolic disorders such as pregnancy toxemia. Despite the high prevalence of FL conditions in prolific sheep, its spontaneous development only late in gestation limits its potential use as a controlled model for investigating the pathophysiology of FL disease, primarily since pregnant ewes represent a highly heterogeneous population of widely varying physiological states. To establish a consistent and sensitive model system for testing FL-related hypotheses using sheep, we have sought to create an inducible model employing lambs, which constitute a homogeneous and controlled population with respect to many relevant endocrine and metabolic parameters.
Method: Over nutrition is strongly associated with hepatic fat accumulation in animals and human, we thus hypothesized that raising lambs on a high-energy diet will induce excess hepatic fat. To test this, we fed 2-months old weaned lambs (n = 31) with two widely different caloric diets, for 4.5 months, and performed post mortem analysis of their livers for fat content. The high-calorie (HC) diet was based on highly digestible carbohydrates, while the low-calorie (LC) diet was based on hay.
Results: The HC group was consistently more hyperglycemic, with up to 41% greater average blood-glucose levels (100 mg/dL for HC vs. 71 mg/dL for LC; $p < 0.0001$). This was rather surprising, considering the fact that the primary source of blood glucose in ruminants originates from hepatic gluconeogenesis. The LC group had higher plasma free fatty acids (FFA), 282 compared to 156 μEq/L ($p < 0.02$) in the HC, indicating a state of enhanced lipolysis. In contrast, the fasting levels of FFA were much higher in the HC group, 1010 vs. 492 μEq/L ($p < 0.0001$), likely due to significant adipose insulin resistance. The HC group developed larger livers averaged at 1.36 Kg compared to 0.625 Kg in the low LC group ($p < 0.0001$). This difference could not be explained by the difference in their body weights, since the liver weight per kilogram of body weight was also significantly higher in the HC group (hepatic index of 1.9 vs. 1.4 in the LC group, $p < 0.0001$). Strikingly, the average hepatic fat content in the HC group was twice that of the low LC (7% vs. 3.6 %, $p < 0.004$). Consistently, histopathological examination also revealed higher hepatic fat in the HC group.
Conclusion: The two different steatotic states, established by this large mammal nutritional model for FL, offer a scheme for sensitive hypotheses testing of the potential of various factors to modulate liver fat content, to help advance NAFLD biomedical research and therapy development.

P04-02YI The effect of carvedilol compared to propranolol to control hepatic venous pressure gradient (HVPG) in cirrhotic patients with portal hypertension: an evident based case report

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Background and aims: Portal hypertension is a clinical syndrome in which there is an increase in portal vein pressure, as one of the consequences of cirrhosis that manifests in esophageal varises and gastropathy. Prophylaxis therapy of portal hypertension that is commonly used nowadays is propranolol, a non-selective beta blocker, acts by inhibiting β1 and β2 receptor. Other drug, carvedilol, has additional effect of inhibiting α1 receptor. The action of inhibiting α1 receptor is said to make it more superior, because of the vasodilation effect on intrahepatic circulation. This study aims to find out carvedilol's effect compared to propranolol to control Hepatic Venous Pressure Gradient (HVPG) in cirrhotic patients with portal hypertension.
Method: Online literature searching was done using 4 databases: PubMed, Cochrane, EBSCO, and ProQuest. Studies were selected according to the inclusion and exclusion criteria. Selected articles are critically appraised according to the Center of Evidence-Based Medicine, University of Oxford.
Results: Two articles were critically appraised. Study by Tong Li et al (2016) shows the number-needed-to-treat (NNT) to cause ≥20% reduction of HVPG or to cause HVPG to fall <12 mmHg is 3. Study by Gupta et al (2017) shows the NNT is 4.
Conclusion: Carvedilol is more effective compared to propranolol to control HVPG in cirrhotic patients with portal hypertension.

Figure:

Article (year)	Type of study	Level of Evidence	Outcome	CER (%)	EER (%)	ARR (%)	RRR (%)	NNT	Conclusion
Tong Li, et al (2016)	Meta-analysis		Hemodynamic response* in 24 hours	37, 5	62, 7	25, 2	67, 2	4	Important
		1	Hemodynamic response* in 24 hours until 13 weeks	38, 2	54, 4	16, 2	42, 4	3	Important
Gupta, et al (2017)**	RCT	2	Hemodynamic response*	48, 28	73, 33	25, 05	51, 88	4	Important
			HVPG decrease below 12 mmHg	6, 90	10	3, 2	44, 93	32	

* Hemodynamic response: HVPG decrease >20% or below 12 mmHg
**using intention to treat analysis
CER: *control event;* EER: *experimental event;* RRR: *relative risk reduction*; ARR: *absolute risk reduction;* NNT: *number-needed-to-treat.*

P04-03YI Association between NAFLD, cardiovascular complications, insulin resistance in obese patients

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Background and aims: The role of NAFLD discuss as a disease, that determines not only the severity of injury, but associated with the progression of cardiovascular disease (CVD) and the development of type 2 diabetes mellitus (DM) and other system injuries. Purpose: to determine the relationship between NAFLD, CVD and insulin resistance in obese patients.
Method: The study involved 156 NAFLD patients with normal, overweight and obese without of type 2 diabetes mellitus and 112 patients with normal, overweight and obese with type 2 diabetes mellitus. Conducted anthropometric survey measured levels of AST, ALT, GGT, the degree of liver fibrosis using elastography (FibroScan), ECG. The stratification of CV risk was evaluated by SCORE scale version for countries with high risk. We determined the level of inflammatory mediators (TNF-α, IL-1, IL-6), markers (CRP, fibrinogen), endothelin -1, the thickness of the intima-media complex, presence atherosclerotic plaque and stenosis of the carotid arteries, insulin resistance index HOMA-IR for all patients.
Results: In both groups was revealed left ventricular diastolic dysfunction and QT prolongation in patients with NAFLD and type 2 DM, that was associated with the severity of disease. Most patients with NAFLD by obesity showed a reduction in endothelium-dependent vasodilation, indicating the presence of endothelial dysfunction. The concentration of pro-inflammatory cytokines in patients with NAFLD was 3-7 times higher than the similar parameters of patients with a similar degree of obesity, but without evidence NAFLD. The concentration of ET-1 in the blood plasma of patients with NAFLD has a strong direct correlation with the degree of cardiovascular risk of surveyed patients. It is found that many inflammatory mediators (TNF-α, IL-1, IL-6) and markers (C-reactive protein, fibrinogen) highly correlate with the degree of obesity, the concentration of ET-1 and markers of insulin resistance, a predictor for cardiovascular risk.
Conclusion: Presents of diastolic dysfunction of left ventricular, disturbances of endothelium-dependent vasodilatation, the concentration of ET-1, mediators of systemic inflammation, increase the values of intima-media thickness, an increase the frequency of cardiac arrhythmias is highly correlated with the degree of CV risk. Inflammatory mediators highly correlate with the degree of obesity. Presence of NAFLD dictates mandatory screening for cardiovascular disease in these patients.

P04-04YI Histidine rich glycoprotein as a hypoxia-inducible factor 2α-dependent pro-fibrogenic mediator in experimental non-alcoholicfatty liver disease

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Background and aims: Hypoxia and hypoxia inducible factors (HIFs) are believed to significantly affect fibrogenic progression of chronic liver diseases (CLD). Recently, we showed that HIF-2α expression is up-regulated in parenchymal cells in either experimental or human non-alcoholic fatty liver disease (NAFLD) and contributes in sustaining liver fibrogenesis in the methionine/choline-deficient (MCD) diet model of NAFLD. In the present study, we provide further insights in the mechanisms by which HIF-2α promotes the progression of experimental NAFLD.
Method: NAFLD was induced by feeding mice carrying hepatocyte-specific conditional deletion of HIF-2α (HIF-2α fl/fl/Alb-Cre mice) and control littermates with choline-deficient L-amino acid refined (CDAA) diet. In vitro studies have been performed using HepG2 cells overexpressing HIF-2α. We analyzed also liver biopsies from NAFLD patients (n = 26) ranging from early disease (staged F0 -F1) to more advanced conditions of fibrosis (staged F2-F3) or cirrhosis (F4) and referring to the Division of Gastro - Hepatology of the University of Turin.
Results: In the dietary model of NAFLD hepatocyte deletion of HIF-2α resulted in: i) a decrease in fatty liver and parenchymal necrosis; ii) amelioration in lobular inflammation and in the hepatic expression of pro-inflammatory cyto/chemokines (TNF-α, IL-12, CCL2, CXCL10); iii) a significant decrease in the expression of pro-fibrogenic genes (collagen 1A1, TGF-β1, α-SMA) as well as in extracellular matrix deposition (Sirius Red staining) and in the number of activated myofibroblasts. Such an improvement in NAFLD evolution in HIF-2α deficient mice was associated with a selective lowering of the hepatic production of Histidine-Rich Glycoprotein (HRGP), a hepatocyte-derived protein recently implicated in sustaining macrophage M1 activation. Accordingly, the fraction of inflammatory F4-80 +/CD11b +/Ly6C+ hepatic macrophages and their capacity to produce IL-12 was significantly lower in HIF-2α fl/fl/Alb-Cre mice receiving the CDAA diet vs control littermates. Furthermore, in vitro experiments confirmed that up-regulation of HIF-2α resulted in enhanced HRGP expression by HepG2 cells. Analyses performed on specimens from NAFLD patients indicated that HRGP was overexpressed in all patients showing hepatocyte nuclear staining for HIF-2α and revealed a significant positive correlation between HIF-2α and HRGP liver transcripts levels in these patients.
Conclusion: These results indicate that activation of HIF-2α in hepatocytes can stimulate the progression of experimental NAFLD through the up-regulation of HRGP production.

P04-05YI Automated quantitation of ballooning, inflammation, steatosis and fibrosis using machine learning in routine histological images of liver biopsies of patients with NAFLD

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Background and aims: Histology remains the gold standard for diagnosing Non-Alcoholic Fatty Liver Disease. The current scores display high intra and inter-observer variability. We developed an automated method based on machine learning for the quantitation of ballooning, inflammation, fat and fibrosis using routine histological images of patients with NAFLD.

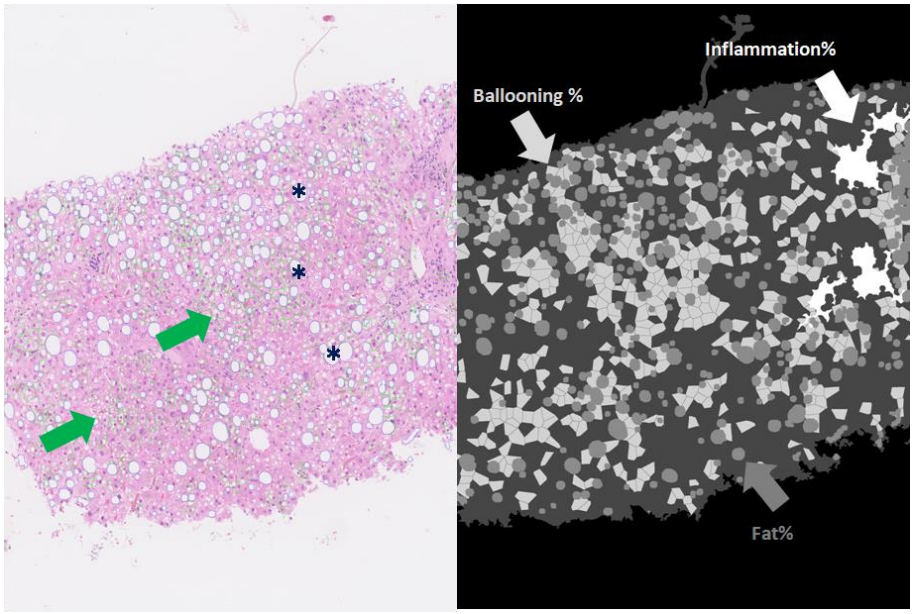
Method: We evaluated consecutive liver biopsies with NAFLD as the diagnosis. Biopsies were stained with HandE and Sirius Red and scored by two liver pathologists for NASH CRN scoring system. Images were digitalized and areas of ballooning and inflammation were annotated manually to facilitate machine learning. Results were expressed as percentages (areas of ballooning and inflammation divided by the whole biopsy area). Concordance between results and manual annotations was reported. Fat % and Collagen Proportionate Area (CPA) were obtained using image analysis as previously published by our group.

Results: We have analyzed 120 liver biopsies. As per CRN scoring system, steatosis grade was mild in 33 (27%), moderate in 65 (54%) and severe in 22 (18%) cases. Fibrosis was staged as F1-F2 in 47 (39%) cases, F3 in 44 (36%) and F4 in 14 (12%). Inflammation was: grade 0 in 28 (24%), grade 1 in 72 (59%), grade 2 in 17 (15%) and grade 3 in 2 (2%). Ballooning was reported as score 0 in 38 (32%), 1 in 49 (41%) and 2 in 33 (27%) cases. 32 (27%) patients had no NASH, 45 (37%) borderline NASH and 43 (36%) definite NASH.

As per image analysis, median Fat% was 3.2 ± 3% for grade 1, 15 ± 9% for grade 2 and 27 ± 8% for grade 3. Median CPA was 1.7 ± 1% in stage 1, 2.7 ± 3% in stage 2, 4.3 ± 3% in stage 3 and 14.6 ± 5% in stage 4. Median ballooning% was 15.1 ± 7% for score 0, 18.8 ± 6% for score 1 and 20 ± 7.4% for score 2. Median inflammation% was 1.5 ± 4% for score 0, 1.9 ± 2% for score 1, 3.3 ± 3% for score 2 and 4.7 ± 0.7% for score 3. Concordance between manual and automatic measurements was excellent with an ICC = 0.95, 95%CI = 0.69-0.98, F value = 13.2, p = 0.001 for ballooning and ICC = 0.98, 95%CI = 0.96-0.99, F value = 103.3, p = 0.0001 for inflammation.

Conclusion: We have developed and validated a novel technique for high-throughput, objective quantitation of all key histological features in liver biopsies of NAFLD. This technique is fully automated and requires no specialised equipment, making it applicable to clinical practice. The ability to assess histological outcomes and differences in NAFLD more objectively would be to great clinical advantage.

Figure: Results of quantitation in HandE: Fat% 27.7, Inflammation 2.1%, Ballooning 16.6%.
On the left, fat droplets (asterisk), ballooning (arrow).
On the right, inflammation in white, ballooning in light grey, fat droplets in dark grey.


Inflammation%
Ballooning %
Fat%

P04-06YI Pharmacological inhibition of Adipose Triglyceride Lipase prevents metabolic disorders in mice

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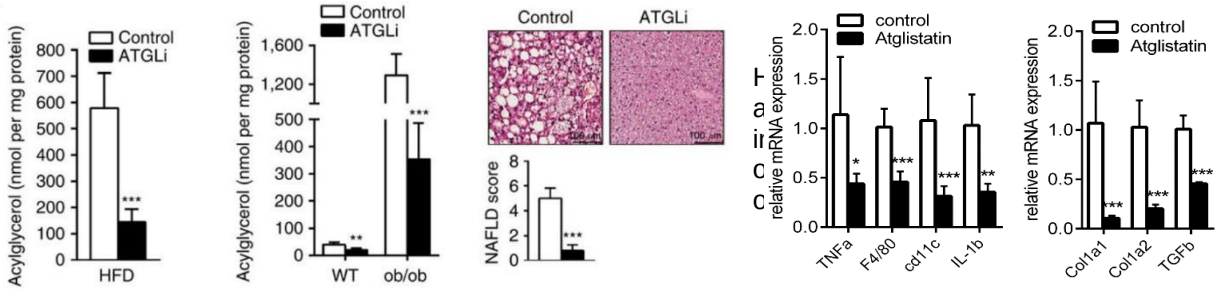
Background and aims: Obesity and associated comorbidities have become a major issue for public health. Adipose tissue expansion is often associated with NAFLD and insulin resistance, hallmarks of metabolic and cardiovascular complications of obesity. Excess calories are temporarily stored as triacylglycerols (TGs) within adipocytes of fat depots. Adipose Triglyceride Lipase (ATGL) initiates the degradation of TGs and hence determines the concentration of free fatty acids in the circulation.
We thus examined the effect of pharmacological ATGL inhibition via the ATGL specific inhibitor Atglistatin in mice to delineate the interconnection between adipose tissue lipolysis, obesity, NAFLD, and insulin sensitivity.
Method: High fat diet induced or genetically induced (Leptin deficient; ob/ob) obese mice were treated with ATGL specific inhibitor Atglistatin. Effects on diverse aspects of metabolic disease were investigated.
Results: Atglistatin treatment reduces obesity and insulin resistance and protects from NAFLD and ectopic lipid accumulation.
Conclusion: Our findings delineate an important contribution of ATGL mediated lipolysis to the development of obesity and metabolic disorders. Hence, modulating lipolysis by pharmacological intervention displays a useful approach to study the interconnection between lipolysis, obesity, and insulin resistance.

