Supplementary Materials and Methods

Cell culture and treatments

The human well-differentiated hepatoblastoma cell line HepG2 and the human hepatocarcinoma cell line Hep3B (from ATCC) were cultured as described.[1] The immortalized human biliary epithelial cell line H69 (kindly provided by Dr. Gregory Gores, Mayo Clinic, Rochester, MN, USA) was grown as reported.[2] Where indicated cells were treated with palmitic acid (PA) (Sigma, St. Louis, MO, USA) dissolved in isopropanol and added to serum-free culture media containing 1% fatty acid free BSA to the desired final concentration as reported,[3] controls received same volume of isopropanol (always <1% in the medium). For DNMT1 knockdown HepG2 cells were transfected with DNMT1 specific siRNAs (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or with control siRNAs (siGL) for 48 h as previously described.[1] 5-aza-deoxycytidine (5AZAdC) was from Sigma. For the study of PPARγ2 gene expression regulation, where indicated HepG2 cells were treated with a lipogenic cocktail (DMI): 0.5 mM 3-isobutyl-1-methyl-xanthine IBMX, 10 µg/ml insulin and 1 µM dexamethasone in serum free medium, with or without FGF19 (50 ng/ml), for 48 h as described. [4,5] Subsequently cells were further incubated in the presence of insulin (10µg/ml) with or without FGF19 (50 ng/ml) for another 60 h, and then lysed for mRNA extraction and analyses. For the analysis of p70S6 kinase (p70S6K) phosphorylation, Hep3B cells were serum-starved for 6h in DMEM medium supplemented with 0.2% bovine serum albumin. Then, where indicated cells were pretreated with the FGFR4 inhibitor BLU9931 (Cayman Chemical, MI, USA) for 1h before being stimulated with ApoA-I, FGF19 or Fibapo for 30 min.

Apoptosis Assay
Nucleosomal fragmentation characteristic of apoptosis was estimated by the determination of soluble histone-DNA complexes using the Cell Death Detection ELISA Assay (Roche Applied Sciences, Germany) as previously described.[6]

**Western blot analyses**

Cells and liver tissues were lysed in RIPA buffer and homogenates were subjected to western blot analysis as reported.[1,7] Antibodies used were: anti-GRP78 (Bip), anti-CHOP, anti-cleaved caspase-3 (Asp 175), anti-p-p70S6K (Thr 389), anti-p70S6K, anti-p-S6 (Ser 235/236), anti-S6, anti-β-ACTIN and anti-α-Tubulin from Cell Signaling Technology (Beverly, MA, USA); anti-PPARγ2 and anti-β-actin from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-FGF19 from R&D Systems (Minneapolis, MN, USA); anti-CD36 from GeneTex (Irvine, CA, USA).

**Sandwich ELISA for the determination of FGF19 and Fibapo protein levels**

Serum samples, tissue homogenates (10 mg in 500 µl of RIPA buffer) and cell culture conditioned media were incubated for 24h at 4 °C with anti-FGF19 antibody (8µg/ml) as capture antibody and for 4h RT with biotinylated anti-FGF19 antibody (100 ng/ml) (Refs. AF969 and BAF969, both from R&D Systems, Minneapolis, MN, USA). A standard curve using recombinant human FGF19 was used to calculate protein concentrations after subtraction of background signal calculated from non-FGF19/Fibapo treated samples (in serum and tissue samples). For the calculation of FGF19 and Fibapo serum t1/2, protein serum concentrations over time were fitted to the equation for one-compartment model after an i.v. bolus administration and the estimated elimination constants were compared with the Extra sum-of-squares F test. GraphPad Prism
software (GraphPad Software Inc., San Diego, USA) version 7.00 was employed for statistical analysis.

**Cloning of FGF19 and Fibapo and construction of AAVs**

FGF19 and Fibapo cDNAs, synthetized by GenScript (Piscataway, NJ, USA), were subcloned into the pcDNA3.1 mammalian expression vector for hydrodynamic injection experiments. FGF19, ApoA-I and Fibapo cDNAs were also cloned in adenoassociated viral vectors (AAV8) flanked by AAV2 WT inverted terminal repeats, and under the regulation of a chimeric liver-specific promoter composed of the human α1-antitrypsin promoter (AAT) with regulatory sequences from the albumin enhancer (Eal) as described before.[1] All rAAV8 viruses were produced by polyethyleneimine-mediated (PEI-mediated) cotransfection with pDP8.ape plasmid (PlasmidFactory GmbH & Co.) in HEK-293 cells.[8] Cells were harvested 72 h after transfections and virus was released from the cells by three rounds of freeze-thawing. Crude lysate from all batches was then treated with RNase and DNase for 1 h at 37°C and kept at -80º C until purification, which was performed by iodixanol gradients as described.[9] The purified batches were concentrated and diafiltered by cross-flow filtration with a molecular mass cutoff of 400 kDa. Batches were then concentrated further by passage through Centricon tubes (YM-100; Millipore) to a final concentration of $1 \times 10^{12}$ vg/ml, as determined by qPCR. Finally, viral batches were filtered (pore size 0.22 mm) and stored at -80 ºC. Mice were intravenously infected with $1 \times 10^9$ vg in the case of Fgf15−/− mice (≈25 g body weight) or $5 \times 10^9$ vg for db/db mice (≈45 g body weight).

