**Detailed Methods**

**Human Studies**

*ABOS cohort:* Peri-operative liver biopsies from patients included in the biological atlas of severe obesity (*Atlas Biologique de l’Obésité Sévère, ABOS)* cohort were used to obtain hepatic transcriptomics data as described in Margerie D et al., BMC Genomics 2019. The ABOS cohort includes patients referred to the Lille University Hospital bariatric surgery unit for evaluation. All patients fulfilling the subsequent criteria for bariatric surgery were prospectively included in the cohort prior to surgery. No specific diet was followed during the days before surgery. Patients were to be 18 years or older at time of evaluation and meeting the criteria for bariatric surgery according to French national guidelines: morbid obesity or severe obesity with at least one comorbidity factor (i.e. arterial hypertension or diabetes mellitus) for at least 5 years and resistant to medical treatment; absence of medical or psychological contra-indications for bariatric surgery; social security insurance coverage; no current excessive drinking (average daily consumption of alcohol of 20 g/d for women and 30 g/d for men), and no past excessive drinking for a period longer than 2 years at any time in the last 20 years; absence of long-term consumption of hepatotoxic drugs; negative screening for chronic liver disease (viral hepatitis, autoimmune hepatitis). The Lille University Hospital ethics committee approved the cohort (NCT01129297). Written informed consent was obtained from all patients. Of the 909 patients profiled in Margerie, et al., those without complete histological characterization and taking statins were excluded for correlation analysis in Figure 1 (N = 551). Clinical characteristics of this selected population are provided in **Supplemental Table 1.**

*UZA cohort:* All patients were consecutively recruited at the Liver Clinic and Obesity Clinic of the Antwerp University Hospital and underwent hepatologic and metabolic work-ups. Blood analysis included blood cell count and white blood cell formula. Exclusion criteria were alcohol consumption >2 U/day for women and >3 U/day for men, liver diseases other than NAFLD, age <18 years, liver cirrhosis. For the baseline gene expression analysis in the liver, patients (n=155, including no liver disease n=27, simple steatosis n=22, NASH n=106) were selected from a cohort recruited since 200616. The study protocol is part of the Hepadip protocol (Belgian registration number B30020071389) and was approved by the Ethical Committee of the Antwerp University Hospital (file 6/25/125). Written informed consent was obtained from all patients. **Supplemental Table 2.**

**RNA/Quantitative PCR analysis**

Total RNA was extracted with TRIzol (Invitrogen) according to manufacturer’s instructions. Equal quantities of RNA were DNAse treated (DNAse I, ThermoFisher) and retrotranscribed (High-Capacity cDNA Reverse Transcription kit Applied Biosystems) according to manufacturer’s protocols. Quantitative RT-PCR analysis was performed using Stratagene Brilliant II SYBR Green qPCR Master Mix with a Stratagene Mx3005P Real-Time PCR detection system (Agilent technologies). The RT-PCR primers are listed in **Table 1**.

**Table 1 : RT-PCR primers for the indicated genes**

|  |  |  |
| --- | --- | --- |
| Gene | Forward primer | Reverse primer |
| Acat2 | GCTGCGTGCTGGTCTTTGAG | GCTGATGCCCTTTCCTCCTCT |
| ApoB | GTGGCTTCTGTCACTGCTAAAG | GTCTGATTTCCCCTCAATGG |
| Apobr | AACTCCAGAGCTAAGTGCCACA | GGATGAGCAAAGCCAAACCCAC |
| Murine ApoF | TGGCCGGACTGTATGGGTG | CTCTCCGTGTGAAGTGGCAT |
| Human ApoF | ACCCCTTGTCCTGCCAATTT | CCAGGGCATTCCTTAGAGCC |
| Ap2a1 | CTCGCCGTGTTCATCTCCGA | AGGCCTTGTCCCCTTTGAAC |
| Ap2a2 | CGGCAGAATTTAGGCCGAATG | CAGTTGGGTCCACGGGCTTA |
| Ap2m1 | TACGAGCTGCTGGATGAGATTCTG | GGTGATCTGGGACTGTTCTTCC |
| Cyclophilin A | GCATACGGGTCCTGGCATCTTGTCC | ATGGTGATCTTCTTGCTGGTCTTGC |
| Dgat1 | GTGGTGCATCAGACACTTCT | GCTCACTAGGTACTCATGGAA G |
| Ext1 | CCAGTACTGTGCGCAGATCAT | CATAGGGCAGAAACCGGCTG |
| Idol | ATGCTGTGCTATGTGACGAGG | TCGATGATCCCTAGACGCCTG |
| Insig1 | TCTCCTCCGCCTGGTGGGTG | AGCGCATAACGCTGGCCC AT |
| Ldlr | CAGGCAGCAGGAACGAGTTC | GGAGTCAGGAATGCATCGGC |
| Lrp1 | GCTGCTGCTGCTGCCGCTGC | CCGTTGCAGAGACGAGACATG |
| Lsr | TACTGCTCCGTGGTCTCAGC | CGACCACAAAGAGCCAATCTTCC |
| Mttp | TATGACCGTTTCTCCAAGAG | TCAATCACCACCTGACTACC |
| Npc2 | CGGAGCCCCTGCACTTC | GGGCTCACATTCACCTCCTTTA |
| Pcsk9 | AGGTGGAGGTGTATCTCTTAGATACCA | CGCTGTTGAAGTCGGTGATG |
| Scap | AGCCACACTCAAAGACTTGCTG | AAGACCAGGGTGATGGTGTTAGG |
| Srebp2 | GTGGAGCAGTCTCAACGTCA | CCGGATGCATGGTAGGTCTC |
| 36B4 | CATGCTCAACATCTCCCCCTTCTCC | GGGAAGGTGTAATCCGTCTCCACAG |