Figure:



P04-07YI Human induced pluripotent stem cell derived hepatocyte like cells-a valuable model for drug testing in Non-alcoholic Fatty Liver Disease

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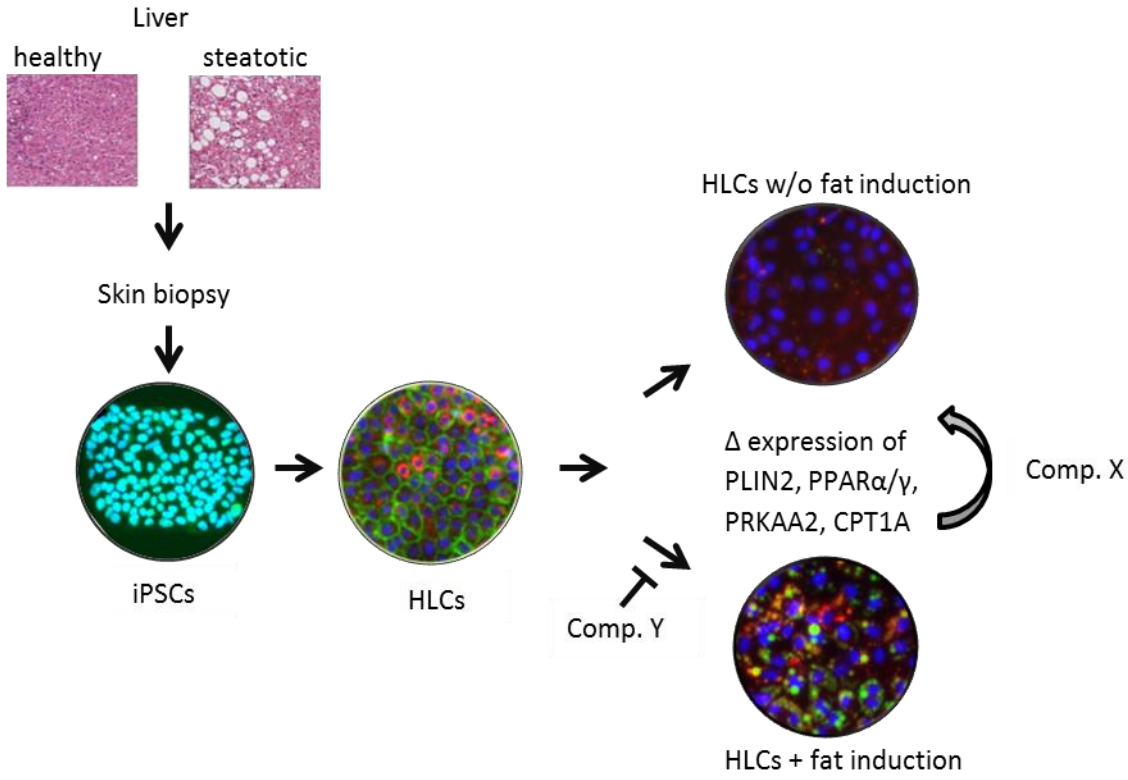
Background and aims: Non-alcoholic Fatty Liver Disease (NAFLD) is a multi-factorial disease with increasing incidence worldwide. It is characterized by the accumulation of lipid droplets (LDs) in hepatocytes and can progress from benign steatosis towards Non-alcoholic Steatohepatitis, cirrhosis and liver cancer. Due to its complex phenotype and a divergence between mouse and human metabolism, it has been difficult to model NAFLD. To circumvent this, we have established a reliable *in vitro* model that allows us to analyse the molecular mechanisms that lead to disease development and progression and also to identify druggable targets in order to interfere with these processes.
Method: We have established several induced pluripotent stem cell (iPSC) lines from healthy donors as well as from NAFLD patients, we then comparatively differentiated these into hepatocyte like cells (HLCs) for modelling the disease with respect to the distinct genetic backgrounds of the donors. We induced fat storage in these cells by adding oleic acid to the medium and observed LD formation by Bodipy493/503 staining. In order to reduce fat incorporation and improve metabolism and insulin sensitivity in the HLCs, we tested two small molecules (compound X and Y) on these cells in short- and long term experiments.
Results: All analysed HLCs were able to incorporate LDs although the pattern of the LD size and distribution varied between cell lines. In general, we observed significant up-regulation of the LD-coating protein perilipin 2 (PLIN2) after fat induction and differential expression of various metabolism associated genes e.g. Peroxisome Proliferator-Activated Receptor (PPAR) α, PPARγ, Protein Kinase AMP-Activated Catalytic Subunit Alpha 2 (PRKAA2), and Carnitine Palmitoyltransferase 1A (CPT1A). We were able to improve overall metabolic functions by applying compound X to the cells while compound Y reduced LD formation.
Conclusion: Our data demonstrate that iPSC-derived HLCs are valuable for modelling NAFLD and enable studying the influence of genetic background on the disease. Furthermore, it is possible to analyse the impact of distinct nutritional cues on the metabolism. NAFLD patient derived iPSCs differentiated into HLCs also provide a novel tool for drug screening and toxicology, thus advancing personalized medicine.


Liver
healthy
steatotic
Skin biopsy
iPSCs
HLCs
HLCs w/o fat induction
Δ expression of
PLIN2, PPARα/γ,
PRKAA2, CPT1A
Comp. X
Comp. Y
HLCs + fat induction

P04-08YI C-MYC overexpression in hepatocytes is responsible for the spontaneous development of murine non-alcoholic steatopatitis

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease associated with obesity, diabetes mellitus, and hyperlipidemia. It can also progress to irreversible liver cirrhosis and hepatocellular carcinoma (HCC); however, the underlying mechanism is still unknown, but *endogenous* (i.e. genetic) factors such as oncogenes have been suggested to play a role.Here we analysed the impact of the proto-oncogene *c-MYC* for the development of murine NASH.
Method: Transgenic mice bearing overexpression of C-*MYC* in hepatocytes (Alb-MYC^{tg}) were studied for metabolic phenotype at baseline. Serum markers of NASH were measured and hepatic steatosis, inflammation and fibrosis scored histologically.
Results: Alb-MYC^{tg} mice develop moderate degrees of obesity and significant increase in blood glucose after fasting, as well as after the glucose load, especially apparent in animals older than 36 weeks old. Moreover, mutant Alb-MYC^{tg} mice exhibit hyperlypidemia and profound hepatic changes at baseline, characterized by significant microvesicular steatosis, hepatocellular ballooning and increased hepatic triglyceride content. Liver injury and inflammation associated with elevated serum transaminases (e.g.: ALT), marked infiltration of CD45 and F4/80 positive cells, mild perisinusoidal fibrogenic changes and compensatory proliferation.
Conclusion: Alb-MYC^{tg}-overexpressing mice develop mild obesity, glucose intolerance, hyperlipidemia and *NASH.* The fact that 70% of transgenic mice develop HCC at the age of 70 weeks suggests the important role of the oncogene C-MYC in the natural history of NASH to HCC progression.

P04-09YI Hepatocyte-specific caveolin-1 in non-alcoholic steatohepatitis mouse models

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Background and aims: Caveolin-1 (CAV1) has been reported to be an important regulator of lipid accumulation and metabolism. Several animal models have shown that global CAV1 deficiency prevents fat accumulation within hepatocytes and hepatic steatosis. However, to date the contribution of hepatocyte CAV1 expression on pathogenic steatosis has not been investigated in NASH development and progression. We therefore aimed to analyze the role of CAV1 in the development of steatosis, fibrosis and inflammation in NASH.
Method: We fed hepatocyte specific CAV1 knockout (KO) male mice with methionine-choline deficient (MCD) for 4 weeks, after which liver tissues were collected. The role of CAV1 in disease progression was analyzed by measuring serum biochemical parameters, quantitative PCR, Western blotting, and immunohistochemistry, e.g. Hematoxylin and Eosin, Sirius red, Masson's Trichrome, and Oil Red O staining. Further, RNA was isolated from livers of male mice, and used for microarray analysis of gene expression changes.
Results: MCD feeding caused a reduction in body weight, obvious hepatic steatosis, slight fibrosis and inflammation. In addition, liver function parameters such as ALT, AST, GLDH, urea and total bilirubin were elevated, while cholesterol and glucose levels were reduced when feeding MCD diet. However, these differences appeared to be independent of CAV1 expression. Microarray analysis identified *Fmo3, Cyp2b9, Sult1e1, Mmp12, Sprr1a* among upregulated genes, and *Moxd1, Serpina4-ps1, Hsd3b5, Mup21* and *Elovl3* among downregulated genes in MCD fed mice. While pathway enrichment analysis showed that metabolic processes are altered independent of CAV1, several pathway changes, e.g. proteoglycans in cancer, focal adhesion, fatty acid elongation, nucleotide excision repair and TGF-beta signaling pathway were prominent in the CAV1 KO setting.
Conclusion: Our results demonstrate that hepatocyte specific CAV1 does not affect liver steatosis and fibrosis in NASH but may influence the response of hepatocytes to pathological accumulation of metabolites.

P04-10YI The association between visceral fat and evolution of patients with non-alcoholic fatty liver disease

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Background and aims: Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD). However, patients with body mass index (BMI)>30Kg/m^2 and NAFLD do not always develop advanced fibrosis (AF). The distribution of body fat predicts the risk of AF in patients with NAFLD. This study investigated the association between visceral fat (VF) and the severity of NAFLD.

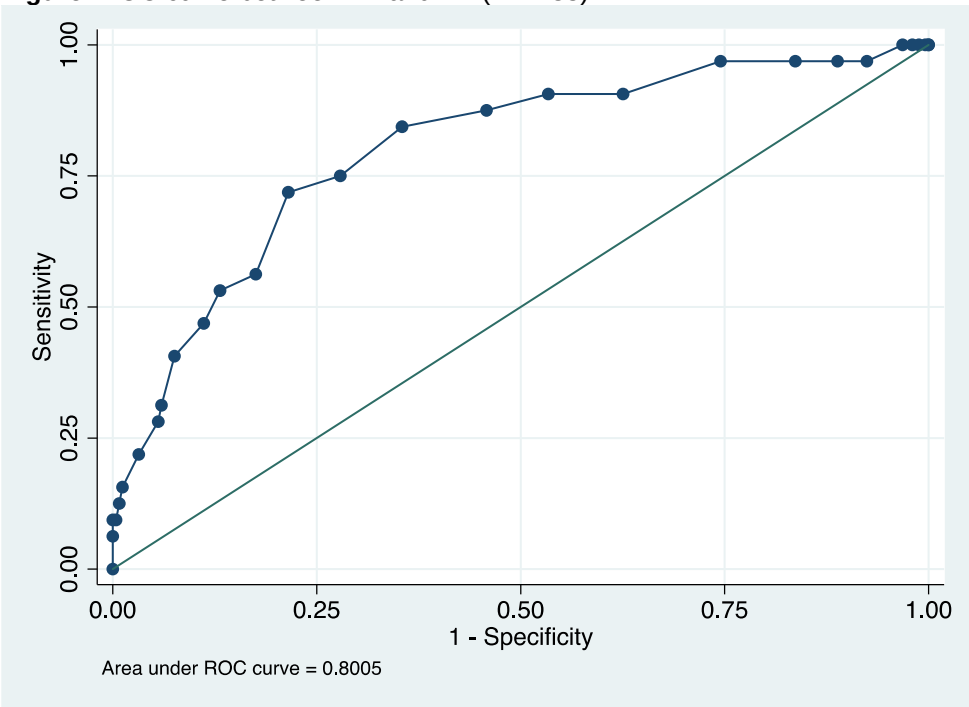
Method: In this cross-sectional study, 319 patients with liver steatosis detected by ultrasound were included. 36 patients with alcohol consumption >20gr/day, other liver comorbidities, prior bariatric or ileal surgery, ingestion of drugs known to produce hepatic steatosis or with malignancy were excluded. All patients underwent an anthropometric evaluation, blood tests and bioimpedance (TANITA DC430PMA). AF was defined by a NAFLD fibrosis score (NFS) >0.675 and Fibroscan® >8.7Kpa. Histological diagnosis was obtained in 71patients

Results: Between September 2017 and May 2018, 283 patients were collected for the study (64.3% male, age 57 years (SD10.3), Metabolic syndrome (MetS) 53% and Obesity 53.4%). 32 (11.3%) patients had AF. Age (65 vs. 56years; p <0.01), BMI (34.7 vs 30.7Kg/m^2; p <0.01), waist circumference (WC) (119 vs. 106.4cm; p <0.01), VF (19 vs. 13; p <0.01), bilirubin (1 vs. 0.7mg/dl; p <0.01), FA (96.1 vs. 81.9IU/L; p = 0.04) and MetS (81.3 vs. 49.4%; p = 0.01) were related to AF. In the multivariate analysis VF (OR 1.3, 95%CI 1.1-1.4; p <0.01) and bilirrubin (OR 5, 95%CI 2.1-11.9; p <0.01) were related to AF. The optimal cutoff point for VF in terms of AF prediction is 16 (Youden´s index = 0.75) (Se 72% and Sp 78%)and an AUROC of 0.8 (95CI0.7-0.9) (Figure 1).

In the cohort of patients confirmed by liver biopsy, 26 (33.6%) patients had AF. Age (65 vs. 53years; p <0.01), VF (19 vs. 14; p <0.01), Fibroscan® (23.5 vs 9.2Kpa; p <0.01), platelets (155.3 vs. 219.7 10F3/microL; p <0.01), albumin (4.2 vs. 4.5g/dl; p = 0.04), INR (1.2 vs. 1.1 RN; p <0.01), HbA1c (6.6 vs. 5.8%; p = 0.02) and MetS (91.7 vs. 54.2%; p = 0.02) were related to AF. The risk of AF was higher in patients with higher VF (OR 1.2, 95%CI 1.1-1.3; p = 0.01) and the optimal cutoff point for VF to predict AF is 14 (Youden´s index = 0.77) (Se 92% and Sp 63%) and an AUROC of 0.8 (95CI 0.7-0.9)

Conclusion: VF is independently associated with AF in patients with NAFLD. VF is a surrogate marker of visceral obesity and should be measured in all patients with high BMI and NAFLD to predict AF

Figure: ROC curve between VF and AF (n = 283)



P04-11YI Combination of GLP-1 agonist and SGLT2-inhibitor improves the non-alcoholic steatohepatitis in a high fat-high fructose mice model for NASH

Katharina Luise Hupa[1], Janine Dywicki[1], Björn Hartleben[2], Benjamin Heidrich[1], Freya Wellhöner[1], Michael P. Manns[1], Matthias Hardtke-Wolenski[1], Elmar Jaeckel[1]
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Background and aims: Non-alcoholic steatohepatitis (NASH) is one of most common cause of chronic liver disease. Sedentary lifestyle, western type nutrition and the metabolic syndrome are the main risk factors. The pathogenesis of NASH is a complex interaction between a disrupted lipid metabolism, which is followed by hepatotoxic accumulation of free fatty acids in the liver, oxidative stress, mitochondrial dysfunction and ER stress, leading then to inflammation, hepatotoxicity and fibrosis. Treatment options for NASH are very rare. GLP-1 was successfully used in initial clinical trials, but the distinct mechanisms are not fully understood. Due to their insulin-independent mechanism, SGLT-2 inhibitors seem to be a promising therapy additional to GLP-1 agonist. The aim of this study was to investigate the combined effect of the new long-acting GLP1- agonist dulaglutid and SGLT-2 inhibitor empagliflozin in a High Fat-High fructose mice model for NASH and the mechanism with special regard to the immune system.
Method: We used a high-fat- high-carbohydrate (HF-HC) diet with a surplus of cholesterol. C57BL/6 mice receiving the diet for sixteen weeks encompass most of the features of the human disease conditions like obesity, glucose tolerance, higher liver transaminases, inflammation and steatosis in different severity. After 12 weeks of HF-HC diet, C57BL/6 mice were treated with dulaglutid (10nmol/kg) and empagliflozin (10mg/kg) for four weeks.
Results: Treatments with long-acting GLP-1 agonist dulaglutid and SGLT-2 inhibitor empagliflozin led to a significant improvement of the metabolic situation and obesity. Analysis of the immune cells show a significant decrease in inflammatory cells, especially in CD4+Foxp3+ regulatory T cells and proinflammatory macrophages. Furthermore, it attenuated inflammatory and fibrotic pathways and improved microbiome dysbiosis.
Conclusion: This is the first study investigating the effects of the combination of new, long acting GLP-1 agonist dulaglutid and SGLT-2 inhibitor empagliflozin in a dietary mice-model of NASH. Here, we could show improvement of the metabolic situation as well as improvement of pro- inflammatory and pro-fibrotic pathways in NASH. Further studies in mice models with polygenetic background for diabetes and metabolic syndrome are needed for validation of these results.

