

**THU-386**

**Protective effect of quercetin treatment on HFD-induced intestinal dysbiosis and barrier dysfunction in an in vivo model of non-alcoholic fatty liver disease**

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**Background and Aims:** Gut microbiota is involved in obesity, metabolic syndrome and nonalcoholic fatty liver disease (NAFLD). Quercetin may have the ability to modulate the intestinal microbiota composition, suggesting therapeutic potential in NAFLD. The present study aims to investigate the beneficial effect of quercetin treatment on dysbiosis, intestinal barrier dysfunction and gut-liver axis alteration in high-fat diet (HFD)-fed mice.

**Methods:** Gut bacterial communities were identified by pyrosequencing of the 16S rRNA extracted from caecal samples of C57BL/6J mice fed with HFD supplemented with or without quercetin for 16 weeks. Hepatic TLR-4-NF-κB signaling pathway activation, inflammasome response, endoplasmic reticulum stress induction, as well as intestinal barrier dysfunction were analyzed.

**Results:** Metagenomic studies revealed differences at phylum, class and genus levels induced by HFD, leading to dysbiosis, characterized by an increase in *Firmicutes/Bacteroidetes* ratio and in Gram-negative bacteria and a lower concentration of the total bacteria. Quercetin blocked gut microbiota imbalance, showing prebiotic capacity. At genus level, we found a dramatically enhanced detection of *Helicobacter* genus, which was reverted by quercetin treatment. NAFLD severity correlated with dysbiosis markers, indicating a microbiota-dependent individual metabolic phenotype. HFD-induced dysbiosis was associated with impaired intestinal SCFAs production (acetate: -32%, propionate: -21%, butyrate: -29%) and barrier integrity (claudin1: -59%, occludin: -23%, IAP: -40%). HFD-related endotoxemia was accompanied by TLR-4-NF-κB signaling pathway activation (nuclear p65: +112%, cytosolic p65: -33%), inflammasome response (NLRP3: +37%, caspase1: +64%) and endoplasmic reticulum stress (GRP78: +78%, CHOP: +101%). Treatment with quercetin restored SCFAs production (acetate: +34%; propionate: +27%; butyrate: +21%) and intestinal barrier function (claudin1: +143%, occludin: +37%, IAP: +40%), reducing the activation of NF-κB (nuclear p65: -47%, cytosolic p65: +24%), and inhibiting over-expression of inflammatory components (NLRP3: -48%, caspase1: -40%) and reticulum stress markers (GRP78: -37%, CHOP: -21%).

**Conclusions:** Our data support the role of dysbiosis in NAFLD development, sustaining the suitability of quercetin as a therapeutic approach for obesity-associated NAFLD. Supported by BFU2013-48141-R, LEU35U13/LE063U16 (JCyL y Fondo Europeo de Desarrollo Regional (FEDER)) y GRS1428/A/16. CIBERehd is funded by ISCIII.

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**Mitochondrial-shaping proteins as specific biomarkers to distinguish alcohol from fat-induced liver toxicity**

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**Background and Aims:** Despite some common features (lipotoxicity, oxidative stress) Alcoholic Liver Disease (ALD) and Non-Alcoholic Liver Disease (NAFLD) differ in many clinical aspects and a clearer understanding of their pathogenesis would improve their management. Hepatic mitochondrial alterations have been observed in both conditions, however morphological enlargements (megamitochondria, MM) are typical of ALD but rarely associated with NAFLD. The shape of mitochondria correlates with their function and adapts rapidly to the needs of the cell with cycles of fusion (organelle binding) and fission (fragmentation). These changes are strictly regulated by the activity of the mitochondrial-shaping proteins (MSP). Our aim is to elucidate the role of mitochondrial dynamics, shape and MSP in ALD/NAFLD in order to identify early disease specific markers.

**Methods:** Human precision cut liver slices and VL-17A cells (positive for ADH/CYP2E1) were cultured with ethanol (EtOH) and/or oleic/linoleic acid (FFA), to induce hepatotoxicity. Post-treatment cell viability, intracellular fat accumulation and mitochondrial functionality were evaluated and changes in the mitochondrial shape were assessed by confocal and electron microscopy. MSP protein or gene expression was analysed in our models and in liver biopsies from patients with ALD (15) or different stages of NAFLD (32) and compared to healthy lean (14) or obese (27) controls.

**Results:** The toxic effect of EtOH/FFA was confirmed by an increase in the percentage of apoptosis or a reduction in ATP levels. The two insults induced a very different mitochondrial phenotype, as well as differential intracellular fat accumulation. Fat was associated with a more pronounced toxicity and mitochondrial dysfunction, while EtOH showed a moderate effect. With EtOH exposure, the surviving cells showed a dramatically increased proportion of MM which were not present in FFA cultures. Moreover, changes in MSP involved in mitochondrial fragmentation were observed only in ALD, but not in NAFLD patients.

**Conclusions:** In conclusion, we demonstrate that hepatotoxic agents such as EtOH or fat affect the mitochondrial dynamics and MSP expression differently. In particular, we show MM formation in hepatocytes as a specific response to EtOH, but not to fat insult. Finally, we suggest the use of MSP involved in mitochondrial fission as disease-specific biomarkers potentially discriminating between ALD and NAFLD.

**THU-388**

**Hypoxia-inducible factor 2alpha drives the progression of experimental non-alcoholic fatty liver disease by stimulating hepatocyte production of histidine rich glycoprotein**

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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-2alpha 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 insides in the mechanisms by which HIF-2alpha promotes the progression of the experimental NAFLD.

**Methods:** NAFLD was induced by feeding mice with hepatocyte-specific conditional deletion of HIF-2alpha (HIF-2alpha fl/fl/Alb-Cre mice) and control littermates with MCD and choline-deficient L-amino acid refined (CDAA) diets. In vitro studies have been performed using HepG2 cells overexpressing HIF-2alpha.

**Results:** In both the dietary models of NAFLD hepatocyte deletion of HIF-2 alpha resulted in: (i) a decrease in fatty liver and parenchymal necrosis; (ii) amelioration in lobular inflammation