**Microarray analysis**

Total RNA was extracted from livers of mice overexpressing GFP or hApoF (6 mice per group) as described above. RNA quantity and quality were assessed using the Agilent 2100 Bioanalyzer (Agilent Biotechnologies). Transcriptome analysis was performed using Affymetrix GeneChip MoGene 2.0 ST arrays as described previously(1). Quality controls were verified using the Affymetrix expression console.

**Microarray data processing**

Microarray data were normalized by robust multi-average method using the package oligo/Bioconductor. For preprocessing, probesets were to symbols selecting the highest expressed probe when multiple probes mapped to a given gene. Differential gene expression analysis was performed on the 12000 highest expressed genes using limma (2). Gene set enrichment analysis was performed in EnrichR (3) using Bioplanet annotations.

**Western blot analysis**

Western blot analysis was performed on 2µL of plasma with antibodies against human ApoF(4) or on 30µg protein for Insig1 (sc-390504) and GAPDH (CST, 5174). Primary antibodies were detected using IRDye secondary antibodies (LI‐COR Biosciences, Lincoln, USA). Band intensity was defined as sum of the pixel intensity in the band after background subtraction using Image Studio Lite v5.2.

**Cell culture studies**

Primary human hepatocytes (Sigma) were cultured in William’s E supplemented with 0,1% BSA, 100 nM dexamethasone, 1% glutamine and antibiotics (gentamycin). In overexpression experiments, hepatocytes were infected with Ad-GFP or Ad-hApoF (described below) and the media was changed after 16h and cells were cultured for an additional 24 hours prior to uptake experiments. In knockdown experiments, hepatocytes were transfected with ApoF siRNA (ON-TARGETplus Human APOF siRNA L-011576-00-0005) or non-targeting siRNAs using Dharmafect 1 transfection reagent (Horizon discovery) according to manufacturer’s instructions.

**Lipid and lipoprotein analyses**

Hepatic lipids were extracted by the Folch method (5). Hepatic and plasma TG, total cholesterol (BioMerieux, France) and plasma FFA (Diasys), were measured using colorimetric assays according to manufacturer’s protocol. Lipoprotein fractions were separated by fast-performance liquid chromatography (FPLC) on a Waters 2695 Acquity pump system with a Superose 6 10/300 GL gel filtration column (GE Healthcare). Total TG and cholesterol measurements were performed using an in-line column reaction module with measurement at 490 nm by a spectrophotometric detector.

**Dil-labeled VLDL uptake assay**

Primary human hepatocytes (Sigma) were incubated with 10 µg Dil-labeled VLDL (CliniSciences) for 16 hours. Cells were washed with PBS, fixed with 4% paraformaldehyde and labeled with 1 µg/mL Hoechst (ThermoFischer Scientific). Fluorescence was measured using a TECAN plate reader. Dil-VLDL uptake was calculated as the ratio of Dil/Hoechst fluorescence.

**Lipoprotein Lipase and Hepatic Lipase activity assay**

After overnight fast, plasma was collected 10 min after heparin injection (CHOAY, 0.1 U/g bodyweight in saline). Post-heparin lipase activity measurements were performed by CEREMET (University of Barcelona, Spain) as described (6,7). Total lipase activity was measured with and without addition of 1M NaCl. LPL-specific activity was estimated as the difference between low salt lipase activity (total) and high salt activity (corresponding to hepatic lipase).

**MTTP activity assay**

Hepatic MTTP activity was measured using a commercial kit (Roar Biomedical). Liver were ground in homogenisation buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1mM EDTA and protease inhibitor cocktail from Roche) and sonicated on ice. Liver lysates (40 µg protein) were combined with 10 μl of donor and acceptor particles in 150 μl of MTP assay buffer and incubated at 37°C. The increase in fluorescence was measured with excitation and emission of 465/535 nm.