P04-12YI Steatotic zebrafish larva to evaluate mechanisms involved in NAFLD progression induced by a mixture of alcohol with an environmental pollutant, benzo[a]pyrene

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Background and aims: The rise in prevalence of non-alcoholic fatty liver disease (NAFLD) constitutes an important public health concern worldwide. Including obesity, environmental factors or alcohol consumption have also been described as risk factors of NAFLD. However, there are very few studies that have explored the combined role of these factors. So, we decided to investigate the influence of a co-exposure to low doses of alcohol and benzo[a]pyrene (B[a]P), a prototype of polycyclic aromatic hydrocarbons notably found in cigarette smoke and diet, in high-fat fed zebrafish larva, a suitable *in vivo* model of steatosis. In this context, we aimed to assess pathological progression and underlying mechanisms.
Method: On 4 days post-fertilization (dpf), zebrafish larvae were fed with a high fat diet to develop steatosis. Then, on 5 dpf, larvae were exposed to sub-toxic doses of B[a]P (25 nM) and ethanol (43 mM) for a chronic treatment of 7 days. After treatment, steatohepatitis was characterized by examining histological liver injury and qPCR analyses. Specific chemical inhibitors were used to decipher mechanisms involved. Taking advantages of larvae transparency, plasma membrane order was analysed by fluorescence microscopy. Transcriptomic analyses were performed on Affymetrix GeneChips. In parallel, mitochondrial oxygen consumption was evaluated *in vivo* using XFe24 Extracellular Flux Analyzer.
Results: In steatotic zebrafish larva, mixture of alcohol and B[a]P induced liver toxicity leading to a steatohepatitis-like state. Using specific inhibitors, several mechanisms were identified as oxidative stress, plasma membrane remodeling (changes in membrane fluidity and lipid-raft characteristics). Next, from transcriptomic analyses—done to identify global mechanisms and pathways—mitochondrial metabolism appeared to be a key player of NAFLD progression in response to xenobiotics. Finally, metabolic disruption was revealed by a decrease in mitochondrial respiratory capacity following toxicant mixture and it was also supported by qPCR validation of several mitochondrial mRNA targets.
Conclusion: Overall, using the suitable larval zebrafish model, it can be concluded that mixture of alcohol and B[a]P can induce NAFLD disease progression via membrane remodeling, oxidative stress and likely through mitochondrial metabolic disruption. Thus, in future, these results could provide biomarkers and be considered for developing combination therapy to deal with steatohepatitis.
Institutional support: ANR-13-CESA-0009; HEC Pakistan.

P04-13YI Restoration of mitochondrial respiratory chain activity by RIP3 depletion in experimental NAFLD

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Background and aims: Hepatocyte cell death, inflammation and oxidative stress constitute key pathogenic mechanisms underlying non-alcoholic fatty liver disease (NAFLD) progression to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. We have shown that Receptor Interacting Protein 3 (RIP3) knockout attenuates methionine and choline-deficient diet-induced liver injury, steatosis, inflammation, fibrosis and oxidative stress. Intriguingly, RIP3 may function as an energy metabolism regulator that increases aerobic respiration. Here, we aimed to evaluate the role of RIP3 depletion on restoration of the activity of mitochondrial respiratory chain (MRC) complexes in experimental NAFLD progressing from steatosis to the development of preneoplastic lesions.
Method: C57BL/6N wild-type (WT) and RIP3$^{-/-}$ mice were fed either a choline-sufficient, amino acid-defined control diet (CSAA; n = 38) or a choline-deficient, amino acid-defined diet (CDAA; n = 38) for 32 and 66 weeks. Liver samples were collected and processed for steatosis, inflammation, fibrosis and hepatocyte proliferation assessment. Citrate synthase and MRC complex I, II, II+III and IV activities were evaluated. RIP3 deficiency ameliorated CDAA-induced inflammation, fibrosis and oxidative stress, while decreasing the NAFLD activity score and the incidence of preneoplastic lesions.
Results: Compared to CSAA-fed WT mice, citrate synthase activity was unaffected at both 32 and 66 weeks of CDAA feeding. At 32 weeks, the CDAA diet resulted in significantly decreased activities of MRC complex I, II, and II+III in WT mice compared to CSAA-fed animals. These CDAA-induced changes in WT mice were less pronounced after 66 weeks, with only MRC complex II activity being significantly decreased. Strikingly, RIP3$^{-/-}$ mice were protected against CDAA-induced impairment of MCR complex activities, particularly at 32 weeks, compared to CDAA-fed WT mice.
Conclusion: In conclusion, impaired MRC complex activity correlates with inflammation, fibrosis and proliferation in experimental NAFLD. RIP3 deficiency leads to MRC complexes protection and halts NAFLD progression.
Funding: PTDC/BIM-MEC/0895/2014; SAICTPAC/0019/2015; EU H2020 Marie Sklodowska-Curie 722619 grant

P04-14YI Non-alcoholic fatty liver disease: the principles of diet therapy

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Background and aims: Evaluate the effectiveness of a personalized diet with controlled protein, fat and carbohydrate content in patients with NAFLD.

Method: The study included 398 patients (men 183, women 215, mean age (44.9 ± 11.8) years) with NAFLD and obesity. All patients after the anthropometric measurements, determination of the amount of fat mass and index of insulin resistance HOMA (IIR HOMA), a personalized diet was prescribed. The control group consisted of 60 patients with NAFLD who did not receive diet therapy.
Results: After 12 months of treatment, 73% had a 10% or more weight loss, 21% had 5-10%, and 6% less than 5% of the baseline, with a reduction in weight in the main group mainly due to fat reduction masses. In control, the weight remained stable in 72%, in 4%-decreased by 3-6% of the initial value, and in 24% there was a weight gain of 2-7%. Thus, the weight of the patients of the main group significantly differed after 12 months of therapy in comparison with the control and amounted to (97.3 ± 6.1) kg and (124.4 ± 7.8) kg, respectively ($p < 0.05$). BMI and waist to hip ratio index significantly differed from the control group at 12 months of therapy ($p = 0.004$ and $p = 0.0032$, respectively). IIR HOMA after treatment in the main group was 3.01 and significantly differed from baseline ($p = 0.005$). In 79% of the patients, it was possible to achieve the target values of the IIR HOMA. In the control group, on the contrary, there was an insignificant increase in the IIR index. When comparing the main and control groups, significant differences were found between the indices of IIR HOMA ($p = 0.0002$) after 12 months of diet therapy.
Conclusion: The use of a personalized diet in patients with NAFLD leads to a significant reduction in body weight due to the fat component, a decrease in BMI, an index of waist to hip ratio, as well as an expression of insulin resistance.

P05-01 Association between metabolic risk factors and histological characteristics in non-cirrhotic NAFLD

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Background and aims: NAFLD is the leading cause of liver disease on a global scale. Metabolic inflammation leads to progressive liver injury, accumulation of hepatic fibrosis and results in increased mortality. The rate of comorbidities in NAFLD is high and components of the metabolic syndrome, such as diabetes, obesity, hypertriglyceridemia and hypertension critically influence the disease activity. The aim of this analysis was to investigate the prevalence of metabolic risk factors and the histological characteristics to distinguish early from advanced fibrosis based on clinical risk factors.
Method: The analyses were carried out on the German collective of the multicentric, pan-European, prospectively enrolling European NAFLD registry. A total, 261 patients with liver biopsy were included in the study and exploratory analyses were carried out.
Results: The median age was 51 years (range 19-93) and the metabolic syndrome was highly prevalent (hypertension 54.4%, diabetes 29.9%, hyperlipidaemia 29.1%). 41 patients exhibited advanced, non-cirrhotic fibrosis (F3). Patients with advanced fibrosis were significant older (57 vs. 50 years, p = 0.003) and had a higher BMI (32.3 vs. 30.5, p = 0.023) than patients with earlier fibrosis. Additional, arterial hypertension (78% vs. 50%, p = 0.001) and diabetes (61% vs. 24.1%, p <0.001) was higher in the advanced fibrosis group. In addition, patients with diabetes had a significantly higher SAF-Score (p <0.01) and a higher rate of NASH (p <0.002) compared to non-diabetics. The risk of developing a NASH was 3 times greater in the diabetes-group.
Conclusion: In this histologically characterized non-cirrhotic NAFLD cohort from Germany, metabolic comorbidities are highly prevalent, especially in patients with advanced fibrosis. In a non-specialized setting these risk factors can help to identify patients for referral and further work up.

P05-02 Modelling NASH for drug discovery using 3D liver microtissues

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease affecting around 30% of the population and can progress to non-alcoholic steatohepatitis (NASH), defined as hepatic steatosis with inflammation. NASH frequently further develops into fibrosis, liver cirrhosis and liver failure. The most frequently used *in vitro* model to study the effect of anti-NASH drugs are simple mono-layer cultures of hepatic stellate cells (HSCs) and hepatocytes, which do not sufficiently reflect the complex mechanisms of the disease. We aimed to develop a human-relevant *in vitro,* 3D Liver NASH model of lipotoxic stress which incorporates key physiological aspects such as steatosis, inflammation and fibrosis, and to characterize this model by comparing it to established mechanisms for pathogenesis of NASH.
Method: 3D Human Liver Microtissues were engineered to incorporate all the relevant primary human liver cell types responsible for the development of the disease: hepatocytes, HSCs, Kupffer cells (KCs) and liver endothelial cells (LECs). Free fatty acids (FFA) plus LPS treatment representing the lipotoxic and inflammatory stress stimuli in NASH have been applied in the diabetic media containing high levels of glucose and insulin. The developed NASH model was characterized on morphological and functional level by Nile-Red staining of incorporated lipids using confocal microscopy, immunohistochemistry (IHC) for detection of ECM deposition, and secreted pro-inflammatory markers using Luminex technology.
Results:
The presence of hepatocytes, HSC, KCs and LECs was confirmed in the 3D liver NASH model using cell-type specific makers. Treatment of the tissues with FFA and LPS in the diabetic medium as compared to the control increased the accumulation of lipids as well as secretion of pro-inflammatory markers such as TNF-α, IL-6, IL-8, MCP-1, MIP-1α, IP-10. Furthermore, lipotoxic stress stimuli increased mRNA and protein expression of pro-fibrotic markers such as the α-smooth muscle actin (α-SMA), a marker of activated HSCs and collagen type I. Sirius Red staining of the liver tissues demonstrated the presence and increased deposition of ECM in the NASH model.
Conclusion:
Our high throughput screening-compatible *in vitro* human 3D liver NASH model recapitulates key biological aspects of NASH such as inflammation, steatosis and fibrosis, reflecting well the mechanisms of action for assessing potential anti-NASH drugs.

P05-03YI High fat high sucrose intake underlies the progression of simple steatosis to non-alcoholic steatohepatitis

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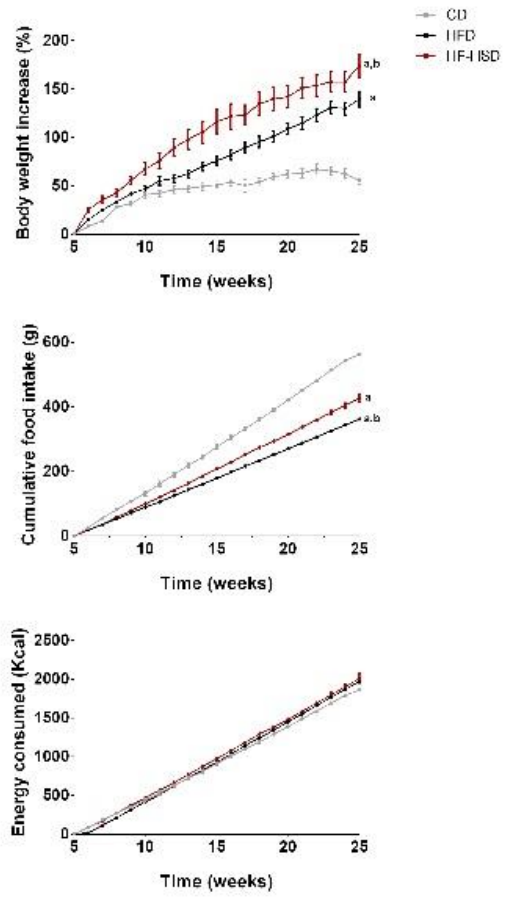
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in Western countries and probably the leading indication for liver transplantation in the years to come. The exact mechanisms of generation and development of NAFLD are still unknown. Although, the over-intake and the composition of the diet same to have a key role in this process. As such, the aims of our study were to better understand the effect of dietetic composition in the develop and procession of NAFLD.
Method: Male C57BL/6 J mice were subjected to dietary intervention: **Chow diet** (CD, 8.4% of kcal derived from fat, 72.4% of kcal derived from carbohydrates, 19.3% derived from protein), **High fat diet** (HFD, 60% of kcal derived from fat, 21% of kcal derived from carbohydrates, 19% derived from protein) and **high fat- high sucrose diet** (44.6% of kcal derived from fat, 40.6% of kcal derived from carbohydrates (sucrose 340 g/kg diet) and 14.7 % protein) (n = 8),) during 20 weeks. We assessed the systemic metabolic effects and performed a detailed liver and epididymal white adipose tissue (eWAT) histological and molecular profiling.
Results: Mice fed a HFD and HF-HSD developed overweight, eWAT hypertrophy, hyperlipidemia and insulin resistance. Surprisingly, these alterations were more evidence in mice fed a HF-HSD. At the hepatic molecular level, mice fed HFD developed early liver steatosis. However, HF-HSD consistently induced steatosis, hepatocyte ballooning and inflammation.
Conclusion: We postulate that excessive dietary sucrose consumption may be underlie the progression of simple steatosis to liver inflammation or NASH and their relationship with metabolic syndrome.

