Aim : In order to use SIA in high-throughput screening of HCC drugs, methods for fast and in depth data analysis are required. Accordingly, we developed SImBA (Spheroid Image Batch Analysis), a FIJI-based software tool which allows to perform high-throughput image segmentation, visualization and quantification of invasive spheroids.
Methods : Spheroids of two HCC cell lines (HEP3B and SNU423) were embedded in a 3D collagen Type I matrix. Phase contrast microscopy images were recorded at different time points to evaluate the invasive process of control and treated spheroids. Since FIJI is commonly used free softwareD, SImBA has been written as an open-source FIJI macro which ensures high user-friendliness and easy customization by the user. SImBA allows batch image processing independent of data set size and is applicable on images of variable and low contrast.
Results : Since SImBA is able to visualize and quantify the invasion data in multiple ways (using total spheroid area, spheroid outlines, area overlaps, montages and a derived invasion index), it allows to robustly demonstrate the effects of various drugs compared to control treatment. Importantly, effects on invasion and cytotoxicity can be linked using SImBA when fluorescent images of cytotoxicity from e.g. Sytox green staining are recorded.
Conclusions : We created a highly functional, easy to use free software tool for the quantitative analysis of high-throughput spheroid invasion and visualization of the invasive capacity of hepatic cancer cell lines. This will contribute to future drug screening in HCC using either 3D SIA or related assays using organoids.

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LONGITUDINAL CHARACTERIZATION OF MUSCLE ALTERATIONS IN A RODENT MODEL OF NASH. H. Louvegny (1), M. Nachit (2), C. Bouzin (3), J. Thissen (4), Y. Horsmans (5), G. Vande Velde (6), I. Leclercq (1) / [1] UCLouvain, Belgium, Institut de Recherche Expérimentale et Clinique, Laboratory of Hepato-Gastroenterology, [2] UCLouvain, Belgium, Institut de Recherche Expérimental et Clinique, Laboratory of Hepato-Gastroenterology, [3] UCLouvain, Belgium, Institut de Recherche Expérimentale et Clinique, Imaging Platform, [4] UCLouvain, Belgium, Institut de Recherche Expérimentale et Clinique, Pôle Endocrinologie, Diabétologie et Nutrition, [5] Cliniques universitaires Saint-Luc, UCLouvain, Brussels, Belgium, Gastroenterology Unit, [6] KUL, Belgium, Department of Imaging & Pathology.

Introduction : Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, represents a spectrum of disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Unlike simple steatosis, hepatocellular lesions and inflammation present in NASH promote fibrosis and the evolution to end-stage liver disease. A substantial body of literature supports that a low muscle mass, low strength or a higher muscle fatty infiltration are associated with NAFLD/NASH presence and severity.
Aim : To investigate metabolic parameters and muscle changes in a widely used NASH rodent model.
Methods : For 8 weeks, we followed C57BL/6J fed a high-fat diet with choline deficiency (CDAA-HFD, n=12) or supplemented in choline (CSAA-HFD, n=11). Dorsal muscle area and density (i.e surrogates for muscle mass and myosteatosis) were assessed non-invasively with micro-computed tomography (micro-CT) at weeks 0, 4, 6 and 8 (W0,4, 6 ,8). Muscle strength was evaluated every two weeks with a grip test. Quadriceps was harvested and weighted and liver examined by histology at W4 and W8. Results are expressed as mean ± SEM.
Results : At W8, CSAA-HFD developed glucose intolerance without steatosis. By contrast, CDAA-HFD had normal fasting glycemia (90.5±7mg/dl), normal glucose tolerance and severe steatohepatitis (panlobular steatosis and severe inflammation) with pericellular fibrosis at W4. However, due to the absence of ballooning, criteria for NASH diagnosis are not met. CDAA-HFD had a significant lower weight than CSAA-HFD from W4 on (21.41±1.07g vs 24.85±1.09g, respectively, $p<0.0001$). While constant in CSAA-HFD, liver density was strikingly lower in CDAA-HFD from W4 on (-0.18±0.11 vs 0.91±0.04 in CSAA-HFD, $p<0.0001$). Dorsal muscle area and quadriceps weight were lower in CDAA-HFD (48±2.83mm², $p<0.0001$ and 175.55±10.13mg, $p=0.05$, respectively) than in CSAA-HFD (58.22±2.24mm² and 196.18±11.79mg, respectively) from W4 on. Muscle density was high in both groups (muscle/spleen density ratio>0.8), albeit significantly lower in CSAA-HFD at W8 than in CDAA-HFD mice with steatohepatitis (0.80±0.03 vs 0.89±0.04, respectively, $p<0.05$). Muscle strength did not change over the study period in both groups.
Conclusions : In CDAA-HFD, loss of muscle mass occurs together with severe steatohepatitis, but with no myosteatosis or metabolic syndrome. Further studies are needed to decipher the respective contribution of dietary choline deficiency, metabolic alterations and liver disease to changes in muscle compartment.