**Apolipoprotein quantification**

Fasting plasma ApoA-I ApoA-II, total ApoB (B100 and B48), ApoC-III and ApoE were quantified by liquid chromatography-tandem mass spectrometry analysis of specific peptides adapted for murine samples (**Supplemental Table 9**) as described previously (8).

**Post-prandial lipid response**

Tail blood samples were taken from 5 hour-fasted mice before and 1, 2, 3 and 4 hours after oral gavage of 400 µL of olive oil and TG levels measured. Data are presented as the delta from T0 for both genotypes.

**Adenovirus Studies**

Ad5-adenovirus vectors (Ad-GFP, Ad-hApoF, Ad-shLacZ and Ad-shApoF) were produced at GeneCust (Luxembourg), amplified and purified by two-step CsCl2 gradient ultracentrifugation as described previously (9). For overexpression, GFP or human APOF cDNA expression was driven by the CMV promoter. The Ad-hApoF virus also includes a cassette for expression of GFP under the EIF1A promoter to mark infected cells. Short-hairpin constructs (shApoF and shLacZ) are expressed under the U6 promoter for RNA pol III-driven expression. Unless otherwise indicated, mice received intravenous injections of 1 x 1010 purified viral particles in 100µL sterile saline. Metabolic analysis was performed on the 4th day following injection. In all experiments, fasting plasma was obtained by retro-orbital bleeding after inhaled isoflurane anesthesia or by tail bleed without anesthesia. All experiments were approved by the Ethical Committee for Animal Experimentation for Hauts de France (APAFIS#5746-2016040109244171v2).

**Hepatic VLDL-TG secretion** **analysis**

Mice were fasted for 5 hours before the assay and tail blood was collected for baseline TG levels. Tail blood was collected at 60, 120 and 180 min after intraperitoneal injection of 250 µL 10% Poloxamer 407 (P407, Sigma 16758-250g) in saline for measurement of plasma TG. Plasma samples at 180 min were analyzed by ELISA for ApoB (ab230932, Abcam). Fractional catabolic rates (FCR) were calculated as TG secretion rate (TGSR) divided by the plasma TG pool size(10).

TG(time): plasma TG concentrations (mg/mL) at given time after P407 injection

**VLDL-like particle uptake assay**

VLDL1-like TG-rich emulsion particles (80 nm) labelled with glycerol tri[3H]oleate (TO) and [14C]cholesteryl oleate (CO) were prepared as described(11).

Briefly, fasted mice (5hrs) were injected intravenously with 200 µL of radiolabeled emulsion. Blood was collected prior to and 2, 5, 10, and 15 min after injection to measure plasma decay of [3H]TO and [14C]CO. The mice were killed by cervical dislocation, perfused with ice-cold PBSand several tissues were harvested. Organ uptake of radioactivity was determined by dissolving organs in Tissue Solubilizer (Amersham Biosciences, Roosendaal, the Netherlands) at 55°C overnight. Radioactive counts were normalized to tissue weight in grams.

**Lipid Droplet Isolation**

Liver samples were dounce homogenized in buffer containing 200 mM Tris-HCl 1 mM EDTA pH 7.5. Samples were first centrifuged at 400g for 15min at 4°C to pellet debris and unbroken cells. Supernatants were then centrifugated at 3000g for 15min at 4°C. This nuclear pellet was discarded and supernatants were again centrifuged at 20 000g for 15min at 4°C to pellet mitochondria. Finally, this supernatant was centrifugated at 100 000g for 15min at 4°C to separate lipid droplets (floating) from the endoplasmic reticulum (pellet). Lipid content of the lipid droplets and endoplasmic reticulum was measured using enzymatic assays and normalized to protein content of each fraction.

**SUPPLEMENTAL MATERIAL**

**Supplemental Table 1. Clinical Characteristics of ABOS cohort used for correlations with hepatic APOF expression**

|  |  |
| --- | --- |
| **Category** | **Median [IQR] or N** |
| N | 551 |
| Sex (% men) | 23% |
| Age (yrs) | 38 [31, 47] |
| BMI (kg/m2) | 45.9 [41.9, 51.2] |
| Fasting Plasma Glucose (mM) | 5.45 [5.02, 6.33] |
| Fasting Plasma Insulin (mU/L) | 13.9 [9.2, 19.7] |
| HOMA-IR | 3.5 [2.22, 5.19] |
| Plasma TG (mM) | 1.34 [1.02, 1.81] |
| Plasma Cholesterol (mM) | 5.01 [4.45, 5.56] |
| LDL-C (Friedwald, mM) | 3.2 [2.66, 3.69] |
| HDL-C (mM) | 1.14 [0.96, 1.29] |
| ASAT (U/L) | 23 [19, 30] |
| ALAT (U/L) | 27 [19, 39] |
| Steatosis Grade, N (0/1/2/3) | 108 / 238 / 122 / 83 |
| Lobular Inflammation, N (0/1/2/3) | 442 / 86 / 23 / 0 |
| Ballooning, N (0/1/2) | 491 / 44 / 16 |
| Fibrosis Stage, N (0/1/2/3/4) | 390 / 85 / 22 / 27 / 0 |