Figure:



P05-04YI The plasma lipidomic signature of paediatric non-alcoholic steatohepatitis

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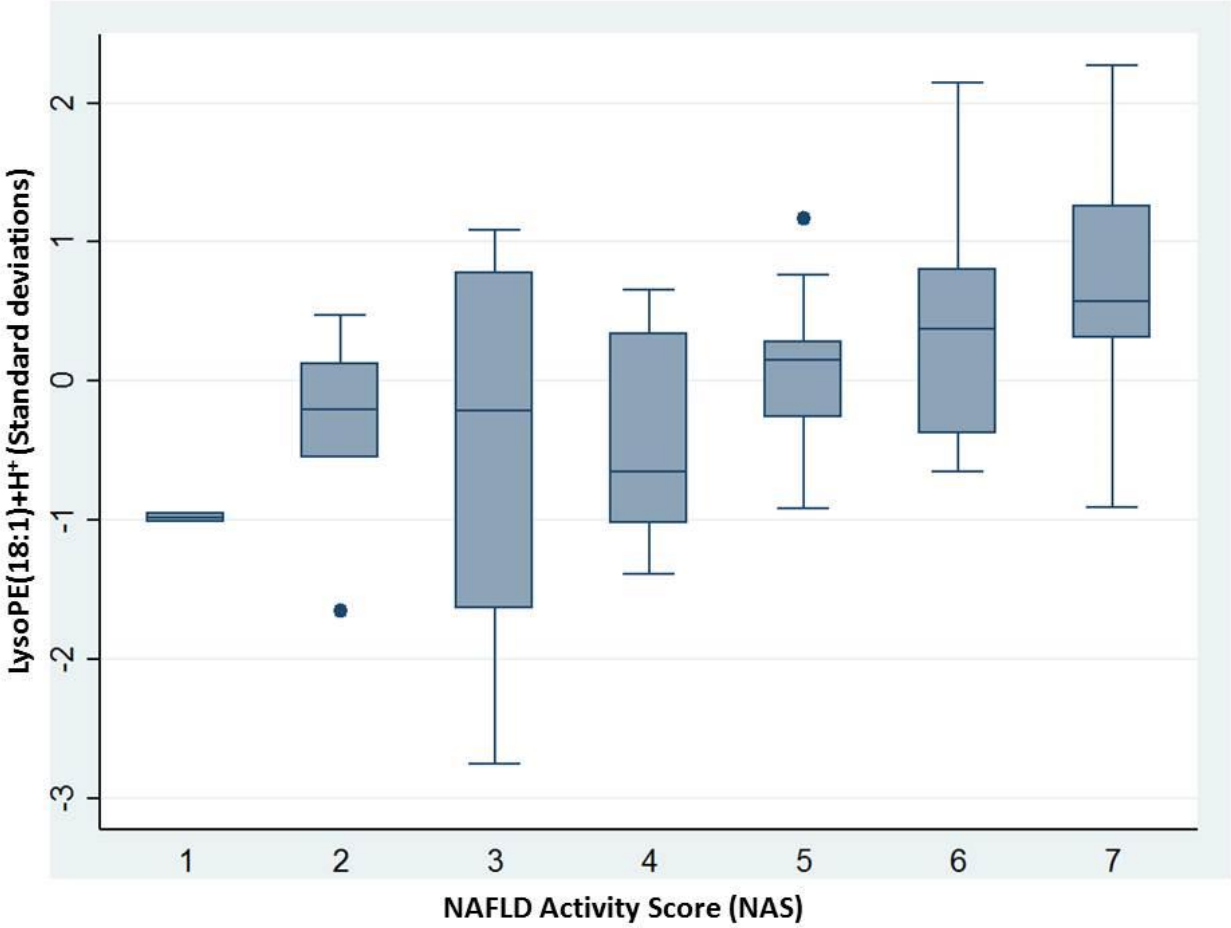
The plasma lipidomic signature of paediatric non-alcoholic steatohepatitis

Background and aims: Examination of the plasma lipid profile in adults has identified species associated with NASH and provided new insights into disease mechanisms. The lipidome of paediatric NAFLD has not been previously reported and poses a significant opportunity for exploration of a novel non-invasive assessment of NAFLD. Therefore, we aimed to: 1) determine the serum 'lipidome' of paediatric NAFL and NASH, and 2) identify whether a lipidomic signature can be used to distinguish NASH and/or moderate-advanced fibrosis.
Method: Children (<16 years) who had undergone liver biopsy for diagnosis of NAFLD were included. Histology was scored according to the NASH CRN, including the NAFLD Activity Score. NASH was diagnosed according to the FLIP algorithm. Participants were genotyped for NASH-associated SNPs in r rs738409 C>G in PNPLA3, rs58542926 C>T near TM6SF2, rs641738 C>T near MBOAT7. Lipidomics was performed on fasting serum using a well-established high-throughput mass spectrometry technique. 293 lipid signals could be identified, including: phosphatidylcholines, cholesteryl esters, ceramides, phosphoethanolamines, and di-/tri-acylglycerols. Ratios between significantly associated lipid species were calculated. The lipidomic correlates of NASH and individual histological features was assessed using linear and logistic regression adjusted for confounding factors and with adjustment for multiple testing.
Results: 67 children were included (mean age 10.4 ± 2.6 years), of whom 42/67 (63%) had NASH. Children with NASH were older (8.6 years vs 11.4 years, p <0.0001) but no other baseline anthropometric or biochemistry measurement differentiated steatosis from NASH.
Diagnosis of NASH by FLIP algorithm was associated with increased CE (20:2)+NH4+ to Cer (41:1)+H+ (OR 2.8 ((CI 95% 1.1-2.8) and DG (33:4)-H2O+H+ (OR 2.3 ((CI 95% 1.1-4.7) after adjusting for age.
Increasing NAS was associated lysoPE (18:1)+H+ (β 0.7, p = 6.7x10-5) after adjusting for PNPLA3 genotype and age (see Figure).
Increasing stage of fibrosis was associated with the ratio of PC (36:1)+H+ to Cer (41:1)+H+ (β -0.06, p = 0.0002) and ratio of PC (42:4)+H+ to DG (24:0)-H2O+H+ (β 0.05, p = 0.0008). PC (36:1)+H+ to Cer (41:1)+H+ ratio was also associated with reduced odds of moderate-severe fibrosis (OR 0.82 (CI 95% 0.69-0.98)).
All associations between lipid species and histology remained independent of genotype, total lipids, BMI, and insulin resistance.
Conclusion: Distinct lipid species are associated with different histological features of NAFLD in children. These markers need further validation in independent cohorts. Exploration of these may also provide insight into disease mechanism and support risk stratification without the need for liver biopsy.


LysoPE(18:1)+H+ (Standard deviations)
2
1
0
-1
-2
-3
1
2
3
4
5
6
7
NAFLD Activity Score (NAS)

P05-05YI Orotic acid-treated hepatocyte carcinoma cells resist steatosis by modification of fatty acid metabolism

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Background and aims: Orotic acid (OA) has been intensively utilized to induce fatty liver in rats. However, the capacity of OA to cause liver steatosis is species-specific, which may be due to the regulation of transcription factors mediating hepatic lipogenic events. Despite this, previous research evaluating OA-mediated hepatocyte lipidosis in cell types other than primary rat hepatocytes remains sparse, and there are no previous data about the detailed fatty acid (FA) composition of the OA-treated cells nor about how the available FA are processed. In this study, by using HepG2 cells, we have re-elucidated the usefulness of OA to investigate the cellular mechanisms in both non-alcoholic fatty liver disease (NAFLD) pathogenesis and cellular protection from lipid accumulation. In addition, alterations in detailed FA profiles of cells and culture media were studied.
Method: In our model, we used HepG2 cells exposed to OA. Bacterial endotoxin, lipopolysaccharide (LPS), alone or in combination with OA, was used to mimic hepatic inflammation. The suitability of the model was assessed by labeling cellular lipids with a Nile red stain and by performing image quantifications. The expression levels of key enzymes involved in de novo lipogenesis (DNL) (acetyl-coenzyme A carboxylase [ACC], fatty acid synthase [FASN]) and inflammatory markers related to the disease development (interleukin [IL] 6 and 8) were studied by qRT-PCR. FA profiles of cells and culture media were determined from total lipids with gas chromatography-mass spectrometry.
Results: OA induced the increased expression of IL-6, ACC-alpha and ACC-beta, but no clear FASN response nor lipid accumulation. FA composition studies revealed that the levels of 18:0 plasmalogen-derived dimethyl acetal derivative were elevated in cells, and 20:4n-6, 22:6n-3 and total polyunsaturated FA (PUFA) in both cells and in their culture medium, suggesting that OA-treated cells could alleviate lipidosis by the secretion of lipoproteins and by releasing extracellular vesicles. Increased n-3/n-6 PUFA ratio could be due to the cells being able to resist lipidosis. Increased delta9-desaturation, which has previously been suggested to act as a protective mechanism against DNL and lipid accumulation, may have caused the decreased 16:0 levels, as supported by high proportions of 16:1n-7 and 18:1n-7.
Conclusion: Our results indicate that OA triggers inflammatory responses and promotes the first stage of DNL, but is not capable of causing actual lipidosis in HepG2 cells. Apart from the transcription-level events reported by previous studies, FA metabolism may also be involved in the prevention of OA-mediated steatosis. Thus, the secretion of lipoproteins and extracellular vesicles as well as delta9-desaturation could provide promising targets of translational research for preventing lipid accumulation and the fatty liver disease.

P05-06 Bergamot polyphenols reverse inflammation and sinusoidal fibrosis in experimental Non-alcoholic Steatohepatitis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) represents a continuum of events characterised by excessive hepatic fat accumulation which can progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and in some severe cases, hepatocellular carcinoma. To date, no approved therapy for NASH exists. Bergamot is an endemic plant growing in Calabria (Southern Italy) and its Polyphenolic Fraction (BPF) has shown anti-oxidative and anti-inflammatory properties in patients suffering from metabolic syndrome. Although different mechanisms have been proposed to explain the possible role of BPF in preventing NAFLD, no evidence exists regarding molecular mechanisms able to counteract the progression of steatosis to NASH.

Method: DIAMOND mice were fed a chow diet and tap water (NC NW) or fat Western Diet (WD SW) for up to 27 weeks. WD SW animals were treated with vehicle or 50 mg/kg/day BPF (gavage; 11 weeks) starting from week 16. NC NW mice did not receive any treatment. The effect of BPF on glucose tolerance and insulin sensitivity was assessed by GTT and ITT tests. Liver histology was assessed using hematoxylin/eosin stains. Fibrosis was assessed using Sirius Red stains. The liver histology was assessed using the NASH CRN and SAF score. The effects of BPF on JNK, p38, AMPK, ACC and PARP were assessed by western blot analysis

Results: Treatment of WD SW-fed mice with 50mg/kg/d BPF significantly improves glucose tolerance and insulin resistance. Although steatosis is not modified, treatment with 50mg/kg/d BPF improved inflammation. Histology score for lobular inflammation and hepatocellular ballooning showed a trend toward significance. the NAS is decreased upon treatment with BPF ($p = 0.0542$) and the activity index is significant decreased ($p < 0.05$). Sinusoidal fibrosis was observed in the vehicle group, whereas it was not detected in WD SW BPF-treated mice with. BPF did not show effect on *de novo* lipogenesis, but it significantly reduced the phosphorylation levels of JNK ($p < 0.05$) and p38 ($p < 0.001$) in the livers of WD SW-fed mice. Finally, BPF significantly reduced PARP expression levels in mice with NASH.

Conclusion: Here we show that BPF improves glucose tolerance as well as insulin resistance and some histologic features associated with NASH by blunting the inflammation in an animal model of diet-induced NAFLD

P05-07YI Significant decline in circulating Mucosal Associated Invariant T (MAIT) Cells with increased terminal activation marker expression in patients with advanced Non-alcoholic fatty liver disease (NAFLD) fibrosis

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Background and aims: MAIT cells are innate-like T lymphocytes, present in peripheral circulation and enriched in the liver. Recent experimental studies have demonstrated a potential role in the stimulation of hepatic stellate cells leading to the promotion of fibrogenesis. Limited data is currently available on the characteristics of MAITs in NAFLD.
Method: Whole blood samples from 39 patients with NAFLD were stained with antibodies specific for CD45, CD3, CD8, CD161, Vα7.2, CD69 and CD95 and were analysed via multicolour flow cytometry, using a BD FACSCanto II (BD Biosciences) and FlowJo software (Tree Star, Asland, OR), these were analysed in conjunction with paired liver biopsies. Patient characteristics: median age 58 *(range: 20-71)*, BMI 34.8 *(25.5-48.9)*, ALT 49 *(10-136)*, AST 33 *(10-136)*. Diabetes 54.8% *(n = 23).*
Results: The percentage of circulating MAITs was significantly reduced in patients with diabetes, *0.90% (0.31-2.07%)* compared to those without: *1.63% (0.19-7.41%), p = 0.026*. There was a weak negative correlation between circulating MAITs and HbA1c levels (Spearman's rank correlation coefficient: *-0.305 (p = 0.05).* The percentage of circulating MAITs amongst patients with Brunt stage ≥2 *(median: 0.96% (0.21-4.57%) was* significantly lower in comparison with Brunt stage 0-1 *(1.63% (0.19-7.41%)), p = 0.445.* Absolute circulating MAIT cell numbers were also significantly reduced in patients with Brunt ≥2 stage NAFLD *(median: 13.1, (3.6-41))* compared to Brunt <2 *(32.1 (3.6-128.9)), p = 0.0497.* The peripheral blood MAIT MFI for the terminal activation marker CD95 was significantly elevated with advanced fibrosis, p = 0.0026, the MFI for CD69 was unaffected *p = 0.53.*No alterations were observed amongst circulating MAITs across different grades of hepatic steatosis or ballooning.
Conclusion: We hypothesize the significant decline in circulating MAITs with increased terminal activation marker expression observed with advanced fibrosis is reflective of chronic MAIT cell stimulation, exhaustion and apoptosis in advanced NAFLD. This is the largest descriptive study on MAIT cells amongst NAFLD patients to date.

P05-08 Nicotinamide, a surtuin modifier, ameliorates hepatic steatosis by alternating bile acid metabolism in the liver but not in the gut and microbiota

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Background and aims: We previously reported that nicotinamide (NAM), a metabolite of NAD catalyzed by Sirtuins (Sirts), ameliorated hepatic steatosis via Sirts activation by inhibition of fatty synthesis (The international liver congress 2018). Recently, it has become widely known that the gut-liver axis including bile acid metabolism and microbiota, plays an important pathophysiological role in NAFLD; however, the effects of NAM on the gut-liver axis have not been elucidated. Therefore, we investigated how NAM affected the bile acid metabolism and microbiota.
Method: C57BL/6J mice (n = 20) were divided into four groups and fed with: normal diet (ND); ND+NAM (NAM mixed with ND to 0.1% wt/wt); high fat diet (HFD); HFD+NAM for 8 weeks. The expression of bile acid metabolism-related genes in the liver and the ileum was evaluated by real-time RT-PCR. Microbiota analysis was performed by T-RFLP analysis using a deep sequencer. Metabolized products by microbiota, such as short-chain fatty acids (SCFC), in caecum contents or in serum were measured by LC/MS.
Results: In the Liver, NAM reduced the HFD-induced gene expression of sterol regulatory element-binding transcription factor 1c, acetyl-CoA carboxylase, and fatty acid synthase; NAM also decreased that of cholesterol 7a-hydroxylase (CYP7A1) and enhanced that of small heterodimer partner, indicating the activation of farnesoid X receptor (FXR). By contrast, in the ileum, adding NAM to HFD did not change the gene expression of apical sodium-dependent bile acid transporter or fibroblast growth factor 15, suggesting the irrelevance of FXR involvement. With regard to microbiota, NAM significantly reduced Firmacutes (Lactococcus lactis), but oppositely increased Actinobacteria (Corynebacterium stationis) at phylum level in ND+NAM; however, in HFD+NAM, no obvious difference in microbiota between HFD and HFD+NAM was found. In a similar manner, NAM increased levels of bile acids (CA, CDCA, and DCA) in serum and SCFA (acetate, propionate, and butyrate) in serum and caecum contents in ND, but these effects were also not found in HFD+NAM.
Conclusion: NAM ameliorated hepatic steatosis through the activation of Sirts, leading to the inhibition of fatty acid and bile acid synthesis probably via FXR activation in the liver. On the other hand, NAM affected bile acid metabolism and microbiota in ND but not in HFD. Our results indicate that NAM potentially effects the gut as well as the liver, and that increasing the dose of NAM might be more effective for treating NAFLD/NASH.

