Accumulation of dihydrosphingolipids and neutral lipids are related to steatosis and fibrosis damage both in human and animal models of NAFLD

BOHDAN BABIY[1,2,3], SILVIA SACRISTÁN[2], RAMOS-MOLINA BRUNO[4], MARTINEZ-BOTAS JAVIER[2,7], REBECA BUSTO[2,7], GEMMA VILLA TURÉGANO[1,2], AGUSTIN ALBILLOS[3,6,8], LUIS OCAÑA[5], LASUNCIÓN MIGUEL A[2,7], OSCAR PASTOR[1,2,7]

[1]UCA-CCM, Servicio de Bioquímica Clínica. Hospital Universitario Ramón y Cajal-IRYCIS. Madrid; [2]Grupo de Lípidos. Servicio de Bioquímica Investigación. Ramón y Cajal-IRYCIS. Madrid; [3]Universidad de Alcalá de Henares. Madrid; [4]Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), Murcia; [5]Servicio de Cirugía General. HCU Virgen de la Victoria. Málaga; [6]Servicio de Gastroenterología. Hospital Universitario Ramón y Cajal-IRYCIS. Madrid; [7]CIBIEROBN; [8]CIBIEREHD

Supervisor email: oscar.pastor@salud.madrid.org

BACKGROUND & AIM:

Non-alcoholic fatty liver disease (NAFLD) is a growing entity related to the epidemic of obesity and diabetes. (Dihydro)sphingolipids are class of lipid molecules whose synthesis respond to the increased burden of circulating free fatty acids associated to NAFLD. Blocking (dihydro)sphingolipid synthesis has been proposed to overcome NAFLD. However, there are conflicting results regarding the relationship of (dihydro)sphingolipids levels and the degree of liver histological damage in patients and animal models. We use a diet induced model of NAFLD and apply lipidomics to study the relationship of lipids with NAFLD and explore which among these molecules are most related with the histological changes throughout its progression.

METHODS:

C57BL/6J mice were fed a high fat diet supplemented with glucose and fructose in drinking water up to 40 weeks (**Figure 1**). A subset of animals were treated with CCl4 (ip.) for 6 and 10 weeks to induce a more advanced fibrosis stage (>F2). Animals were sacrificed at different time points to reproduce the spectrum of histological damage found in human disease: NAFL, NASH (<F2) and NASH-fibrosis (>F2). The liver histology was grading following the SAF classification system (steatosis, ballooning/inflammation, fibrosis).

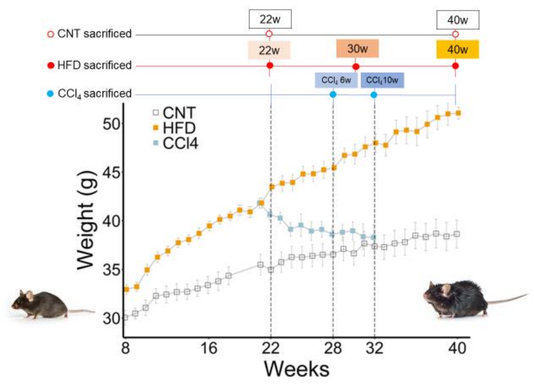

Figure 1. Animal model body weight acumulation inducen by the HFD diet. Mouse were sacrificed at weeks 22, 30 and 40 in order to study development of the disease. CCl4 treatment started at week 22.

In a complementary study, plasma and liver tissue were obtained from 200 patients whose diagnosis and NAFLD severity was evaluated by liver biopsy (**Figure 2**). Lipidomic analysis was performed using RPLC-tandem mass spectrometry following a quality control strategy to reduce potential quantification biases. The molar concentrations are reported in nmol/mg (liver) and nmol/mL (plasma).

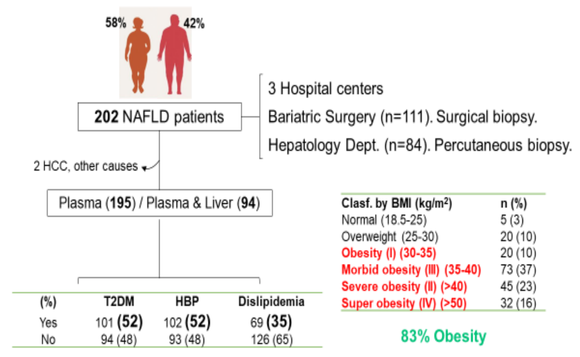

Figure 2. Demographic and clinical parameters of included patients. All the patients were diagnosed with NAFLD disease, 170 of them present obesity and 111 patients undergo bariatric surgery.