**Supplemental Table 2: Top 20 transcripts positively (left) and negatively (right) correlated with hepatic steatosis.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **ρ** | **p-value** |  | **Gene** | **ρ** | **p-value** |
| *FAT1* | 0.499 | 4.60E-36 |  | *DNAJC12* | -0.471 | 1.06E-31 |
| *PRAMEF10* | 0.450 | 8.12E-29 |  | *NUDT13* | -0.458 | 5.80E-30 |
| *KRT8* | 0.443 | 7.23E-28 |  | *VIL1* | -0.429 | 4.74E-26 |
| *SULF2* | 0.435 | 7.31E-27 |  | ***APOF*** | **-0.426** | **1.05E-25** |
| *LPL* | 0.424 | 1.90E-25 |  | *SLC16A10* | -0.404 | 5.30E-23 |
| *TMEM19* | 0.412 | 5.20E-24 |  | *P4HA1* | -0.394 | 6.06E-22 |
| *KRT18* | 0.410 | 9.37E-24 |  | *CENPV* | -0.389 | 2.21E-21 |
| *MACROH2A2* | 0.407 | 2.02E-23 |  | *PDIA5* | -0.389 | 2.42E-21 |
| *TMEM154* | 0.402 | 8.07E-23 |  | *TNIK* | -0.385 | 7.03E-21 |
| *ZMAT3* | 0.401 | 1.09E-22 |  | *PELI2* | -0.377 | 4.74E-20 |
| *ME1* | 0.400 | 1.28E-22 |  | *RGS5* | -0.374 | 9.47E-20 |
| *FCAMR* | 0.400 | 1.42E-22 |  | *UHRF2* | -0.374 | 9.66E-20 |
| *ANKRD18A* | 0.399 | 1.74E-22 |  | *RBMS1* | -0.372 | 1.59E-19 |
| *GNA12* | 0.398 | 2.56E-22 |  | *MIR4785* | -0.372 | 1.59E-19 |
| *FABP4* | 0.397 | 3.33E-22 |  | *CD82* | -0.372 | 1.69E-19 |
| *SATB2* | 0.393 | 7.93E-22 |  | *TCF7L1* | -0.368 | 4.07E-19 |
| *SERPINB8* | 0.393 | 9.71E-22 |  | *PACSIN3* | -0.367 | 5.38E-19 |
| *MIR622* | 0.392 | 1.24E-21 |  | *PTPRD* | -0.366 | 7.39E-19 |
| *CDKN1A* | 0.386 | 4.93E-21 |  | *ECE1* | -0.364 | 9.66E-19 |
| *TP53I3* | 0.385 | 6.49E-21 |  | *RAB30-DT* | -0.358 | 4.31E-18 |

**Supplemental Table 3 : Correlation of hepatic *APOF* expression with clinical parameters of the ABOS cohort.**

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Spearman ** | **p-value** |
| Age | 0.015 | 0.7292 |
| BMI | -0.067 | 0.1192 |
| Weight | -0.126 | **0.0032** |
| TC | -0.051 | 0.2328 |
| HDL-C | 0.190 | **<0.0001** |
| LDL-C | -0.002 | 0.9544 |
| TG | -0.308 | **<0.0001** |
| Fasting plasma glucose | -0.161 | **0.0002** |
| HOMA2-IR | -0.051 | 0.2583 |
| HbA1c | -0.234 | **<0.0001** |
| Total bilirubin | 0.038 | 0.3783 |
| ASAT | -0.308 | **<0.0001** |
| ALAT | -0.385 | **<0.0001** |
| GGT | -0.323 | **<0.0001** |
| alpha2-macroglobulin | -0.094 | **0.0276** |
| Haptoglobin | 0.101 | **0.0185** |
| ApoA-I | 0.049 | 0.2556 |
| Ultrasensitive CRP | 0.094 | **0.0455** |