P05-10YI In overweight patients with non-alcoholic fatty liver disease Saroglitazar is able to improve transaminases but not liver stiffness measurement and controlled attenuation parameter unless accompanied by weight reduction

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Background and aims: Saroglitazar is a novel dual peroxisome proliferator-activated receptors-alfa/gamma agonist and is being investigated for treatment of non-alcoholic fatty liver disease (NAFLD). We aimed to see the response in hepatic steatosis after three month treatment with Saroglitazar.
Method: Consecutive overweight (BMI >23 kg/m^2) patients of NAFLD, diagnosed on the basis of controlled attenuation parameter (CAP) >248 dB/m, and attending the outpatient department of Sir Ganga Ram Hospital, New Delhi, were enrolled. Patients with cirrhosis (liver stiffness measurement, LSM >13.5 kPa) and those with concomitant liver disease due to other etiologies (alcohol, viral etc.) were excluded. All patients received saroglitazar 4 mg/day; in addition, they were advised to reduce weight and were counseled regarding diet and exercise. At 3-month follow-up, patients were categorized into those who were able to reduce ≥5 kg weight and those who couldn't; and both these groups were compared.
Results: A total of 91 patients (median age 45 [range 18-66] years; 80% males) were included in the study. The median BMI was 29.3 (23.6-42.2) kg/m^2. The baseline AST, ALT, GGT, LSM and CAP values were 40 (22-144) IU/dL, 48 (13-164) IU/dL, 42 (4-171) IU/dL, 6.7 (3.6-13.1) kPa, and 308 (249-400) dB/m. All patients tolerated Saroglitazar well. At 3-month 49 (54%) patients were able to reduce ≥5 kg weight while in rest 42 (46%) the weight reduction was <5 kg. The comparison of various parameters in these two groups is shown in the Table. Transaminases values improved in both the groups, however, liver stiffness measurement and controlled attenuation parameter improved only in patients who reduced weight.
Conclusion: In overweight patients with NAFLD, a 3-month therapy with saroglitazar is able to improve transaminases but not LSM and CAP unless accompanied by weight reduction of at least 5 kg.

Figure:

	Weight reduction ≥5 kg achieved (n = 49)			Weight reduction ≥5 kg not achieved (n = 42)		
	Baseline	3 months	P value	Baseline	3 months	P value
AST, IU/dL	43 (23-144)	36 (23-88)	<0.01	36 (22-110)	35 (21-102)	0.016
ALT, IU/dL	57 (17-164)	45 (20-102)	<0.01	34 (13-125)	32 (17-102)	0.001
GGT, IU/dL	46 (4-169)	43 (17-86)	0.073	40 (15-171)	35 (11-80)	0.076
LSM, kPa	6.7 (3.6-13.1)	5.9 (3.1-11.2)	<0.01	6.4 (3.6-13.1)	6.1 (4.1-14.1)	0.141
CAP, dB/m	315 (251-400)	264 (210-354)	<0.01	285 (249-367)	288 (206-396)	0.031

P05-11YI Influence of hypothyroidism on insulin resistance and adypokine profile in patients with Non-alcoholic Fatty Liver Disease

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Background and aims: In recent decades, significant increase in the prevalence of non-alcoholic fatty liver disease (NAFLD) is observed. The abovementioned is associated with the growing number of individuals with obesity, dyslipidemia, type II diabetes and metabolic syndrome that are combined with insulin resistance. Except the recollected factors, there are other conditions, which association with NAFLD are now actively discussed. Among them an important role plays hypothyroidism, which even in subclinical form, is associated with increased risk of NAFLD development.
The aim of the study was to investigate influence of different forms of hypothyroidism on insulin resistance and adypokine profile in NAFLD patients.
Method: The study involved 188 NAFLD patients (average age 53, 6 ± 12, 34 years), among them in 20 patients except NAFLD subclinical hypothyroidism and in 24 patients manifest hypothyroidism was diagnosed. 144 NAFLD patients with normal functional activity of the thyroid gland formed comparison group. The control group consisted of 45 healthy individuals representative by age and gender to the patients of studied groups. Biochemical blood parameters, insulin, leptin, adiponectin blood levels in observed patients and healthy individuals were investigated.
Results: The NAFLD patients were shown to develop insulin resistance syndrome, which becomes more severe in the case of combined subclinical or manifest hypothyroidism. The HOMA IR index in NAFLD patients in 4, 17 times (p <0, 001) prevailed, while the J.F. Caro index was at 44, 4% (p = 0, 04) lower in comparison with practically healthy persons. In NAFLD patients with subclinical and manifested hypothyroidism J.F. Caro index was in 4, 15 times (p <0, 001) and 3, 38 times (p <0, 001) lower as compared to NAFLD patients without hypothyroidism. At the same time, in NAFLD patients with manifested hypothyroidism HOMA IR index was at 67, 7% (p = 0, 04) higher in comparison with patients with normal thyroid activity. The patients with subclinical and manifested hypothyroidism were found to have a higher leptin level at 35, 7% (p = 0, 04) and 72, 1% (p = 0, 009), and those with NAFLD combined with manifested hypothyroidism also in 2, 1 time (p = 0, 004) lower adiponectin content in the blood as compared to patients without hypothyroidism.
Conclusion: NAFLD patients with hypothyroidism were characterized by decreased syndrome of insulin resistance and adipokine imbalance.

P05-12YI RNase MCPIP1 regulates hepatic PPAR gamma via TXNIP/PGC 1-alpha pathway

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Background and aims: Monocyte chemoattractant protein-1-induced protein-1 (MCPIP1) acts as an endonuclease that degrades selected mRNAs, viral RNAs and pre-miRNAs. Through the cleavage of proinflammatory cytokines transcripts MCPIP1 functions as a negative regulator of inflammation. It also inhibits adipogenesis by degradation of CCAAT/enhancer-binding protein beta mRNA and adipogenesis-related miRNA. In the course of hepatic steatosis progression, excessive accumulation of lipids in hepatocytes and development of inflammation are important features. The aim of our work was to analyze if MCPIP1 is involved in regulation of lipid metabolism in hepatocytes.
Method: C57BL/6J mice were fed high-fat diet for 2-20 weeks, to reproduce features of the human non-alcoholic fatty liver disease (NAFLD) and next primary hepatocytes were isolated for further analysis. Development of NAFLD in C57BL/6J mice was confirmed by histological stains and glucose tolerance test. Additionally, visceral and subcutaneous adipose tissue from these mice was analyzed. Experiments *in vitro* were performed using HepG2 cell line and murine primary hepatocytes stimulated with 0.5 mM sodium oleate. In this set of experiments we compared cells overexpressing MCPIP1, cells transduced with vector coding for silencing sequence for MCPIP1 and control cells.
Results: We have found that Mcpip1 level was lower in primary hepatocytes starting from 4 weeks of high-fat diet, when compared to control cells. Moreover, level of Mcpip1 was also depleted in subcutaneous and visceral adipose tissue isolated from obese and glucose intolerant mice characterized by fatty liver disease. Our *in vitro* experiments showed that MCPIP1 overexpression in HepG2 cells treated with oleate induces level and activity of Peroxisome proliferator-activated receptor γ (PPARγ) through the Thioredoxin-interacting protein (TXNIP)/Peroxisome proliferator-activated receptor γ coactivator 1- α (PGC1-α) pathway. Silencing of MCPIP1 expression reverted observed effect.
Conclusion: MCPIP1 contributes to lipid metabolism in hepatocytes by regulating TXNIP/PGC1-α/PPARγ pathway.

P05-13YI Insulin secretion is directly related to NASH, fibrogenesis and fibrosis in Non-diabetic patients with Non-Alcoholic Fatty Liver Disease

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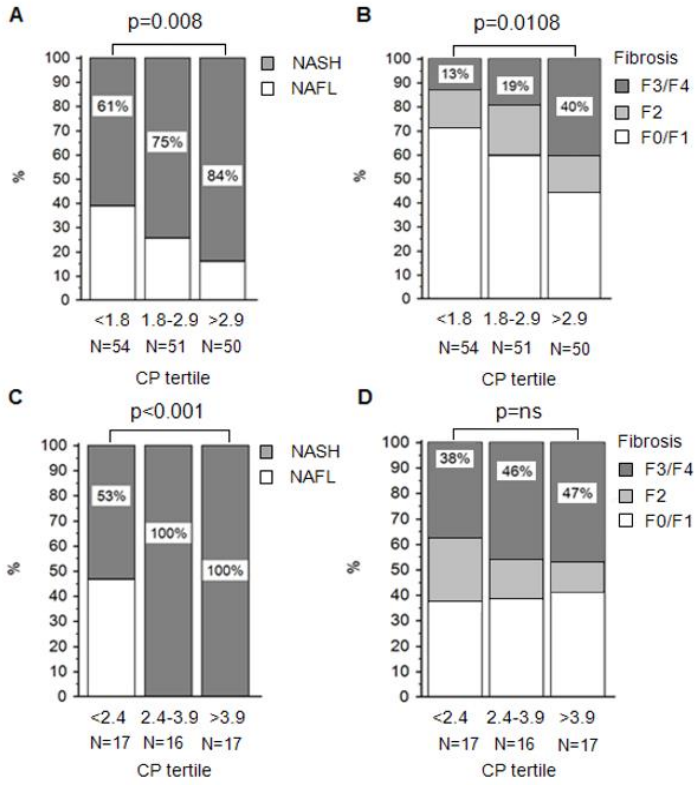
Background and aims: Liver damage in NAFLD has been related to the degree of insulin resistance (IR). IR is associated with compensatory hyperinsulinaemia until beta cell function is preserved. The effect of insulin per se on liver damage is still unknown although cellular studies suggest it can activate hepatic stellate cells promoting fibrosis. Procollagen type III is up-regulated in the early development of liver fibrosis and its N-terminal neo-epitope (PRO-C3) is a non-invasive marker of fibrogenesis. We sought to investigate the association between increased pre-hepatic insulin by C-peptide (CP) and the degree of liver fibrosis in Non Diabetic (ND) vs Type 2 Diabetic (T2D) patients with NAFLD.
Method: We analyzed 205 biopsy-proven NAFLD patients (T2D 24%). Plasma CP was assessed by chemiluminescence assay. Plasma PRO-C3 levels were measured by competitive ELISA. PNPLA3 polymorphism was determined by real-time PCR. NASH was defined according to the joint presence of steatosis, ballooning and lobular inflammation.
Results: In ND subjects, fasting CP levels were increased with BMI ($r = 0.32$, $p < 0.001$), waist circumference ($r = 0.44$, $p < 0.001$), fasting glucose ($r = 0.20$, $p = 0.011$) and fasting PRO-C3 levels ($r = 0.38$, $p < 0.001$), while in T2D both CP and PRO-C3 were higher but no correlations with anthropometric and biochemical parameters were found. When grouped according to CP tertiles for specific group, in ND subjects PRO-C3 levels had a stepwise increase (from7.7 to 9.6 to 12.1 ng/ml, $p < 0.001$) while no significance was found in T2D patients. Hepatic fat significantly increased according to CP tertiles (from 28% to 37% to 42%, $p = 0.021$) in ND subjects while no relation was found between the amount of steatosis and insulin secretion in T2D. The presence of NASH was directly related to CP tertiles in ND subjects ($p = 0.008$) while most patients with T2D had NASH (Figure 1A-C). Similarly, the stage of fibrosis progressively increased according to CP tertiles in ND ($p = 0.012$) but it was unchanged in T2D (Figure 2B-D). Overall, at multivariable logistic regression analysis adjusted for age, sex, BMI, waist, PNPLA3 polymorphism and the presence of T2D, both CP and PRO-C3 were significantly associated with severe fibrosis (OR = 1.5, 95%CI = 1.1-2.0, $p = 0.006$ and OR = 1.1, 95%CI = 1.0-1.2, $p = 0.003$) while in ND subjects, they were significantly associated to severe fibrosis and NASH, respectively (OR = 1.7, 95%CI = 1.7-2.7, $p = 0.014$ and OR = 1.2, 95%CI = 1.0-1.5, $p = 0.042$).
Conclusion: Insulin secretion is associated with severe fibrosis and with increased liver fibrogenesis assessed by PRO-C3 in ND subjects with NAFLD, suggesting its important role in the onset and progression of NASH independently of T2D. *Funded by Horizon2020 under grant agreement no.634413, EPoS*

Figure: Non-alcoholic steatohepatitis and fibrosis distribution according to c-peptide tertile in nondiabetic (A-B) and diabetic (C-D) subjects.


A
p=0.008
NASH
NAFL
61%
75%
84%
%
<1.8 1.8-2.9 >2.9
N=54 N=51 N=50
CP tertile
B
p=0.0108
Fibrosis
F3/F4
F2
F0/F1
13%
19%
40%
%
<1.8 1.8-2.9 >2.9
N=54 N=51 N=50
CP tertile
C
p<0.001
NASH
NAFL
53%
100%
100%
%
<2.4 2.4-3.9 >3.9
N=17 N=16 N=17
CP tertile
D
p=ns
Fibrosis
F3/F4
F2
F0/F1
38%
46%
47%
%
<2.4 2.4-3.9 >3.9
N=17 N=16 N=17
CP tertile

P05-14 Role of Adipocyte fatty acid binding protein (AFABP) in the diagnosis of NAFLD

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Background and aims: NASH diagnosis and the need for reliable non-invasive methods to distinguish it from simple steatosis and allow for grading and staging of disease are of high necessity. Adipocyte fatty acid binding protein (AFABP) is adipokine involved in the inflammation and insulin resistance. To evaluate the role of AFABP in diagnosis and quantification of histopathological status of NAFLD in Egyptian patients.
Method: A total of 90 subjects (45 male and 45 female) were included in the study and were divided into 3 groups: NASH, steatosis and control. All participants were subjected to calculation of HOMA-IR, NAFLD score, BARD score, complete blood counts, lipid profile, fasting and post-prandial blood glucose level, liver function tests, HCV-Abs, HBsAg, serum AFABP, liver biopsy and abdominal ultrasound.
Results: A significant increase in fasting insulin level, HOMA-IR, serum AFABP level and NAFLD score was detected in NASH group ($p < 0.01$) and there was significant increase in BMI in both NASH and steatosis groups compared to control ($p < 0.05$).
The AUROC, cut off level (pg/ml), sensitivity, specificity and accuracy of AFABP in diagnosis of fibrosis according to Brunt score in NASH were (0.838, 10.6, 75, 72.7 and 83.8 respectively) ($p = 0.005$ and 95% CI: 0.686-0.991). Also, AUROCs of non-invasive markers for diagnosis of NASH have been showed highly significant value of BARD score, NAFLD score and HOMA-IR.
While in steatosis, AUROCs showed highly significant value of BARD score, NAFLD score, HOMA-IR and AFABP. As regards AFABP, the AUROCs, cut off level (pg/ml), sensitivity, specificity and accuracy were (0.928, 9.36, 100, 80 and 92.8 respectively) ($p = 0.003$ and CI: 0.830- 1).
Conclusion: AFABP might be a promising marker in predicting fibrosis stages, histological grades and activity.

P06-01 Assessment of NIS4 clinicalutility for identification of patients with active NASH (NAS≥4) and significant fibrosis (F≥2) in patients at risk of NASH

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Background and aims: NASH is dramatically under-diagnosed largely because a liver biopsy is needed for diagnosis confirmation. Given the globel burden of NASH, development of new non-invasive tools are needed to identify millions of patients at higher risk of developing liver related outcomes. We recently described a new non-invasive score, NIS4. The aim of this study was to set-up clinically useful cut-offs for NIS4 to efficiently rule-out patients with no or mild disease and rule-in patients with active NASH and significant fibrosis (NAS≥4 with F≥2).

