Supplemental EXPERIMENTAL PROCEDURES and DATA

Patients

The experimental procedures were performed according to the guidelines of the charitable state-controlled foundation Human Tissue and Cell Research, with the informed patient's consent approved by the local Ethical Committee of the Charité University School of Medicine Berlin (EA2/135/08). All subjects gave written informed consent at least 24 hours prior to surgery. Forty-nine patients were enrolled between February 2009 and March 2010. Details regarding inclusion and exclusion criteria have been published {Docke, 2013 #1022}. This trial is registered at the German Clinical Trials Register: DRKS00005450.

Preparation and cultivation of primary human hepatocytes

Tissue samples from liver resections were obtained from patients undergoing partial hepatectomy. The experimental procedures were performed according to the guidelines of the charitable state-controlled foundation Human Tissue and Cell Research, with the informed patient's consent approved by the local Ethical Committee of the Charité University School of Medicine Berlin (EA2/007/13). The cell isolation process was performed using a two-step collagenase P perfusion technique and subsequent density gradient centrifugation for separation of the hepatocytes from non-parenchymal liver cells. Isolated hepatocytes were cultured in Williams' medium E containing 10% FCS, 1% (v/v) antibiotics (10U/µg penicillin, 10ug/µl streptomycin), 100nM dexamethasone, 0.5nM insulin for 4 h on collagen-coated culture plates (10^6 cells/35mm-plate). When co-culture experiments were performed, the density gradient centrifugation step was omitted and the liver cell suspension was cultured as described. Cells were allowed to attach to the substratum for 4 h, and then the medium was replaced with modified Williams' medium E without FCS. Experiments were carried out after approximately 36 h of culture in modified Williams' medium E, including a change of medium after 16 h.

Animals

Non-human primates

Studies were performed as described {Jimenez-Gomez, 2013 #1304}. Briefly, adult (7–13 years old) male rhesus monkeys (Macaca mulatta) were housed at the NIH Animal Center, Poolesville, MD, a center fully accredited by the American Association for Accreditation of Laboratory Animal Care. All procedures were approved by the Animal Care and Use Committee of the NIA Intramural Program. Details of animal care have been described in detail {Jimenez-Gomez, 2013 #1304}.

Mice

Generation of mINDY-KO mice and IL-6Rα$^{L\text{-}KO}$ mice have been described previously {Birkenfeld, 2011 #849;Wunderlich, 2010 #1309}. Liver specific mINDY KO mice were generated using mIndy fl/fl mice, which we described and generated previously {Birkenfeld 2011}. Animals were cared for within the Institutional Animal Care Committee guidelines, and all animal procedures were approved by local government authorities (Landesamt für Gesundheit und Soziales, Berlin, Germany and Bezirksregierung Köln, Cologne, Germany).

Hepatocyte preparation and cultivation

Density gradient-purified hepatocytes were prepared without the use of collagenase, as described previously {Neuschafer-Rube, #1508}. Hepatocytes were plated on 35-mm tissue culture plates (0.7×10^6 cells/plate; $0.5x10^6$ cells/plate for transfected cells) in M199 medium containing 1% (v/v) antibiotics (10U/µg penicillin, 10ug/µl streptomycin), 100nM dexamethasone, 0.5nM insulin and 4% (v/v) newborn calf serum. After an initial 2 h cultivation to allow cells to attach, the medium was changed to William´s medium E containing 1% (v/v) antibiotics, 100nM dexamethasone and 0.5nM insulin.

Transfection of primary hepatocytes was performed as described previously {Neuschafer-Rube, #1508}. Twenty hours later, cells were maintained in modified William´s medium E and subjected to a broad range of experiments. Then, the hepatocytes were washed and snap-frozen for subsequent RNA preparation, luciferase assays or used to determine [^{14}C]-citrate uptake and [^{14}C]-lipid synthesis.

Real-time PCR.

RT-PCR has been performed as described previously {Birkenfeld, #968}.

Cloning of human *mINDY* and uptake studies

The cDNA encoding human *mIndy* was cloned using a PCR-based approach. The *Indy*-human primer pairs forward (5'-TTAATGCGATGTATTCGGCG-3´) and reverse (5´-GTGCAAGTGGTGTGCCAAGAAGG-3´) were used with sscDNAs originating from human liver as template. Amplified cDNA fragments were cloned into the expression vector pCDNA3.1(+), resulting in the plasmid p*Indy*-human.31 and the sequences were verified.

HEK293 cells were transfected with p*Indy*-human.31 using Effectene transfection reagent (Qiagen, Hilden, Germany). After geneticin selection (800 µg/ml), single colonies were characterized for *mIndy* mRNA expression by QRT-PCR analysis. Clones with the highest *mINDY* mRNA expression were further characterized by transport assays, as described {Birkenfeld, 2011 #849}.