**Supplemental Table 4: Top 20 transcripts positively (left) and negatively (right) correlated with plasma TG.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **ρ** | **p-value** |  | **Gene** | **ρ** | **p-value** |
| *FAT1* | 0.379 | 3.79E-20 |  | ***APOF*** | **-0.315** | **4.07E-14** |
| *ZMAT3* | 0.340 | 2.37E-16 |  | *DNAJC12* | -0.314 | 5.41E-14 |
| *SATB2* | 0.324 | 7.21E-15 |  | *P4HA1* | -0.298 | 9.45E-13 |
| *WDPCP* | 0.310 | 1.13E-13 |  | *SOCS2* | -0.297 | 1.18E-12 |
| *DEFB1* | 0.303 | 3.70E-13 |  | *SERPINA1* | -0.290 | 4.25E-12 |
| *MAMDC4* | 0.295 | 1.59E-12 |  | *TENM3* | -0.276 | 4.56E-11 |
| *TMEM19* | 0.292 | 2.88E-12 |  | *C9* | -0.276 | 4.98E-11 |
| *SQLE* | 0.290 | 4.46E-12 |  | *DIPK2A* | -0.273 | 7.35E-11 |
| *ANKRD18A* | 0.284 | 1.21E-11 |  | *PC* | -0.272 | 9.75E-11 |
| *ME1* | 0.279 | 2.78E-11 |  | *CD82* | -0.264 | 3.23E-10 |
| *ANKRD18B* | 0.278 | 3.29E-11 |  | *SERPINA3* | -0.262 | 4.56E-10 |
| *HMGCS1* | 0.276 | 4.89E-11 |  | *NUDT13* | -0.261 | 4.93E-10 |
| *PDE11A* | 0.272 | 9.21E-11 |  | *SOCS2-AS1* | -0.256 | 1.23E-09 |
| *PTPN11* | 0.271 | 1.10E-10 |  | *IGF1* | -0.255 | 1.31E-09 |
| *DDB2* | 0.269 | 1.38E-10 |  | *PTPRD* | -0.251 | 2.37E-09 |
| *ACLY* | 0.269 | 1.41E-10 |  | *C8A* | -0.246 | 5.56E-09 |
| *TARBP1* | 0.266 | 2.51E-10 |  | *MYOM1* | -0.245 | 6.05E-09 |
| *FASN* | 0.263 | 4.15E-10 |  | *TIMD4* | -0.243 | 7.74E-09 |
| *MEAF6* | 0.263 | 4.18E-10 |  | *PHF8* | -0.241 | 1.12E-08 |
| *NSDHL* | 0.260 | 6.48E-10 |  | *IGFALS* | -0.240 | 1.29E-08 |

**Supplemental Table 5. Clinical Characteristics of UZA cohort used for correlations with hepatic APOF expression**

|  |  |
| --- | --- |
| **Category** | **Median [IQR] or N** |
| N | 155 |
| Sex (% men) | 35% |
| Age (yrs) | 44 [34, 52] |
| BMI (kg/m2) | 39.5 [36.7, 42.6] |
| Fasting Plasma Glucose (mM) | 4.61 [4.39, 4.94] |
| Fasting Plasma Insulin (mU/L) | 16.5 [11.2, 22.5] |
| HOMA-IR | 3.52 [2.38, 5.05] |
| Plasma TG (mM) | 1.63 [1.24, 2.24] |
| Plasma Cholesterol (mM) | 5.25 [4.55, 5.76] |
| LDL-C (Friedwald, mM) | 3.08 [2.66, 3.75] |
| HDL-C (mM) | 1.14 [0.98, 1.4] |
| ASAT (U/L) | 20.8 [17.1, 28.4] |
| ALAT (U/L) | 32.0 [23.0, 48.1] |
| Steatosis Grade, N (0/1/2/3) | 27 / 54 / 37 / 37 |
| Lobular Inflammation, N (0/1/2/3) | 34 / 80 / 31 / 10 |
| Ballooning, N (0/1/2) | 40 / 72 / 43 |
| Fibrosis Stage, N (0/1/2/3/4) | 92 / 36 / 15 / 11 / 1 |

**Supplemental Table 6. Coefficients and p-values for multiple linear regression of hepatic *APOF* expression and plasma TG adjusted for age, BMI, sex and glucose homeostasis in the ABOS cohort.**

|  |  |  |  |
| --- | --- | --- | --- |
| **R2: 0.124; P < 2.2 x 10-16** | |  |  |
|  | **** | **Std. Error** | **Pr(>|t|)** |
| Plasma TG | -0.0616 | 0.0124 | 8.66E-07 |
| Age | 0.0044 | 0.0018 | 0.0152 |
| BMI | 0.0015 | 0.0025 | 0.5499 |
| Sex | -0.1297 | 0.0429 | 0.0026 |
| NGT/IGT/T2D | -0.1554 | 0.0286 | 7.54E-08 |