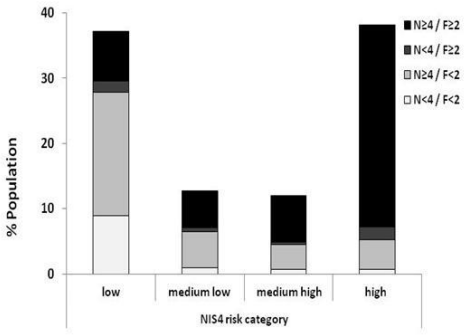
Method: 710 patients were screened for inclusion in GOLDEN and RESOLVE-IT trials. Blood samples and a liver biopsy were collected during the screening periods. Histological diagnosis and scoring were centralized. Each component of NIS4 (i.e. miR-34a, alpha2-macroglobuline, YKL-40 and HbA1c) were measured for calculation of NIS4. A ROC analysis was performed to determine the optimal cut-off for detection of patients with NAS≥4 and F≥2. We then set a low cut-off at 85% sensitivity (Sens) and a high cut-off at 85% specificity (Spec) defining 4 score ranges: low, low-medium, medium-high and high probability of having NAS≥4 and F≥2. Diagnostic metrics (Sens, Spec, negative predictive value and positive predictive value) were derived as well as the % of patients in the 4 score ranges.

Results: Ninety % of patients had signs of liver damage (elevated LFT's) and one or more cardio-metabolic risk factor. The cohort covered the complete histological spectrum of NAFLD (NAS = 0-8 and F = 0-4) and 51% had NAS≥4 with F≥2. The AUROC for detection of these patients was 0.83 [0.80-0.87] for NIS4 vs 0.75 [0.71-0.78] for FIB-4 (p <0.001). At optimal cutoff of NIS4, Sens was 74% [69-78] and Spec was 75% [71-80]. At a low-cut-off set at 85% Sens, Spec was 61% [55-66] while NPV was 80%. 37% of patients (n = 264) had NIS4 values below the low cut-off (Figure 1). The vast majority of patients with no or mild histological lesions (80 %) were well classified in this low probability range. At high cut-off set at 85% Spec, Sens was 61% [55-66] for NIS4 while PPV was 81%. 38% of patients (n = 271) had NIS4 value above the high cut-off. In this range, almost all patients had histological lesions since 98 % of patients had active NASH (NAS≥4) and/or F≥1.

Conclusion: Using a large cohort of patients screened for suspicion of NASH, this study illustrates the clinical utility of NIS4 to eliminate patients at low probability of having active NASH and significant fibrosis and to accurately identify those at high probability of progressive disease who should be considered for therapeutic intervention.

Figure:

Figure 1: Distribution of patients in NIS4 ranges



P06-02 Comparison of Hepatorenal Index and Hepatorenal Echogenicity Difference in B-Mode Ultrasound using Liver Biopsy as 'Gold Standard' for the diagnostic approach of NAFLD and NASH

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Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD) is one the most widespread type of chronic liver disease in the Western Countries. NAFLD may lead to Non-Alcoholic Steatohepatitis (NASH) and, if not opposed, to Cirrhosis and liver failure. Ultrasound (US) is widely used for the disease diagnosis and staging as it is a low cost and radiation-free non-invasive method. The most widely used method in NAFLD detection is the calculation of Hepatorenal Index which is the ratio between Right Kidney Cortex Echogenicity (RKE) and the adjacent Liver Parenchyma Echogenicity (LPE).
The aim of this study is to evaluate B-Mode Ultrasound in quantification of Liver Steatosis using Hepatorenal Index and Hepatorenal Echogenicity Difference and Liver Biopsy (LB) as 'Gold Standard'.
Method: 153 consecutive patients (44 normal (S0) and 109 NAFLD or NASH (57 S1, 40 S2, 12 S3)) with LB and histologic examination were included in the study. The histologic diagnosis classified patients according to Kleiner Score (S0-S3). A B-Mode Ultrasound examination was performed on the Right Liver Lobe of each patient including view of the Right Kidney at the same depth allowing measurement of RKE and LPE. Hepatorenal Index (LPE/RKE) and Difference (LPE-RKE) were calculated. ROC analysis followed for accuracy comparison between the two parameters.
Results: ROC analysis showed a Hepatorenal Index AUC of 0.6297, 0.759, 0.9385 and Hepatorenal Difference AUC of 0.5564, 0.7147, 0.8717 for S≥S1, S≥S2 and S≥S3 respectively. The corresponding cut-off values obtaining maximum accuracy were for Hepatorenal Index: 1.4091, 1.4326, 1.8537 and for Hepatorenal Difference: 3.7, 4.5, 6.2.
Conclusion: This study shows that B-Mode Ultrasound liver echogenicity can be correlated with Steatosis grade in a Fatty Liver and can be measured in an objective way. Additionally, it can differentiate well patients with Significant Steatosis (S2) and Severe Steatosis (S3). However, it cannot differentiate healthy subjects (S0) to Mild Steatosis patients (S1). More subjects should be included so that the Hepatorenal Index and Hepatorenal Difference can be evaluated as a valuable non-invasive tool for estimating liver steatosis of NAFLD patients.

Figure:

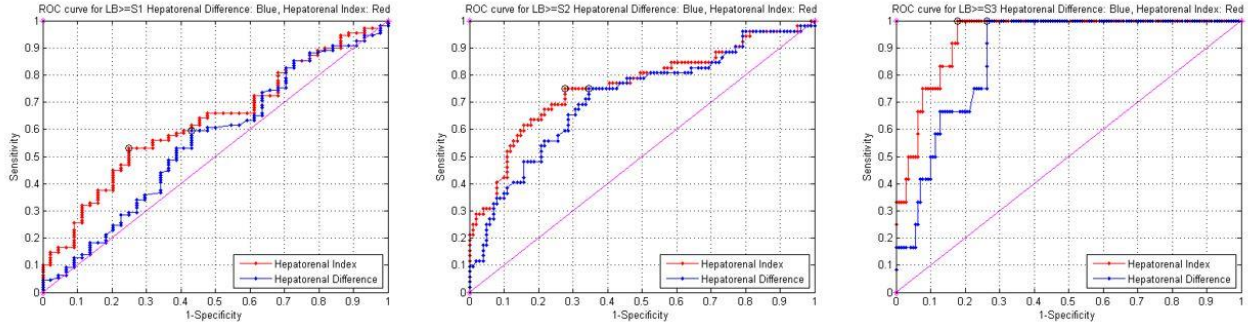

Figure 1. ROC curves of Hepatorenal Index (Red) and Hepatorenal Difference (Blue). Left: S≥S1, Center: S≥S2, Right: S≥S3.

P06-03YI The TM6SF2-E167K variant promotes liver cancer through hepatic lipid accumulation, tissue damage and inflammation

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a risk factor for hepatocellular carcinoma (HCC), an increasingly common malignancy. The TM6SF2 rs58542926 (E167K) single nucleotide polymorphism has been clearly associated with NASH and advanced fibrosis. More recent data indicates an association with HCC risk. This study sought to determine if the association is due to genuine causality and to explore the mechanisms through which the E167K variant promotes HCC development *in vivo* and *in vitro*.
Method: An *in vivo* targeted Tm6sf2-E167K knock-in (E167K/K) mouse was generated using CRISPR-Cas9 technology. E167K/K and WT mice were fed either a chow diet or a high fat-high fructose and glucose (HFHF+G) diet and exposed to diethylnitrosamine (DEN), a widely adopted HCC model (n = 12-15). Findings were validated *in vitro* in primary hepatocytes isolated from E167K/K and WT mice cultured in exogenous lipid (n = 3); and in a human cell line (PLCPRF5) that had been genetically modified by CRISPR-Cas9 to introduce the E167K SNP (n = 3). Cells were cultured in media supplemented with lipid ± DEN, with or without N-acetylcysteine (NAC) antioxidant treatment, to investigate the role of oxidative stress in promoting a pre-cancerous niche. Read outs include Oil-red-O staining, immunohistochemical analysis, ROS quantification and qPCR.
Results: E167K/K mice developed significantly greater levels of steatosis ($p < 0.01$). Similarly, *in vitro,* E167K/K variant cells accumulated more intracellular lipid than WT cells when metabolically stressed. Both *in vivo* and *in vitro*, homozygous carriers of the E167K variant exhibited increased levels of liver injury, increased oxidative stress and higher levels of inflammation. Importantly, DEN exposed, HFHF+G diet fed E167K/K mice also exhibited significantly more tumours, which were of a higher grade, than wildtype. Tumour number was correlated with the number of quantified lipid droplets. Treatment *in vitro* with the antioxidant NAC ameliorated levels of oxidative stress and inflammation, suggesting that the E167K variant promotes hepatic lipid accumulation and subsequently elevated levels of oxidative stress and inflammation, both known drivers of HCC.
Conclusion: This study provides the first mechanistic evidence that the E167K variant not only induces hepatic lipid dysregulation, but also promotes oxidative stress, tissue damage and inflammation. These factors contribute to the genesis of a pre-cancerous niche, which aids HCC development in NAFLD.

P06-04 Treatment with Obeticholic Acid in Patients with NASH Does Not Show Increased Markers of Liver Toxicity Based on Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH)

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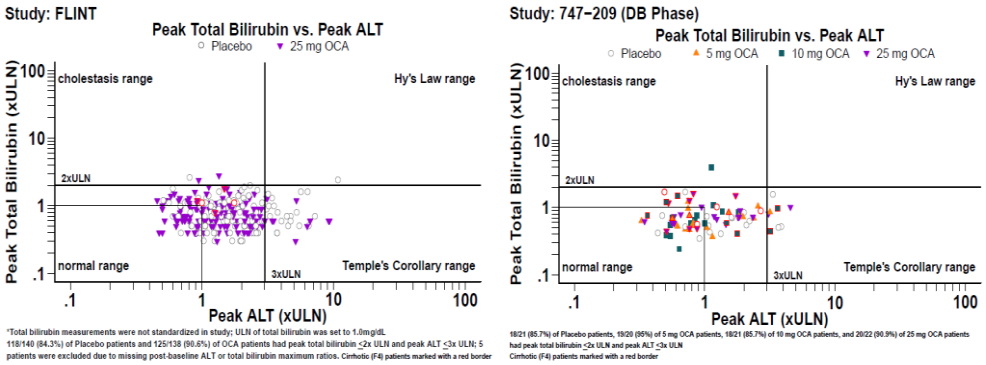
Background and aims: Evaluation of drug-induced serious hepatotoxicity (eDISH) is a tool used to assess and identify potential cases of drug-induced liver injury. eDISH was used to evaluate obeticholic acid (OCA) and placebo profiles in 2 double-blind, placebo-controlled studies in patients with non-alcoholic steatohepatitis (NASH). FLINT was a 72-week study, which demonstrated statistically significant improvements in hepatocellular ballooning, steatosis, lobular inflammation, and fibrosis in patients treated with OCA compared to placebo. CONTROL was a 16-week study, which showed that the addition of low-dose atorvastatin reversed OCA-associated changes in LDL-C. The objective of this analysis was to use eDISH to determine if patients with NASH treated with OCA show increased markers of liver injury or whole liver dysfunction.
Method: eDISH methodology was applied to 278 patients treated with placebo (n = 140) or 25mg OCA (n = 138) from FLINT and 84 patients treated with placebo (n = 21), 5mg OCA (n = 20), 10mg OCA (n = 21), or 25mg OCA (n = 22) from CONTROL. Individual subject peak values of alanine aminotransferase (ALT) and total bilirubin throughout the double-blind treatment phase were plotted on an x-y chart as logarithm$_{10}$ values of multiples of elevations above the upper limit of the normal reference ranges (x ULN).
Results: Overall, no OCA-treated patients were in the Hy's law quadrant (>3x ULN for ALT and >2x ULN for total bilirubin) compared with 1 placebo-treated patient in FLINT. The proportion of patients with peak ALT and total bilirubin values in the lower left quadrant (representing normal or near normal range) was higher in OCA-treated patients compared with placebo (FLINT: 91% OCA vs 84% placebo; CONTROL: 91% OCA vs 86% placebo). 8% of OCA-treated patients from both FLINT and CONTROL presented in the Temple's corollary quadrant (>3x ULN for ALT and <2x ULN for total bilirubin) vs 14% (in both studies) for the placebo-treated patients. Across both studies (n = 362), 4 patients were in the cholestasis quadrant (>2X ULN total bilirubin and <3X ULN for ALT); 1 placebo-treated patient and 3 OCA-treated patients, including 1 patient with Gilbert's syndrome.
Conclusion: In these 2 placebo-controlled, double-blind NASH studies, the eDISH analysis showed no trend for liver injury with OCA at doses up to and including 25 mg.

Figure: Evaluation of Drug-Induced Serious Hepatotoxicity



P06-05YI NAFLD in newly diagnosed Type 2 Diabetes: prevalence and risk factors from a South Asian population

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Background and aims: A rising prevalence of type 2 Diabetes (T2DM) has been observed in Asia, which is a risk factor for Non -Alcoholic Fatty Liver Disease (NAFLD). Periodic screening of diabetics for diabetic nephropathy, retinopathy and neuropathy is a common practice, but does not include surveillance for NAFLD. Hence, many patients with NAFLD remain undiagnosed. In this study we aim to assess the prevalence and risk factors of NAFLD among individuals recently diagnosed with T2DM.
Method: This was a prospective, hospital based cross sectional study conducted during 2008 to 2013 at the outpatient clinics of The Aga Khan University Hospital, Pakistan. Consecutive patients ≥18 years of age diagnosed with T2DM during last six months were enrolled after informed consent. Ultrasound liver was performed to identify NAFLD. Anthropometric measurements and laboratory investigations were carried out.
Results: Out of 203 patients with newly diagnosed diabetes, NAFLD was found in 146 (71.9%) cases. On multivariate analysis dyslipidemia (OR 2.38, 95%CI 1.06-5.34, p0.035), higher LDL (OR 1.02, 95%CI 1.01-1.03, p0.003), HbA1c (OR 1.27, 95%CI 0.97-1.68, p0.045) and diastolic BP (OR 1.05, 95%CI 1.01-1.10, p0.009) were significantly associated with NAFLD. While physical activity (OR 0.23, p <0.0001) and higher HDL (OR 0.92, p <0.0001) were protective factors. A combination of physical inactivity, HTN, dyslipidemia, waist circumference, BMI, HbA1c, TG, HDL, LDL and ALT predicts the highest odds of 10.8 for NAFLD (95%CI 4.9-24, p0.001).
Conclusion: We found a high prevalence of NAFLD in Pakistani patients with newly diagnosed T2DM. Dyslipidemia, higher LDL, HBA1c and diastolic BP were significantly associated with NAFLD. Higher HDL and physical activity were found protective. A rising trend in odds of having NAFLD was observed with increasing number of risk factors.