Lipid synthesis in primary mouse hepatocytes and livers

Isolated mouse hepatocytes (10^6/well) were cultivated as described above. After 36 h of culture in a humidified incubator (5% CO_2 / 95% O_2), cells were washed with PBS and then incubated with William´s medium E, 10 nM insulin and 0.1µCi [^{14}C]-citrate (Hartmann Analytical, specific activity 71,9 mCi/mmol) for 32 h in the absence or presence of the

indicated doses of IL-6 (Fisher Scientific, human IL-6). Cells were washed twice with PBS, lysed and then homogenized in 300 µl of PBS (3 plates per experimental condition). Protein determination was carried out with an aliquot of the homogenate. Then, the homogenate was mixed with 150 µl of 10N NaOH, heated at 70^{o}C for 2 h followed by the addition of 200µl 100% ethanol. Two hours later, hexane extraction was performed twice, and the combined upper layer was transferred into a scintillation vial, dried, and the amount of [^{14}C]-sterols was counted by liquid scintillation. The lower layer was first mixed with 300µl of 10M H_2SO_4 and then extracted with hexanes twice. The resultant upper layer was then transferred into a scintillation vial, dried, and counted to determine [^{14}C]-fatty acids. The production of [^{14}C]-lipids was normalized to the amount of protein. For total lipid determination, the counts associated with sterols and fatty acids were added.

Cultivation of HEK293 cells and lipid synthesis

HEK293 cells were cultured with DMEM containing 1% penicillin/streptomycin and 10% FCS. Cells stably overexpressing mIndy were maintained in medium supplemented with G418 (0,025 U/ml). The cells were seeded on 35mm-diameter culture plates and cultivated until 90% confluence. After a washing step with PBS, cells were incubated with 10nM insulin and 0.1 µCi [^{14}C]-citrate, followed by extraction of [^{14}C]-lipids as described above.

Microarray analysis

Microarray data was analyzed using DIANE 6.0, a spreadsheet-based microarray analysis program based on JMP 7.0 from SAS system, as mentioned in our previous studies {Birkenfeld, 2011 #849}.

Identification of transcription initiation sites by 5'-RACE

Total RNA from two different primary human hepatocyte cultures was used with 5'-RACE system 2.0 (Life Technologies, Eggenstein, Germany) as described previously {Neuschafer-Rube, #1508}.

Generation of human *mINDY* promoter constructs

Fragments containing 621 bp or 379 bp of the putative human *mIndy* promoter were generated from human genomic DNA by PCR using specific primers. Fragments were cloned in the forward orientation into pGL3-basic (Promega, Mannheim, Germany). Luciferase-based reporter assays with transfected primary mouse hepatocytes were performed as described previously and compared against empty vector control transfected hepatocytes {Neuschafer-Rube, #1508}. The following oligonucleotide primers were used:

hIndyprom-600-Xho-F: gcggcgctcgagCACCTAGAGTGGGGCGGACGTGGCG

STAT3-1mut-F:

gcggcgctcgagCACCTAGAGTGGGGCGGACGTGGCGAGGAAGCCAGGGGGGACCGG

GTTGCGAGGCCCGCGGT

STAT3-2mut-F: gcggcgctcgagCTTACGCCTTAGCGGGCAGCACC

STAT3-2mut-R:GGTGCTGCCCGCTAAGGCGTAAG

hINDYprom-HindIII-R2: gcggcgaagcttAGGGAGACTGGCGGGCGAGAC

The following plasmids were used:

pGL3-basic-hINDYprom-376-STAT3prox-mut: STAT3-2mut-F/ hINDYprom-HindIII-R2

pGL3-basic-hINDYprom-618-STAT3prox-mut: PCR1: hINDYprom600-Xho-F/hIndyprom-STAT3-2R, hIndyprom-STAT3-2F/ hINDYprom-HindIII-R2; PCR3: hINDYprom600-Xho-F/ hINDYprom-HindIII-R2; pGL3-basic-hINDYprom-618-STAT3dist-mut: hINDYprom-STAT3-1mut-F/ hINDYprom-HindIII-R2; pGL3-basic-hINDYprom-618-STAT3prox/dist-mut: PCR1: hINDYprom-STAT3-1mut-F/hIndyprom-STAT3-2R, hIndyprom-STAT3-2F/ hINDYprom-HindIII-R2; PCR3: hINDYprom-STAT3-1mut-F / hINDYprom-HindIII-R2

Statistical Analysis.

The presence or absence of normal distribution of data was verified, and natural logarithmic transformation was performed if required. Depending on data distribution, the following statistical methods were used: Spearman's rank correlation coefficient, one-way ANOVA with Bonferroni post hoc adjustment for multiple comparisons, a two-tailed Student's t-test or Mann-Whitney U test. Linear relationships were calculated by using least-square regression analysis for linear models. A two-tailed Student's t-test was used to test differences between two groups. Values are presented as mean ± standard error; $P<0.05$ was considered statistically significant.