**Supplemental Table 7. Coefficients and p-values for multiple linear regression of hepatic *APOF* expression and histological steatosis score adjusted for age, BMI, sex and glucose homeostasis in the ABOS cohort.**

|  |  |  |  |
| --- | --- | --- | --- |
| **R2: 0.200; P < 2.2 x 10-16** | |  |  |
|  | **** | **Std. Error** | **Pr(>|t|)** |
| Steatosis Score | -0.1940 | 0.0202 | < 2.00E-16 |
| Age | 0.0035 | 0.0017 | 0.0436 |
| BMI | 0.0035 | 0.0024 | 0.1414 |
| Sex | -0.1324 | 0.0404 | 0.0011 |
| NGT/IGT/T2D | -0.0926 | 0.0283 | 0.0011 |

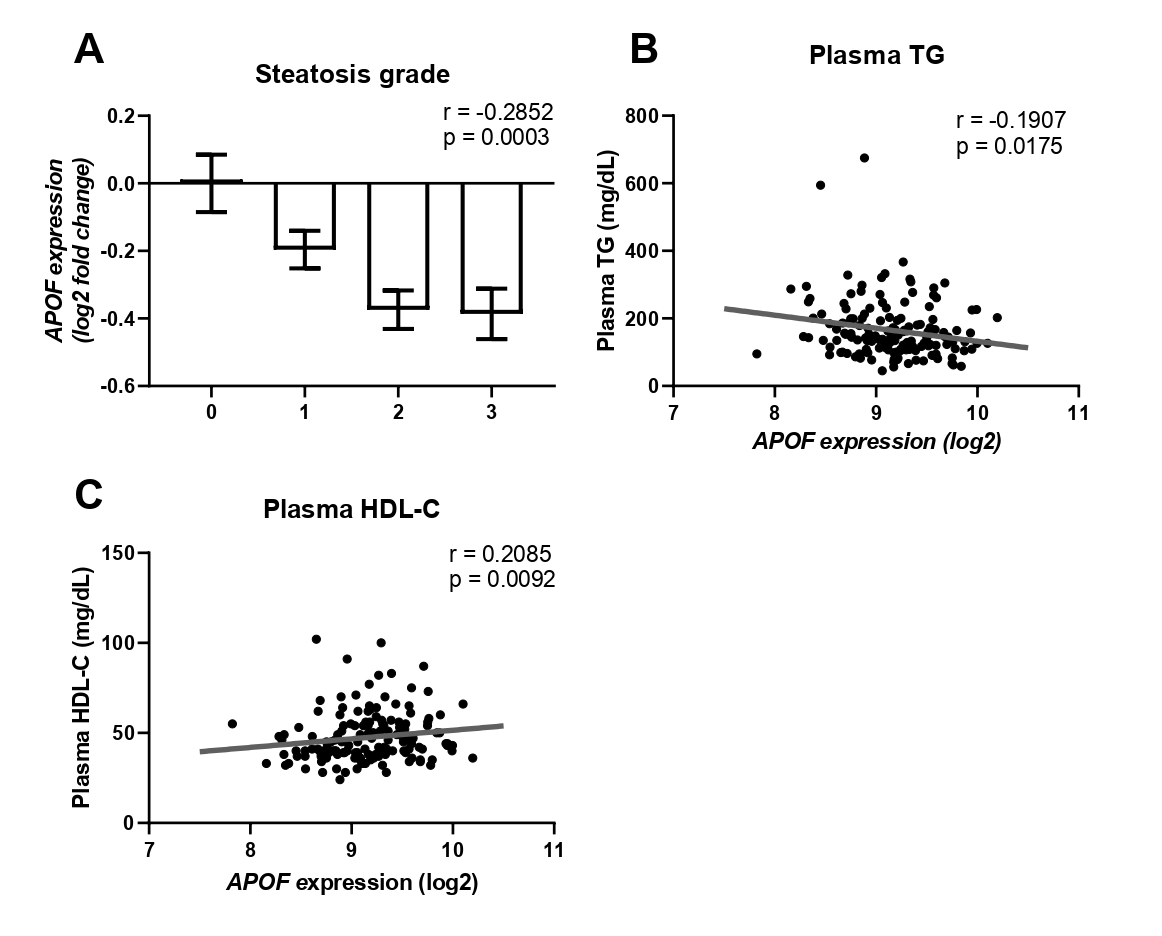
**Supplemental Table 8 : Gene set enrichment analysis of the 250 most upregulated genes by hepatic *APOF* overexpression.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Term** | **P-value** | **Adjusted P-value** | **Odds Ratio** | **Combined Score** | **Genes** |
| Cholesterol biosynthesis | 3.3E-10 | 2.0E-07 | 40.8 | 890.2 | *FDPS, SQLE, PMVK, MSMO1, HMGCR, HSD17B7, LSS, TM7SF2* |
| Metabolism | 1.8E-06 | 5.4E-04 | 2.4 | 31.8 | *PDXK, ACSS2, ALAS1, ACSM1, HAAO, PON1, MSMO1, GLYAT, HMGCR, HSD17B7, PIK3C2A, ACACB, TM7SF2, HSD11B1, DPP4, C1GALT1C1, PMVK, RDH16, PLCG1, PCK1, PRODH, GLUL, ATP6V0A1, HS3ST3B1, FDPS, CYB5A, PHYH, GFPT1, URAD, UPB1, ACADSB, MUT, LSS, SQLE, ETNK1, DPYD, PCCB, POLR3G, ALDOC, LAP3, LPIN2, SLC27A5, GNE* |
| Lipid and lipoprotein metabolism | 3.8E-06 | 7.6E-04 | 3.6 | 44.6 | *FDPS, STARD4, PHYH, STARD5, ALAS1, MSMO1, HMGCR, HSD17B7, PIK3C2A, ACACB, MUT, LSS, TM7SF2, HSD11B1, SQLE, ETNK1, PCCB, PMVK, LPIN2, SLC27A5* |
| Steroid biosynthesis | 1.6E-05 | 0.002 | 19.2 | 212.3 | *SQLE, MSMO1, HSD17B7, LSS, TM7SF2* |
| Angiogenesis | 1.8E-04 | 0.021 | 16.9 | 146.1 | *KDR, PLCG1, TEK, VEGFA* |
| Gamma-carboxylation. transport. and amino-terminal cleavage of proteins | 3.0E-04 | 0.028 | 30.0 | 243.6 | *F7, PROS1, PROZ* |
| Activation of chaperones by IRE1 alpha | 3.2E-04 | 0.028 | 9.4 | 75.2 | *TSPYL2, DNAJC3, GFPT1, DNAJB9, PDIA5* |
| Vitamin B12 metabolism | 4.7E-04 | 0.035 | 8.6 | 65.5 | *CRP, F7, INSR, SOD2, MUT* |
| Response to elevated platelet cytosolic calcium | 6.1E-04 | 0.040 | 6.3 | 46.5 | *APP, PROS1, HGF, HRG, KNG1, VEGFA* |
| Propanoate metabolism | 7.3E-04 | 0.043 | 11.1 | 79.8 | *ACSS2, PCCB, ACACB, MUT* |
| Terpenoid backbone biosynthesis | 7.9E-04 | 0.043 | 20.0 | 142.8 | *FDPS, PMVK, HMGCR* |