P06-06YI N-3 polyunsaturated fatty acids in NAFLD (a double-blind randomised placebo-controled study)

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Background and aims: NAFLD represents the most common chronic liver disease in western countries with no established pharmacological treatment. Weight reduction and lifestyle modification with increased physical activity stay the only effective therapeutic measures, but they are difficult to achieve and sustain. It has been reported that n-3 polyunsaturated fatty acids (PUFA) are able to ameliorate hepatic steatosis and insulin resistance, whereas a diet deficient in PUFA with a high n-6/n-3 ratio could induce fatty liver. Up to date published papers using PUFA have yielded contradictory results.
The aim of the study was to assess the effects of administration of PUFA in development of NAFLD in patients during one-year follow-up.
Method: We have examined 60 patients with metabolic syndrome and NAFLD in different stage of disease (steatosis/NASH (n = 55)/liver cirrhosis (n = 5)). Patients were randomized into two groups: 30 used PUFA in daily dose 1.8 g of eicosapentaenoic acid and 1.36 g of docosahexaenoic acid in four divided doses; 30 patients used placebo in the same scheme. During one-year follow-up were patients periodically examined-anthropometry (weight, waist circumference, BMI), biochemistry (liver enzymes, glucose metabolism etc.), blood count, abdominal ultrasound, liver stiffness measurement using ARFI®, nuclear magnetic resonance spectroscopy (MRS; at the start and end of follow-up). After one-year follow-up results were evaluated and statistically processed.
Results: Of the 60 patients enrolled in the study were 45 men and 15 women, the mean age was 51.9 ± 12.2 years, the weight was 97.1 ± 15.2 kg and the mean BMI was 31.25 ± 4.25. There was no significant difference in any of these parameters among the monitored groups (PUFA versus placebo) at the beginning of the study. Similarly, between both groups there was no significant difference in other key parameters-ALT, GGT, or the percentage of fat in the liver tissue determined by MRS. After one-year follow-up, no changes in anthropometric data (weight, waist circumference, or BMI) were observed in the patients enrolled in the study. On the other hand, there was a significant decrease in GGT activity in the PUFA group (2.27 ± 2.71 vs. 1.43 ± 1.55 ukat/L, P = 0.0397), without any change in the placebo group (2.31 ± 3.37 vs. 2.03 ± 2.8 ukat/L, P = 0.064). Other observed biochemical parameters remained unchanged in both groups. During the follow-up liver elastography did not change in either group, as well as percentage of fat in hepatic tissue measured by MRS in both groups (PUFA 13.44 ± 7.7%, placebo 13.24 ± 9.1%).
Conclusion: We observed significant decrease in GGT serum activity after 12 months of PUFA administration, the total amount of liver fat remained unchanged. We conclude that PUFA could represent a potential agent in preventing the development of NAFLD in patients with metabolic syndrome.
Supported by grants AZV 15-28745A, SVV 260370/2018

P06-07YI B-lymphocytes contribute to the evolution of non-alcoholicfatty liver disease (NAFLD)

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Background and aims: Recent evidence implicates adaptive immunity as a key player in the mechanisms supporting hepatic inflammation during the progression of non-alcoholic fatty liver disease (NAFLD). In these settings, patients with NAFLD often show an increase in the titres of circulating antibodies against oxidative stress-derived epitopes (OSEs). Nonetheless, the actual role of humoral immunity in NAFLD is still unclear. This study investigates the contribution of B-lymphocytes to NAFLD evolution.

Methods:

B cell responses were investigated in 42 NAFLD/NASH patients and in mice with NASH by feeding with a methionine-choline-deficient (MCD) diet up to 4 weeks or with a choline- deficient amino acid defined (CDAA) diet for 24 weeks.

Results: B-lymphocyte immunostaining of liver biopsies from NAFLD/NASH patients showed that B-cells were evident within cell aggregates rich in T-lymphocytes. In these subjects, B/T-lymphocyte infiltration positively correlated with both circulating anti-OSE IgG and interferon-γ (IFN-γ) levels. Furthermore, a high prevalence of lymphocyte aggregates identified patients with more severe lobular inflammation and fibrosis. In mouse models of NASH, the onset of steatohepatitis was characterized by hepatic B2-lymphocytes maturation to plasma cells and by an elevation in circulating anti-OSE IgG titres. B-cell responses preceded T-cell activation and were accompanied by the up-regulation in the hepatic expression of B-cell Activating Factor (BAFF). Selective B2-cell depletion in mice over-expressing a soluble form of the BAFF/APRIL receptor Transmembrane Activator and Cyclophilin Ligand Interactor (TACI-Ig) prevented plasma cell maturation and Th-1 activation of liver CD4+ T-lymphocytes. Furthermore, TACI-Ig mice showed milder steatohepatitis and a decreased progression to fibrosis. Similarly, mice treatment with the BAFF-neutralizing monoclonal antibody Sandy-2 prevented hepatic B2-cell responses and ameliorated steatohepatitis.

Conclusions:

B2-lymphocyte activation is an early event in NAFLD evolution and contributes to the disease progression through the interaction with T-cells. Furthermore, combined clinical and experimental data suggest that elevated circulating anti-OSE IgG can identify a subset of NAFLD patients in whom adaptive immunity has a role in the disease evolution and who might benefit from available drugs interfering with B-cell functions.

P06-08 DNA damage and repair in Non-Alcoholic Steatohepatitis (NASH)

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Background and aims: Non−alcoholic fatty liver disease (NAFLD) becomes an increasingly common cause of persistently abnormal liver function tests. Its most progressive form known as non−alcoholic steatohepatitis (NASH) is usually observed in middle−aged obese women with insulin−independent diabetes mellitus, hyperlipidemia and asymptomatic hepatomegaly on physical examination. Oxidative stress is associated with the mechanism of aging, carcinogenesis and progression of atherosclerosis. Excessive oxidative stress induced by mitochondrial, peroxysomal and microsomal reactive oxygen species in NASH induces apoptosis and damage to both nuclear and mitochondrial DNA. The cellular reaction to the DNA-damaging agents may modulate individual's NASH susceptibility. This reaction is mainly determined by the efficacy of DNA repair, which in turn, may be influenced by the variability of DNA repair genes, expressed by their polymorphism. The hOGG1 gene encodes a glycosylase of base excision repair and RAD51 specifies a key protein in homologues recombination repair. Both proteins can be involved in the repair of DNA lesions, which are may contribute to NASH.
Method: In the present work we determined the extent of basal DNA damage and the efficacy of removal of DNA damage induced by hydrogen peroxide and N-methyl-N'-nitro N-nitrosoguanidyne (MNNG) in peripheral blood lymphocytes of 30 NASH patients and 30 individuals with healthy liver. The results from DNA damage and repair study were correlated with the genotypes of two common polymorphisms of the hOGG1 and RAD51 genes: a G>C transversion at 1245 position of the hOGG1 gene producing a Ser → Cys substitution at the codon 326 (the Ser326Cys polymorphism) and a G>C substitution at 135 position of the RAD51 gene (the 135G>C polymorphism). DNA damage and repair were evaluated by alkaline single cell gel electrophoresis and genotypes were determined by restriction fragment length polymorphism PCR.
Results: We observed a strong association between NASH and the C/C genotype of the 135G>C polymorphism of the RAD51 gene. Moreover, there was a strong correlation between that genotype and NASH occurrence in subjects with a high level of basal DNA damage. We did not observe any correlation between the Ser326Cys polymorphism of the hOGG1 gene and NASH.
Conclusion: Our result suggest that the 135G>C polymorphism of the RAD51 gene may be linked to NASH and can be considered as an additional marker of this disease.

P06-09 Inhibition of DGAT2 improves hepatic steatosis, inflammation and cardiovascular risk factors in the LDLr-/-.Leiden mouse

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The central cause of Non-alcoholic Fatty Liver Disease (NAFLD) and Non-alcoholic Steatohepatitis (NASH) is abnormal hepatic and systemic metabolism. In support of this concept is the fact that insulin resistance, hyperinsulinemia, Type-2 diabetes and cardiovascular complications all are associated with NAFLD and NASH. Hepatic steatosis is an important causative feature found in early NAFLD and advanced NASH. Reinforcing the importance of hepatic metabolism in the causation of NAFLD and NASH is the observation that therapies such as gastric bypass and sustained weight loss reverse hepatic steatosis, inflammation and fibrosis along with improving systemic insulin sensitivity, hyperlipidemia and other cardiovascular risk factors. Therefore, therapies that normalize hepatic metabolism and steatosis may not only improve liver health in patients with NASH but may also improve overall mortality by decreasing risk factors for cardiovascular disease and other related metabolic diseases. To test this hypothesis an inhibitor of Diacylglycerol O-transferase 2 (DGAT2), PF-D1, was administered to LDLr-/-.Leiden mice fed a western diet. The LDLr-/-.Leiden model was used because of the similarities in inflammatory, metabolic and fibrotic gene expression with human NASH biopsy samples. LDLr-/-.Leiden mice were maintained on a High Fat/High Carbohydrate (HFHC) diet for 16 weeks to allow for the development of early NAFLD. From week 16 to 38 mice were gavaged 80 mg/kg/day BID of PF-D1 or vehicle. Weight loss was observed in both vehicle and PF-D1 treated animals initially due to acclimation to BID gavage, which rapidly reversed by week 20. Despite this initial weight loss no differences in body weight were observed between vehicle or PF-D1 treated animals throughout the remainder of the study. Treatment with PF-D1 reduced both hepatic steatosis and inflammation suggesting that PF-D1 treatment could directly improve hepatic metabolism in NASH. Additionally, plasma biomarkers of hepatic inflammation and fibrosis, ALT, CK18 and Timp1 were reduced by PF-D1 treatment and not vehicle. Consistent with the majority of NASH patients, LDLr-/-.Leiden mice fed HFHC diet developed hypertriglyceridemia and hypercholesterolemia.. Treatment with PF-D1 reversed both parameters after only 4 weeks of treatment. Finally, plasma adiponectin levels increased in mice treated with PF-D1, suggesting that overall systemic metabolism improved.
Conclusion: Inhibition of DGAT2 may provide therapeutic benefit to patients with NASH and other metabolic diseases as multiple metabolic risk factors for NASH and cardiovascular disease were improved.
All procedures performed on these animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process

P06-10 Dietary cholesterol mitigates liver bile acid toxicity by activation of phase 1 regenerative response

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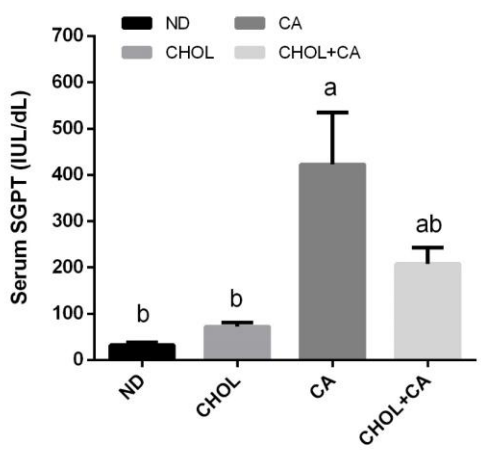
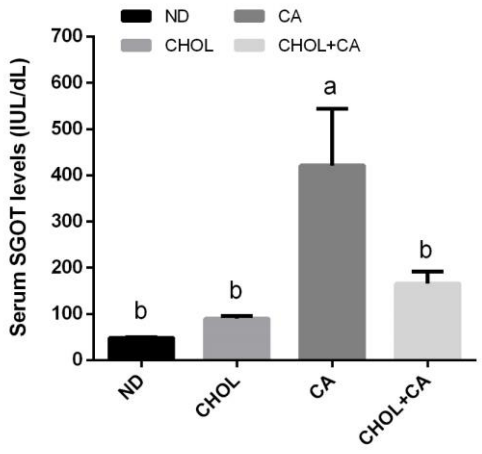
Background and aims: It has been a long standing observation from liver pathology that hepatic cholestasis is associated with proliferation of hepatocytes. In our study we aimed to determine whether the proliferative and regenerative effects that were observed are due to inflammatory response mediated by cholesterol or bile acids.
Method: The study was performed for 3 weeks. Male C57BL mice were assigned to 4 groups (n = 32 mice) and were fed with one of the following diets: ND (standard AIN-93G diet, Control group), ND + cholesterol 1%, ND + Cholic Acid 0.5%, ND + Cholesterol 1% + Cholic Acid 0.5% (atherogenic diet). After 3 weeks, mice were euthanised and blood serum and liver tissue were collected. Plasma serum was analysed for lipid profile, liver enzymes and blood glucose.
Liver tissue was analysed for presence of inflammatory parameters. In addition, immune inflammatory response represented by macrophages infiltration was determined by histologic analysis of the tissue. Furthermore, hepatocytes first and second phase regeneration was analysed by determination of regeneration markers.
Results: It was observed that mice fed cholesterol enriched diet, maintained normal hepatic phenotype, with minor lipid droplet accumulation and hepatocyte proliferation. However dietary administration of cholic acid promotes liver injury and liver necrosis without considerable inflammatory response. The combination of cholesterol and cholic acid (in atherogenic diet group) resulted in high inflammatory response and induced hepatocyte regeneration, suggesting the protective effect of dietary cholesterol on hepatic function due to activation of phase 1 regeneration.
Conclusion: We are showing for the first time that dietary cholesterol may prevent the bile acid-induced cholestasis and liver necrosis.
Figure:
3 weeks dietary treatment: ND, Normal diet, CA, cholic acid, CHOL, free cholesterol.

Liver damage markers:
Serum levels of SGPT and SGOT





P06-11 Transcriptomic profiles of transplanted livers that developed NAFLD

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Background and aims: Liver transplantation leads to NAFLD/NASH in up to 40% of graft recipients. The aim of our study was to assess the transcriptomic profiles of liver grafts and to contrast the hepatic gene expression between the patients after transplantation with vs. without NAFLD.
Method: Total RNA was isolated from liver graft biopsies of 91 recipients (46 men, 45 women). The transcriptomic profile was assessed using Affymetrix®HuGene2.1ST Array Strips processed in Affymetrix GeneAtlas. Data were analyzed using Partek Genomics Suite 6.6 and Ingenuity Pathway Analysis by standardized methods including hierarchical clustering, gene ontology, upstream regulators and mechanistic causal networks.
Results: The individuals with hepatic steatosis showed higher indices of obesity including weight, waist circumference, BMI and increased volumes of different fat depots measured by MR methods (percent of liver fat, volume of abdominal fat). The two groups were comparable in measures of insulin sensitivity and cholesterol concentrations. After correction for multiple comparisons (FDR, false discovery rate <0.05) we have identified 889 transcripts (403 upregulated and 486 downregulated in steatotic samples compared to controls) significantly differentially expressed between grafts with vs. without NAFLD. Among the most downregulated genes in steatotic samples were *P4HA1 (prolyl 4-hydroxylase subunit alpha 1)*, *IGF1 (*insulin like growth factor 1) or fetuin B while the most upregulated were *PLIN1* (perilipin 1) and *ME1* (malic enzyme 1). Most influential upstream regulators included *HNF1A* (Hepatocyte Nuclear Factor 1-Alpha), insulin receptor (*INSR*) and transcription factor *TCF7L2* (transcription factor 7 like 2). The canonical pathways dysregulated in liver grafts showing NAFLD comprised FXR/RXR, PXR/RXR and LXR/RXR activation. We observed significant enrichment in categories of liver necrosis, cholestasis and steatosis together with depletion of liver glutathione. The derived mechanistic network underlying the major transcriptome differences between NAFLD samples and controls featured PPARA (Peroxisome proliferator-activated receptor alpha) and NKFB (Nuclear factor kappa B) as main connecting nodes.
Conclusion: Although there is certain overlap between the results in current study and published transcriptomic profiles of non-transplanted livers with NAFLD, we have identified discrete characteristics of NAFLD in liver grafts potentially utilizable for establishment of predictive signature.
Supported by Czech Health Research Council project 15-26906A

P06-12 Elafibranor and nitazoxanide synergize to reduce fibrosis in a NASH model

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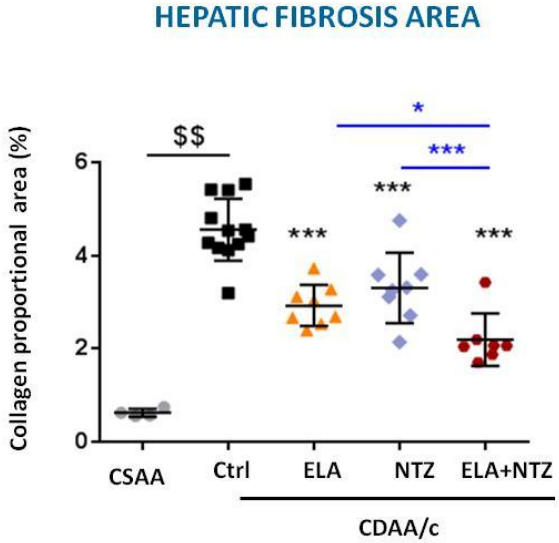
Background and aims: Drug combinations are increasingly required for the successful treatment of complex liver diseases. In NASH, drug combination has recently emerged as a new treatment paradigm to increase the proportion of patients that reach all treatment goals. The ideal NASH medication will both reverse NASH histology and stop the progression of fibrosis in patients at high risk of cirrhosis and complications.
Elafibranor (ELA), a PPARα/δ agonist can reverse NASH histology and decrease fibrosis, especially in patients with advanced disease (GOLDEN-505 phase 2b trial), and is currently being evaluated in the RESOLVE-IT phase 3 trial. We have recently identified nitazoxanide (NTZ), a phase-2 ready drug candidate with a good safety profile in man as a potent anti-fibrotic agent, by using an unbiased phenotypic screening approach.
The aim of this proof of concept study is to assess ELA/NTZ combination in a disease model of NASH to establish its potential value for treating human disease.
Method: Human primary stellate cells (HSC) were activated with TGFβ. αSMA was measured by ELISA. A NASH phenotype was induced in C57Bl/6J mice by feeding a cholesterol-supplemented CDAA diet (CDAA/c) for 12 weeks. Animals received ELA alone (1 mg/kg/day), NTZ alone (100 mg/kg/day) or ELA/NTZ combination for the entire study period of 12 weeks. Histological evaluation of NASH and fibrosis was performed in a blinded fashion.
Results: Nitazoxanide (NTZ) potently interfered with TGFβ-induced HSC activation in vitro. IPA analysis of transcriptional data showed that profibrotic signaling pathways were attenuated in HSC cells that received NTZ. At low doses, ELA and NTZ showed a modest inhibition (20-30%) of HSC activation, when used separately but this effect was doubled when both drugs were used as combination.
Histological examination in vivo revealed a severe NASH phenotype accompanied by perisinusoidal fibrosis. Liver fibrosis area was reduced by 41% and by 32% in mice treated with ELA and NTZ, respectively, whereas ELA/NTZ combination reduced the proportional fibrosis area by 60%. Transcriptomic analysis showed that ELA/NTZ combination created a synergistic beneficial action on multiple pathological mechanisms, involving liver cell death, inflammasone activation, immune cells recruitment and fibrosis.
Conclusion: Elafibranor and nitazoxanide synergize *in vitro* and *in vivo* to reduce liver fibrosis, opening interesting perspectives for further clinical development.