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DISCOVERY OF THE GUT MICROBIAL SIGNATURE DRIVING THE EFFICACY OF PREBIOTIC INTERVENTION ON LIVER STEATOSIS.
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Introduction : Dietary supplementation with inulin as a prebiotic has been shown to lessen obesity and related metabolic disorders in some individuals. Previous work highlighted the interest of prebiotic supplementation in the management of hepatic steatosis in preclinical models. However, the efficiency of such nutritional interventions in humans remains unclear.
Aim : Using a cohort of obese individuals treated with inulin versus placebo (FOOD4GUT cohort), and a model of mice transferred with the fecal microbiota from obese patients, we have addressed the following question : do the characteristics of the gut microbiota in obese individuals influence the effect of inulin on hepatic disorders?
Methods : The gut microbiota of four-week old mice was depleted using antibiotics. Four groups of mice were then colonized with stools from four human obese patients (hum-ob) selected for different gut microbiota composition, presence of hepatic steatosis and response to inulin in an interventional study. Conventional and hum-ob mice were then fed with a high-fat diet (HFD) during four additional weeks, supplemented or not with native inulin (Cosucra) (6-9 mice per group).
Results : We demonstrated a different response to inulin supplementation in the four groups of mice inoculated with different fecal material. Inulin significantly reduced the hepatic lipid content in mice inoculated with the faeces of two out of the four donors. The decrease in hepatic lipid accumulation in “responder” mice was associated with a reduced activation of nuclear expression of markers involved in lipids synthesis (SREBP1 and SREBP2 proteins).. Despite an increased cecal content – signing gut fermentation - observed in all humanized mice, the regulation of the gut microbiota by inulin also differed between donors. Interestingly, some bacterial genera were positively correlated with liver lipids and triglycerides (Barnesiella, Bilophila, Butyricimonas…) whereas some others ones are negatively associated with these parameters (such as Akkermansia or Clostridium XIVa). The different regulation of these bacteria by inulin may explain the observed difference on hepatic lipid metabolism in responder and non-responders in humans.
Conclusions : Our work using a model of gut microbiota transfer from patients into mice highlighted the differential response of humanized mice to dietary supplementation with inulin. We propose that the gut microbiota is an important component to take into account for personalized nutrition related to prebiotic dietary fibers in the future and the management of metabolic disorders including hepatic steatosis.

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CARBON NANOPARTICLE C60 FULLERENE AS A TREATMENT OF HEPATOCELLULAR CARCINOMA. H. Kuznietsova (1), N. Dziubenko (1), T. Herheliuk (2), O. Lynchak (1), Y. Prylutskyy (1), U. Ritter (3) / [1] Taras Shevchenko National University, Kyiv, Ukraine, Institute of Biology and Medicine, [2] Institute for Problems of Cryobiology and Cryomedicine, Kyiv, Ukraine, Department of Biotechnical Problems in Diagnostic, [3] Technische Universität Ilmenau, Ilmenau, Germany, Institute of Chemistry and Biotechnology.

Introduction : Hepatocellular carcinoma (HCC) accounts for 75–85% of primary liver cancers and occupies the 4th position in ranks of cancer death in the World in 2018. It is poorly diagnosed, has an extremely unfavorable prognosis and lack of effective medication therapy. HCC development is characterized by oxidative stress - the main cause and trigger of malignant degeneration, progression and metastasis. Carbon-based nanoparticle pristine C60 fullerene has the powerful antioxidant properties and demonstrates anti-inflammatory and antifibrotic activity under liver inflammation and fibrosis and antitumor activity under colon cancer.
Aim : To discover the impact of C60 fullerene on HCC development on rat model and the possible mechanisms of realization of that.
Methods : 64 male Wistar rats with initial body weight 120±10 g were used in experiment. HCC was initiated by single N-diethylnitrosamine (DEN, 200 mg/kg) intraperitoneal injection. After two weeks tumor promotion was achieved by subcutaneous injection of CCl4 (0.1 ml/100g) twice/week continuously for 20 weeks. In 15 weeks after HCC initiation cell malignant degeneration and well-developed cirrhosis were confirmed and applications of C60 and reference 5-fluorouracil (5FU) were started. Pristine C60 fullerene aqueous colloid solution (C60FAS; 0.15 mg/ml, size of aggregates 1.2-100 nm) was administered daily intraperitoneally at dose of 0.25 mg/kg. 5FU (15 mg/kg) was administered weekly intraperitoneally. At the 22nd week half of the animals were sacrificed, liver injury was evaluated visually (according to 13-point scale), by histological (HE staining) and biochemical (liver and plasma blood markers) methods. Another half was left for survival estimation (up to 53 weeks). Metastasis rates were assessed in autopsies at the 22nd and 53rd weeks. To disclose the mechanisms of C60 action we additionally used HepG2 cells (human HCC). C60 effects on cell proliferation (MTT-test), pan-cytokeratin, vimentin and p53 expression (immunohistochemistry), redox and metabolic state (biochemical markers) were assessed.