**Supplemental Table 9. Multiple reaction monitoring (MRM) parameters used for the detection of apolipoprotein peptide biomarkers by mass spectrometry.**

|  |  |  |  |
| --- | --- | --- | --- |
| Apolipoprotein | Peptide | MRM transition (*m/z*) | Cone/collision (V) |
| ApoA-I | VAPLGAELQESAR | 671.4 → 586.3 (y112+) | 40/23 |
| ApoA-I (IS) | VAPLGAELQESA-[13C6. 15N4]R | 676.4 → 591.3 (y112+) | 40/23 |
| ApoA-II | THEQLTPLVR | 597.7 → 955.8 (y8+) | 40/30 |
| ApoA-II (IS) | THEQLTPLV-[13C6. 15N4]R | 602.7 → 960.8 (y8+) | 40/30 |
| ApoB | INIDIPLPLGGK | 625.9 → 681.5 (y7+) | 30/24 |
| ApoB (IS) | INIDIPLPLGGK-[13C6. 15N2]K | 629.9 → 689.5 (y7+) | 30/24 |
| ApoC-III | GWMDNHFR | 532.1 → 819.8 (y6+) | 30/25 |
| ApoC-III (IS) | GWMDNHF-[13C6. 15N4]R | 537.1 → 829.8 (y6+) | 30/25 |
| ApoE | TANLGAGAAQPLR | 620.8 → 840.5 (y9+) | 30/25 |
| ApoE (IS) | TANLGAGAAQPL-[13C6. 15N4]R | 625.8 → 850.5 (y9+) | 30/25 |

*IS. internal standard.*

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**Supplemental Figure 1. Hepatic *APOF* expression is negatively correlated with steatosis and dyslipidemia in a cohort of obese patients.**

(**A**) Normalized hepatic *APOF* mRNA expression in relation with stage of steatosis in obese patients. Relationship of plasma TG (**B**) and HDL-C (**C**) with hepatic *APOF* expression in a cohort of obese patients (n=155). r, Spearman’s correlation coefficient. Data from (A) are shown as the log2 fold change compared to grade 0 ± SEM.



**Supplemental Figure 2. Hepatic APOF expression is negatively correlated with steatosis and plasma TG in women and in men in a cohort of obese patients.**

(A) Normalized hepatic APOF mRNA expression in women (left) and men (right) in relation with stage of steatosis in obese patients. Relationship of plasma TG (B), non-HDL-C (C) and HDL-C (D) with hepatic APOF expression in women (left) and men (right) in a cohort of obese patients (n=551; 128 males and 423 women). r, Spearman’s correlation coefficient. Data from (A) are shown as the log2 fold change compared to grade 0 ± SEM.