RESULTS:

Both animal and human liver neutral (CE and TG) concentrations were significantly increased with the steatosis burden (**Figure 6, 7)** and were downregulated by advanced fibrosis (**Figure 3**). On the contrary, (dihydro)sphingolipids concentrations were increased both by steatosis and fibrosis stage (**Figure 4**). Concentrations of CE, TG and (dihydro)sphingolipids in plasma of patients follow a similar trend to that observed in the liver (**Figure 8)**.

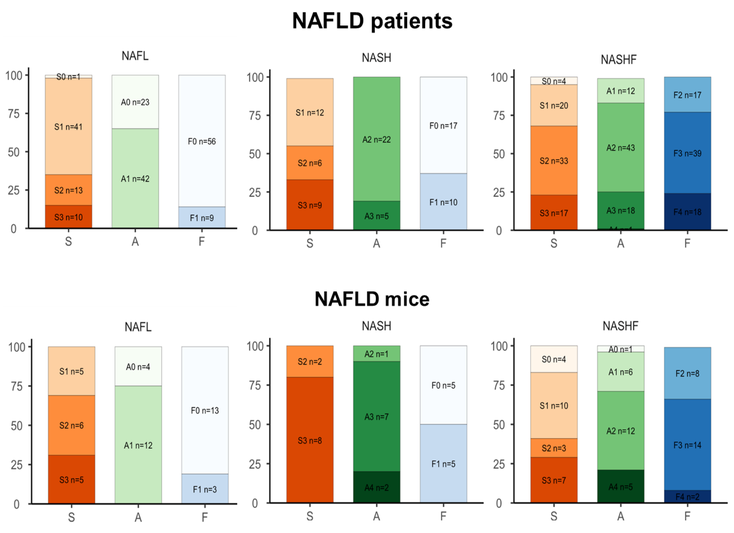

Figure 3. Liver lipid accumulation and inflammatory activity increase with steatosis progression but decrease on significant fibrosis development. Advanced fibrosis classified as >F2 is related to decline in lipid accumulation and inflammatory activity both in patient and animal models.

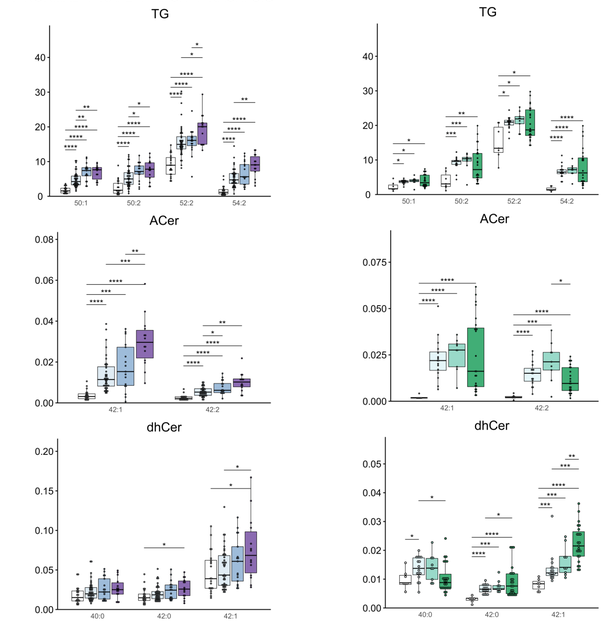

Figure 4. Long chain lipids accumulation in liver through the steato-hepatitis progression. Triacylglycerol (TG), acylceramides (ACer) and dihydroceramides (dhCer) lipid species exhibit a relationship with fibrosis progression both in human patients and animal model.