**Production of recombinant FGF19 and Fibapo proteins**

FGF19, Fibapo and ApoA-I were synthesized upon request by GenScript (Piscataway, NJ, USA). For Fibapo synthesis FGF19 sequence was fused to the N-terminus of human apolipoprotein A-I
with a short linker (GAP). To facilitate purification of the proteins a His-tag was added to the N-terminus end of both proteins. The amino acid sequences of FGF19 and Fibapo are provided in supplementary figure S5. The recombinant proteins were expressed in E. coli and purified from inclusion bodies by Ni²⁺-affinity chromatography by the manufacturer. Endotoxin levels were below 1EU/µg.

**Isolation of HDL**

HDL isolation from serum was performed by differential ultracentrifugation in sodium bromide gradient as described.[10]

**RNA isolation and qPCR**

Total RNA from liver and ileal tissues, and cell lines, was extracted using the automated Maxwell system from Promega (Madison, WI, USA). Reverse transcription was performed as described.[1] Real-time PCRs were performed with iQ SYBR Green supermix (BioRad) in a CFX96 system from BioRad as previously described.[1] Primers are described in Supplementary Table 1.

**Hepatic triglyceride content determination**

Intrahepatic TG concentration was measured by saponification in ethanolic KOH as described [11,12] by determining triolein equivalents using a commercially available kit (BQ029A-CR, BQ Kits, San Diego, CA, USA).

**Total bile acid determination**
Liver tissues were homogenated in water (5 mg/500 µl), sonicated and centrifuged at 10,000g for 10 min. BAs concentrations were determined in supernatants by an enzymatic/colorimetric method using a commercially available kit (Randox Laboratories, Crumlin, UK).

**Histological determinations**

Liver tissue samples were formalin-fixed and paraffin-embedded for H&E staining and the degree of tissue necrosis was evaluated and scored as previously reported.[7] For lipids staining in frozen tissue sections Oil Red O (Sigma) was used as previously published.[13]

**Ki67 immunohistochemistry**

Positive Ki67 hepatocyte number was determined by immunohistochemistry as previously described [7] in 5 liver fields per mice (5x amplification) using ImageJ software (NIH, Bethesda, Maryland, USA).

**Serum biochemistry and BA determinations**

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and BA were measured as previously reported.[7,14]

**Patients description and analyses**

The control and obese patients analyzed in this study have been described previously.[15] Briefly, liver tissue samples from 69 obese subjects (16 males and 53 females) were obtained from patients attending the Departments of Endocrinology & Nutrition and Surgery at the Clínica Universidad de Navarra, Pamplona, Spain. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis and co-morbidity evaluation by
a multidisciplinary consultation team. BMI was calculated as weight in kilograms divided by the
square of height in meters and body fat (BF) was estimated by air-displacement-plethysmography
(Bod-Pod®, Life Measurements, Concord, CA).[16] The waist-to-hip ratio (WHR) was measured
as the quotient between the circumference of the waist (at the midway level between the margin
of the lowest rib and the iliac crest) and the hip (at the widest trochanters). Obese patients were
further subclassified according to the established diagnostic thresholds for diabetes
[normoglycemia (NG): fasting plasma glucose (FPG) concentration < 100 mg/dL and PG < 140
mg/dL 2-h after an oral glucose tolerance test (OGTT) and T2D: FPG > 126 mg/dL or PG ≥ 200
mg/dL 2-h after OGTT].[17] T2D subjects were not on insulin therapy or on medication likely to
influence endogenous insulin levels. The intraoperative liver biopsy was obtained from obese
patients during bariatric surgery for morbid obesity treatment to establish a histological diagnosis
of the hepatic state. The diagnosis of non-alcoholic fatty liver disease was established following a
histopathologic evaluation.[18] Liver tissue samples were also obtained from lean healthy
controls (BMI ≤ 25 kg m⁻²) undergoing liver biopsies (when hepatic tumor was suspected and
results were negative, or during hiatal hernia repair; a total of 18 patients). Tissue samples were
immediately frozen in liquid nitrogen and stored at -80 °C for subsequent analyses. Serum was
obtained from blood drawn from patients after a 12 h overnight fast. The study was approved by
the University of Navarra Ethical Committee and the written informed consent of participants was
obtained. The biochemical and hormonal characteristics of the obese subjects included in the
study are shown in Supplementary Table 2.

Serum FGF19 and hepatic PPARγ2 mRNA levels were determined as reported [15] using the
primers indicated in Supplementary Table 1. Since no significant differences were found in the
circulating FGF19 levels, nor in liver PPARγ2 mRNA levels, between the two groups of obese
patients (normoglycemic and T2D+impaired glucose tolerance), these data were combined in our
analysis.
Statistical analysis

Data are means ± SEM. Data were compared using the Student t test. A p value of <0.05 was considered significant. For the analysis of mouse survival in the 85% PH study p-value was calculated using the Mantel-Cox (log-rank) test. Data analyses were performed using GraphPad Prism software (GraphPad Software Inc., San Diego, USA) version 7.0 was employed for statistical analyses.

References


14 Garcia-Irigoyen O, Carotti S, Latasa MU, *et al.* Matrix metalloproteinase-10 expression is induced during hepatic injury and plays a fundamental role in liver tissue repair. *Liver Int* Published Online First: 4 October 2013. doi:10.1111/liv.12337