**Supplemental Figure 3. *APOF* overexpression doesn’t modify plasma and hepatic cholesterol levels.**

(**A**) Plasma TC and fractionated pooled plasma cholesterol by FPLC in 5-hour fasted Ad-GFP or Ad-hApoF mice (n=8-9 per group). (**B**) Plasma ApoA-I, ApoA-II in 5 hours fasted Ad-GFP or Ad-hApoF mice (n=8 per group). (**C**) Hepatic TC content in 5-hour fasted Ad-GFP or Ad-hApoF mice (n=8-9 per group). (**D**) Hepatic *Apob*, *Mttp* and *Dgat1* mRNA in 5-hour fasted Ad-GFP or Ad-hApoF mice (n=8-9 per group). (**E**) Hepatic MTTP activity in 5-hour fasted Ad-GFP or Ad-hApoF mice (n=8-9 per group). (**F**) Plasma FFA in 5-hour fasted Ad-GFP or Ad-hApoF mice (n=8-9 per group).

\*p<0.05, \*\*\*p<0.001 as compared to the control by t-test or Mann-Whitney test as appropriate. All data are shown as the means ± SEM.



**Supplemental Figure 4. Lowering ApoF expression doesn’t modify plasma and hepatic cholesterol levels.**

(**A**) Plasma TC and fractionated pooled plasma cholesterol by FPLC in 5 hours fasted Ad-shLacZ or Ad-shApoF mice (n=8-9 per group). (**B**) Hepatic TC content in 5 hours fasted Ad-shLacZ or Ad-shApoF mice (n=8-9 per group). (**D**) Hepatic *Apob*, *Mttp* and *Dgat1* mRNA in 5-hour fasted Ad-shLacZ or Ad-shApoF mice (n=8-9 per group). (**E**) Hepatic MTTP activity in 5-hour fasted Ad-shLacZ or Ad-shApoF mice (n=8-9 per group). (**F**) Plasma FFA in 5-hour fasted Ad-shLacZ or Ad-shApoF mice (n=8-9 per group).

All data are shown as the means ± SEM.



**Supplemental Figure 5. *APOF* overexpression doesn’t modify plasma ApoC-III and ApoE levels.**

Plasma ApoC-III (**A**) and correlation of plasma TG with plasma ApoC-III (**B**) in in 5 hours fasted Ad-GFP or Ad-hApoF mice (n=8 per group). Plasma ApoE (**C**) and ApoC-III/ApoE (**D**) ratio in 5 hours fasted Ad-GFP or Ad-hApoF mice (n=8 per group). r, Spearman’s correlation coefficient. All data are shown as the means ± SEM.



**Supplemental Figure 6. *APOF* overexpression mice leads to accumulation of LDL-C in ApoE2-KI mice.**

(**A**) Hepatic *APOF* mRNA in ApoE2 KI mice in Ad-GFP or Ah-hApoF mice (n=8 per group). Plasma ApoB (**B**) TG and TC (**C**) and in 5 hours fasted Ad-GFP or Ad-hApoF ApoE2 KI mice (n=8 per group). (**D**) Fractionated pooled plasma TG and cholesterol by FPLC in 5 hours fasted Ad-GFP or Ad-hApoF ApoE2 KI mice (n= 1 pool of 8 mice per group). (**E**) Hepatic TG and TC content in Ad-GFP or Ah-hApoF ApoE2 KI mice (n=8 per group). (**F**) Hepatic MTTP activity in Ad-GFP or Ah-hApoF ApoE2 KI mice (n=8 per group). \*p<0.05, \*\*\*p<0.001 as compared to the control by t-test or Mann-Whitney as appropriate. All data are shown as the means ± SEM.



**Supplemental Figure 7.** **Characterization of SREBP2 in ApoF overexpression mouse model.** (**A**) Hepatic *Srebp2, Scap* and *Insig1* mRNA in 5-hour fasted Ad-GFP or Ad-hApoF (n=7 per group) mice. (**B**) Western blot for cytoplasmic Insig1 in livers from Ad-GFP or Ad-hApoF (n=7 per group) mice. (**C**) Free cholesterol content in lipid droplet and endoplasmic reticulum in 5-hour fasted Ad-GFP or Ad-hApoF (n=7 per group) mice (**D**) Relative expression of genes related to SREBP2 pathway analyzed by microarray . \*\*\*p<0.001 as compared to the control by t-test or Mann-Whitney as appropriate. All data are shown as the means ± SEM.

\*p<0.05, \*\*\*p<0.001 as compared to the control by t-test or Mann-Whitney as appropriate. All data are shown as the means ± SEM.

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