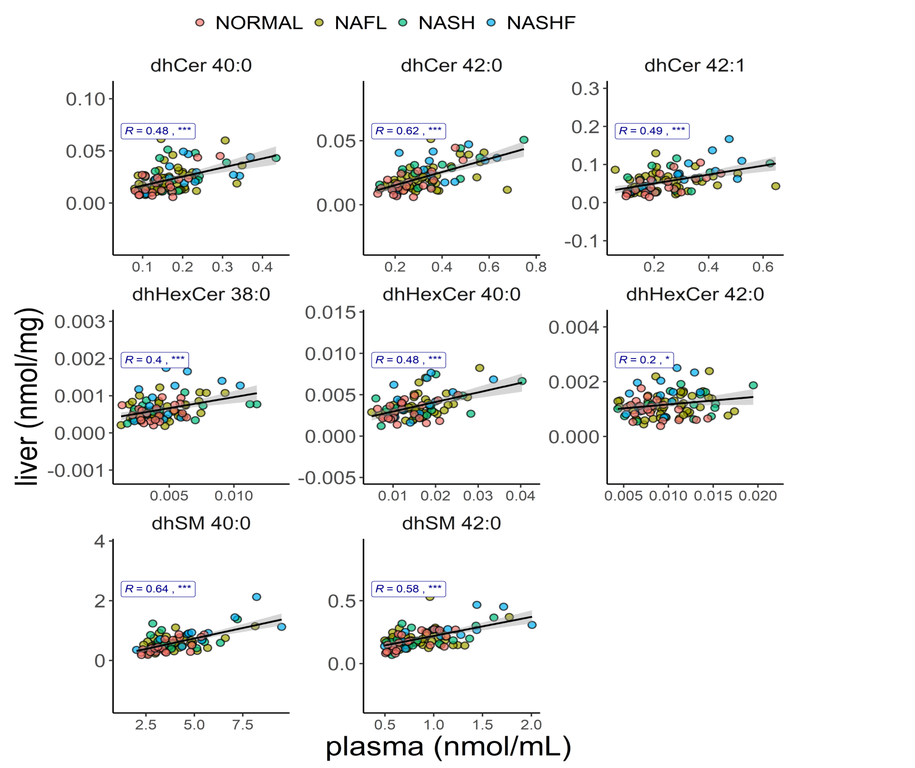

Figure 5. Correlation of dihydrosphingolipids concentrations in liver and plasma of patients. Long chain dihydrosphyngolipids concentrations correlation between both liver and plasma of the patients stresses its relationship with NAFLD progression.

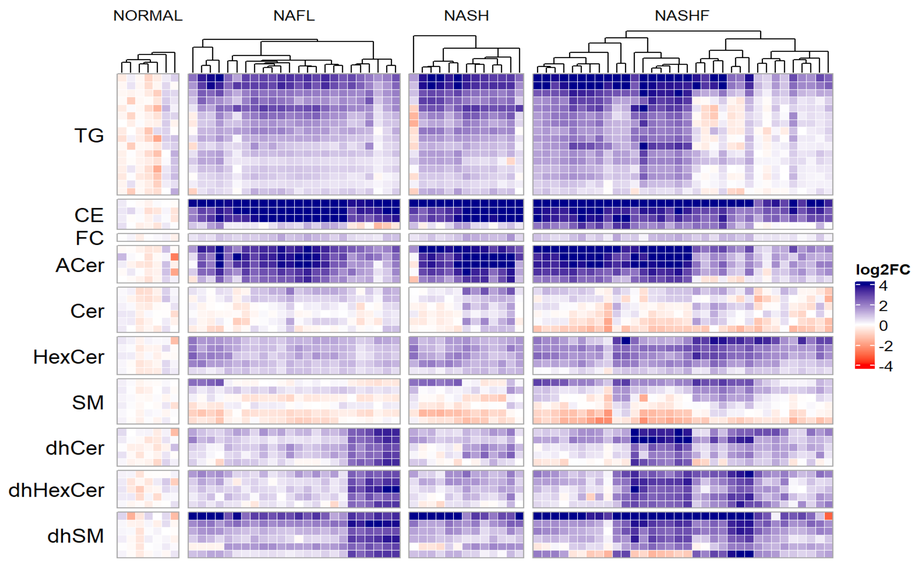

Figure 6. Dihydrosphingolipids accumulation in mouse liver with steato-hepatitis progression. Histological criteria described in Bedossa et.al. 2012, were applied for classification.