P06-13YI The influence of tumour necrosis factor on early fibrogenesis in a diet-induced NAFLD mouse model-a histological description

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Background and aims: Non-alcoholic liver disease (NAFLD) and its complications have been described to be a result of chronic low-grade inflammation due to excessive lipid accumulation in the liver. Tumour necrosis factor (Tnf) is known to play a major role in the initiation of the inflammatory response to injurious stimuli, though its exact role in liver fibrogenesis, a consequence of obesity, has yet to be elucidated. This study investigates the role of Tnf in the regulation of inflammation and development of fibrosis in a high fat diet (HFD) -induced model of NAFLD. In particularly, we are focussing on the effects of Tnf deletion on signs of early fibrogenesis, with high fat diet acting as a mild and more physiologically relevant fibrosis model, compared to chemically induced fibrosis models.

Method: Eight-week old, male Tnf-knockout (Tnf-/-) or C57Bl/6J (B6 control) mice were given a high fat diet (45%kcal fat) or standard chow diet (12%kcal fat) ad libitum for 12 weeks. At termination, serum was analysed for liver transaminases. Whole liver was taken for analysis of collagen deposition, hepatic stellate cell (HSC) activation, hepatocyte necrosis, lipid accumulation, liver architecture and inflammation, determined by histopathological techniques. Image analysis was performed using FIJI software per blinded 5 random fields of view per animal at 10x magnification.

Results: Knockout mice are healthy, viable and exhibit no off-target effects compared to B6 control. Tnf-/- mice were significantly heavier in body weight ($p < 0.05$) and liver weight ($p < 0.05$), with the HFD group having a significantly higher ALT ($p < 0.05$) compared to control. Collagen deposition (by picrosirius red staining) is reduced in Tnf-/- animals undergoing HFD treatment compared to control. Smooth muscle-actin staining for HSC activation was decreased in both chow and HFD treated Tnf-/- mice versus B6 controls, though not to significant levels. Control levels of hepatocyte necrosis was decreased in Tnf-/- mice, but not in HFD. Furthermore, Tnf-/- livers exhibited ballooning of hepatocytes in all zones and significantly higher numbers of both microvesicular lipid accumulation and macrovesicular lipid along portal tracts, whereas B6 control mice on HFD showed dispersed micro- and macrovesicular fat in the liver. Interestingly, leukocyte aggregates were observed in HFD-fed B6 mice, associating with surrounding smooth muscle-actin staining, while this was not seen in Tnf-/- HFD-fed mice.

Conclusion: Tnf has a role in inflammatory and fibrotic liver injury by ablating leukocyte aggregates and minimising HSC activation and downstream collagen deposition. However, the absence of Tnf did not prevent the development of fatty liver, nor reduce overall liver injury.

P06-14YI Autoimmunity and NAFLD: clinical characteristics and long-term outcomes of patients with antinuclear antibody positivity. a longitudinal multicentric european study

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Background and aims: Autoimmunity tests are part of the first diagnostic workup of patients with NAFLD. In these patients, the clinical significance of ANA positivity is uncertain and controversial. We aimed at evaluating clinical features and long-term outcomes of patients with NAFLD and ANA alterations.

Method: 961 patients underwent a liver biopsy for clinical suspicion of NAFLD in centres in Italy (Turin, Milan, Rome, Palermo) and the United Kingdom (Newcastle). Clinical and biochemical data were collected at the time of biopsy. Patients have then undertaken regular visits for routine care and clinical events were recorded by clinicians.

Results: 160 (16.6%) patients reported ANA positivity, with no significant difference between UK and Italian centres [titres: 1:40 (n = 27, 16.9%), 1:80 (n = 68, 42.5%), 1:160 (n = 38, 23.8%), 1:320 (n = 15, 9.4%), 1:640 (n = 10, 6.3%), 1:1280 (n = 2, 1.3%)]. ANA pattern was available for 79/160 patients and showed a predominance of the speckled subgroup [homogeneous (26, 6%), speckled (65.9%), nucleolar (7.6%)]. At the multivariate analyses, no clinical or histological differences were detected between ANA pos. and neg. patients. Interestingly, 98 ANA-neg. patients had a history of autoimmune disease (12.9%), while the prevalence among ANA-pos. patients was 17% (n = 26), with no significative difference between the two groups (p = 0.183). On the other hand, there was an interesting significant difference considering PNPLA3 C>G polymorphism, as the rate among ANA-pos. patients was 85.2% (n = 75), while it was 70.5% (n = 220) among the ANA-neg. group (p = 0.006). After a median follow-up of 90 months, the rate of liver events was the same between ANA-pos. and neg. patients (8.4% vs 9.9% respectively, p = 0.568). Four ANA-pos. patients (2.6%) developed hepatocellular carcinoma (HCC), while the incidence of HCC was 3.2% (n = 24) among ANA-neg. patients (p = 0.705). Considering cancer, pulmonary and cardiovascular events no significant difference was seen between the two groups during follow-up (p = 0.176, p = 0.387, p = 0.768 respectively). Finally, at the Kaplan-Meier analysis, no significant survival difference was detected when compared the two groups [Log-Rank (Mantel-Cox) 0.579].

Conclusion: The long-term data of our study confirmed the non-specific and benign role of ANA positivity in NAFLD. Nonetheless, the association with the PNPLA3 C>G polymorphism may suggest an interaction between these two components, requiring further investigations.

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At Pfizer, we apply science and our global resources to bring therapies to people that extend and significantly improve their lives. We strive to set the standard for quality, safety, and value in the discovery, development, and manufacture of health care products. Our global portfolio includes medicines and vaccines and many of the world's best-known consumer health care products. Every day, Pfizer colleagues work across developed and emerging markets to advance wellness, prevention, treatments, and cures that challenge the most feared diseases of our time.

VLVbio

Hästholmsvägen 32
Nacka 13130
Sweden
www.vlvbio.com

At VLVbio we offer unique assays for accurate and non-invasive detection of liver damage and disease.

Our biomarker assays, M30 and M65, measure apoptosis and total cell death respectively, and are used globally by academia, industry and healthcare providers.

Measuring apoptosis and levels of total cell death is of interest in the diagnosis and treatment follow-up of patients suffering from liver diseases such as NASH and ASH, in the development of new drugs and when assessing toxicological effects of pharmaceuticals and other substances.

Come and say “hi” and let us tell you more about our products!

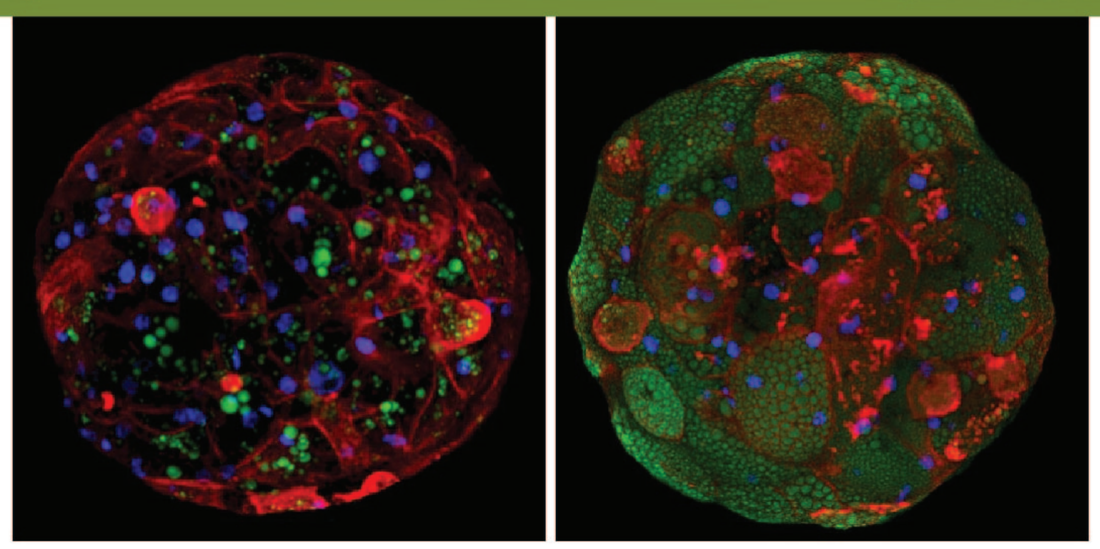
Meet Radina. She works for you

to bring safer, more effective drugs to market faster.

Radina Kostadinova, PhD
Liver Microtissue Product Manager

Radina is one of the many InSphero scientists working tirelessly to develop advanced, reliable 3D *in vitro* models and services for drug efficacy and safety testing. Our 3D platforms, which include specially formulated media and certified assay protocols, are engineered to help you:

- **Leverage proven 3D microtissue technology** that ensures uniform model size, robust functionality, and reproducible data. Our models and services are used by top pharma and biotech companies worldwide
- **Accelerate drug discovery** with physiologically relevant, preclinical models for steatosis, NASH, fibrosis, diabetes, and oncology
- **Reduce animal testing** with standardized *in vitro* toxicology models that predict DILI with twice the sensitivity of conventional 2D models
- **Implement scalable, automation-friendly technology** for experimental continuity



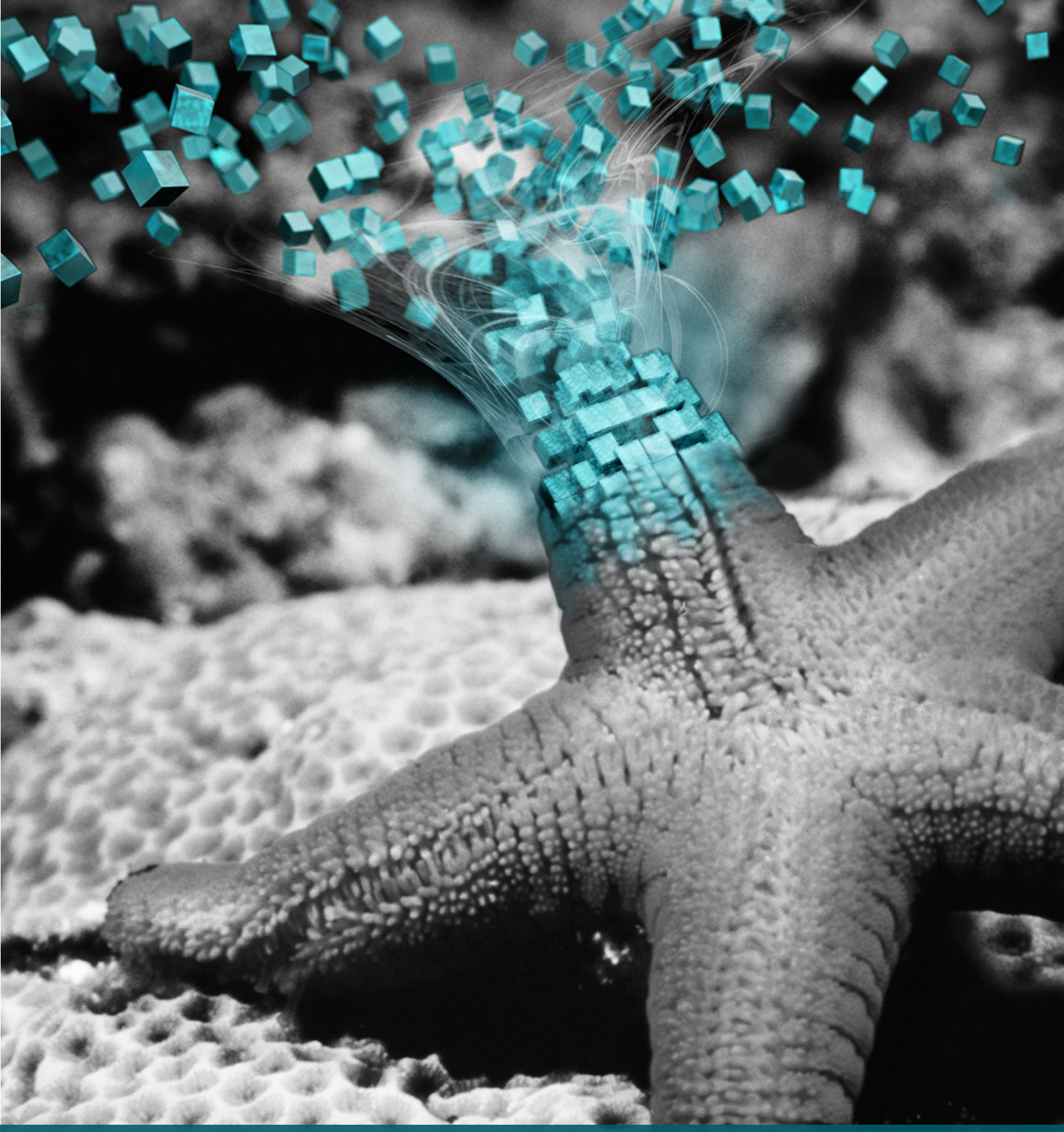
A sample of our team's work: *The 3D InSight™ Human Liver Steatosis Model mimics the pathophysiology of human liver steatosis. The control microtissue (left), treated with BSA, has normal liver microtissue biology, whereas the liver disease model (right), induced by lipid-loading with free fatty acids, develops micro and microvascular steatosis. Fluorescent staining: lipids (green), nuclei (blue), and plasma membrane (red).*

InSphero at EASL NAFLD SUMMIT 2018

- **Attend Poster Session 5, Poster P05-02:** *Modeling NASH for drug discovery using 3D liver microtissues*
- **Visit Booth 5** discuss your NAFLD research projects with our liver platform team
- **Read more** about our liver disease solutions online at insphero.com/event/nafld2018/

Innovative 3D Platforms for Drug Discovery and Toxicology

InSphero AG +41 44 515 04 90 Info@insphero.com www.insphero.com



Intercept

A company inspired by

REGENERATION

We are Intercept. Our mission is to harness the unique regenerative powers of the liver and build a healthier tomorrow for patients with progressive non-viral liver diseases.

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September 2018