Supplemental Data:
Figure S1A

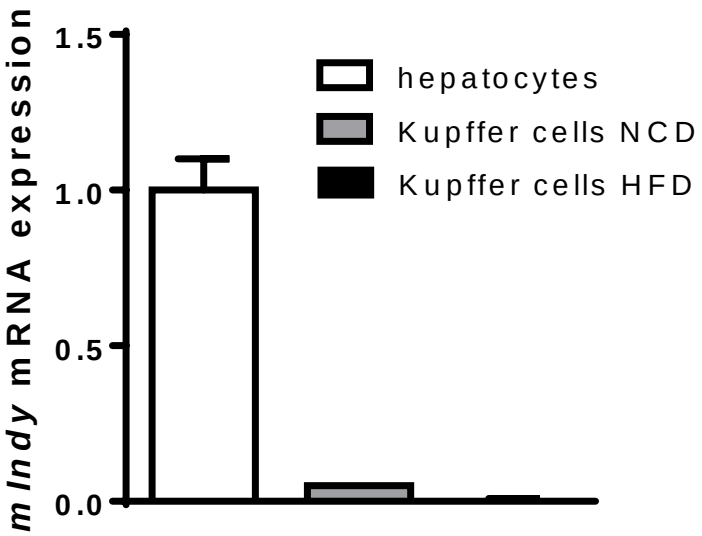
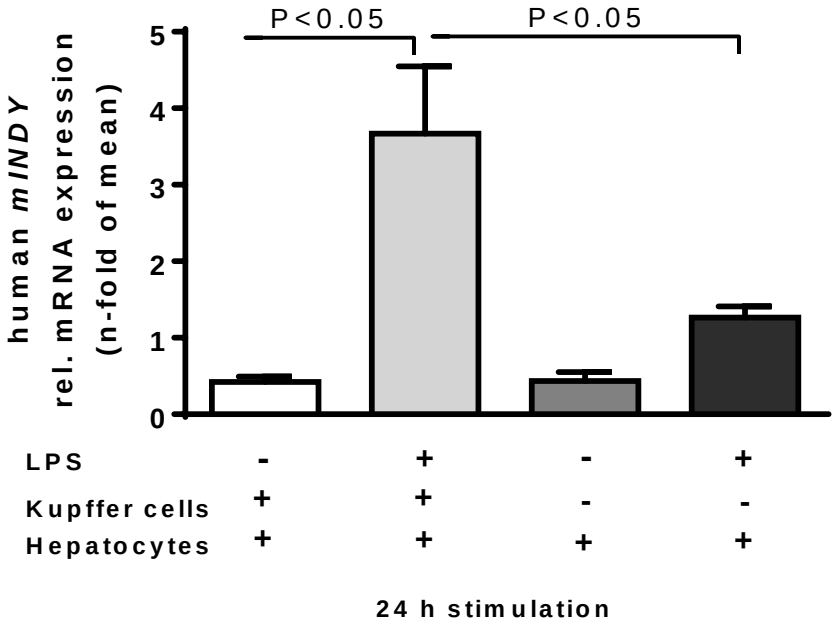
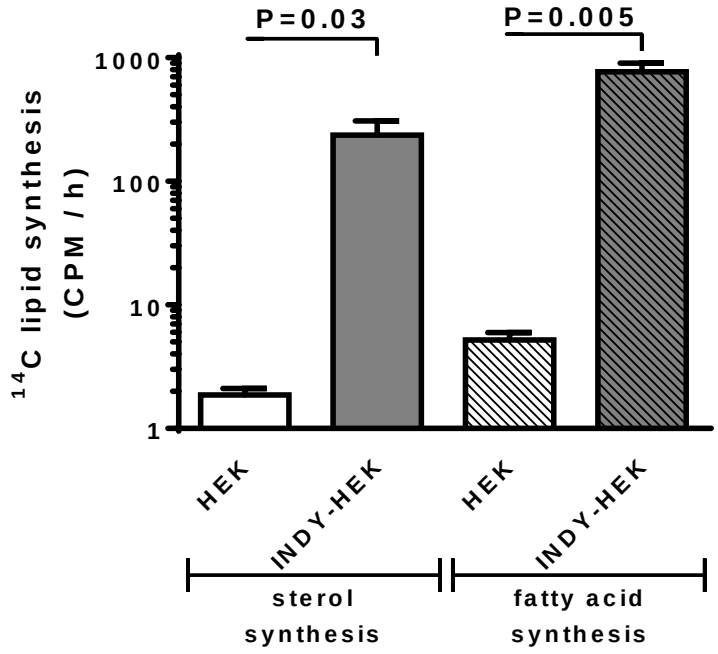


Figure S1B



Supplemental Figure S1: A) Hepatocytes and Kupffer cells were sorted from livers of animals fed either a normal chow diet or a high fat diet for 12 weeks. In both conditions, Kupffer cell-*mIndy* mRNA expression was > 50 fold lower in Kupffer cells compared to NCD or HFD fed hepatocyte mRNA expression, respectively. B) Human primary hepatocytes were either cultured separately or co-cultured with non-parenchymal liver cells, such as Kupffer cells and treated with LPS. When hepatocytes were not co-cultured with non-parenchymal cells, the stimulatory effect of LPS on *mIndy* mRNA expression was reduced compared to co-cultured cells.

Figure S2:



Supplemental Figure S2: In a stably mIndy-overexpressing HEK293 cell line, fatty acid and sterol synthesis from citrate was increased