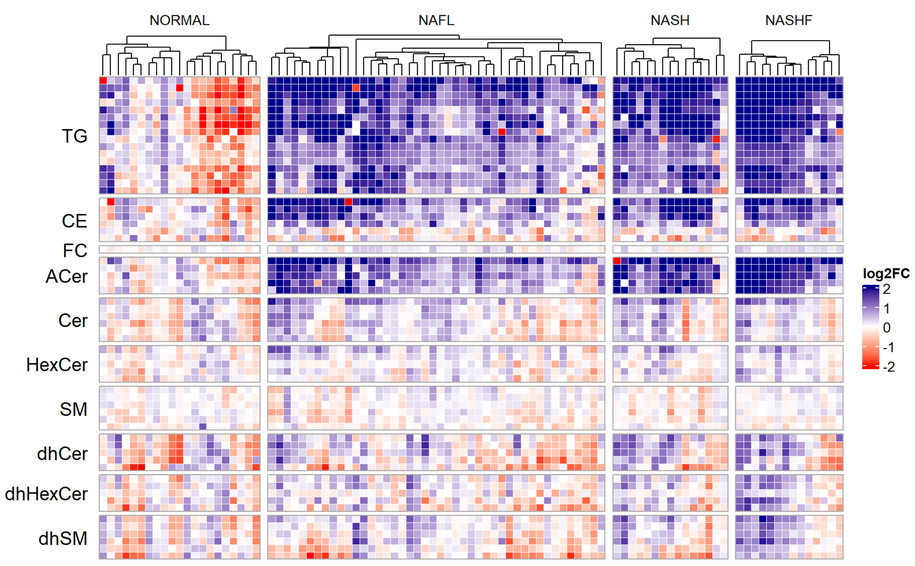

Figure 7. Dihydrosphingolipids accumulation in the patients' liver with steato-hepatitis progression. Histological criteria described in Bedossa et.al. 2012, were applied for classification.

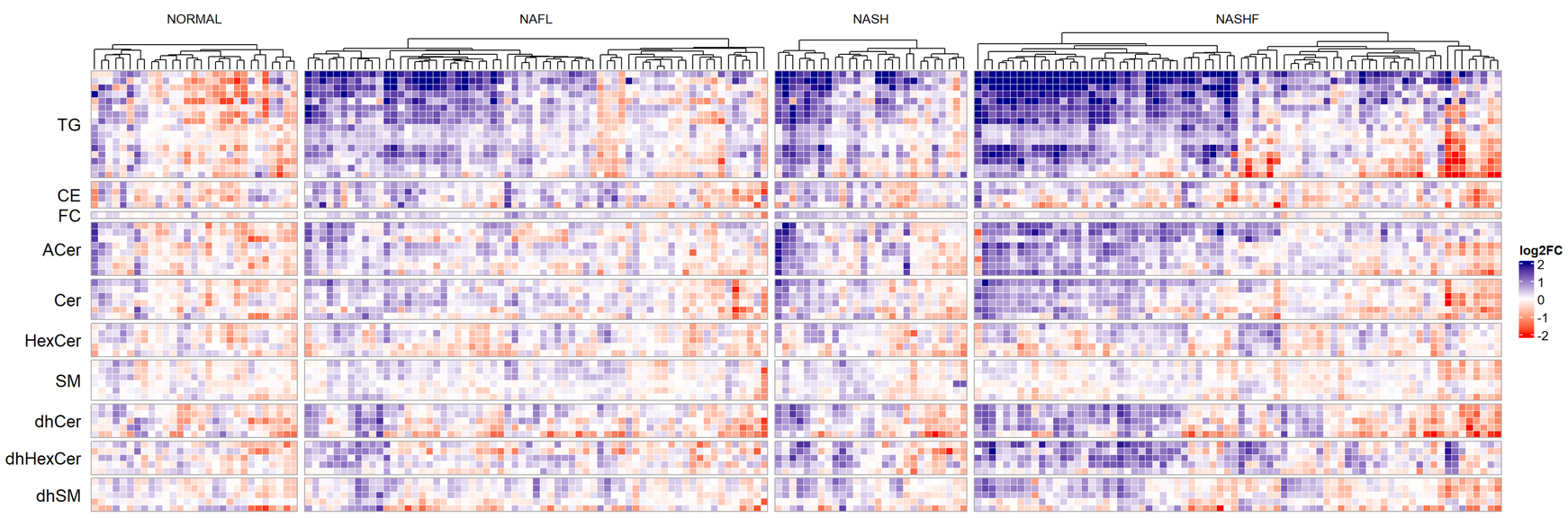

Figure 8. Dihydrosphingolipids accumulation in the patients' plasma with steato-hepatitis progression. Histological criteria described in Bedossa et.al. 2012, were applied for classification. Patient plasma exhibit similar trend to that observed in both human and mouse liver, although within group variability is still an issue.

CONCLUSIONS:

Our study demonstrates that (dihydro)sphingolipids accumulate in liver of NAFLD and correlate with the histological degree of steatosis both in mice and humans (**Figure 6 and 7)**. However, the appearance of fibrosis modulates the concentration of these lipids in the liver. Of note, plasma concentrations of lipid species demonstrate a good correlation with liver findings stressing its relationship with liver metabolism (**Figure 5)**.

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