University of Groningen

Investigating fibrosis and inflammation in an ex vivo NASH murine model

Gore, Emilia; Bigaeva, Emilia; Oldenburger, Anouk; Jansen, Yvette J M; Schuppan, Detlef; Boersema, Miriam; Rippmann, Joerg F; Broermann, Andre; Olinga, Peter

Published in:

American Journal of Physiology. Gastrointestinal and Liver Physiology

DOI:

[10.1152/ajpgi.00209.2019](https://doi.org/10.1152/ajpgi.00209.2019)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Final author's version (accepted by publisher, after peer review)

Publication date:

2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Gore, E., Bigaeva, E., Oldenburger, A., Jansen, Y. J. M., Schuppan, D., Boersema, M., Rippmann, J. F., Broermann, A., & Olinga, P. (2020). Investigating fibrosis and inflammation in an ex vivo NASH murine model. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 318(2), G336-G351. <https://doi.org/10.1152/ajpgi.00209.2019>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Investigating fibrosis and inflammation in an *ex vivo* NASH murine model

Emilia Gore¹, Emilia Bigaeva¹, Anouk Oldenburger², Yvette J. M. Jansen¹, Detlef Schuppan^{3,4},
Miriam Boersema¹, Jörg F. Rippmann², Andre Broermann² and Peter Olinga^{1#}

¹Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands

²CardioMetabolic Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

³Institute of Translational Immunology and Research Center for Immunotherapy, University Medical Center, Johannes Gutenberg University, Mainz, Germany

⁴Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Running head: Investigating an *ex vivo* NASH model

[#]Correspondence to:

Prof P. Olinga

University of Groningen, Department of Pharmaceutical Technology and Biopharmacy,

Antonius Deusinglaan 1, 9713AV Groningen, the Netherlands

Tel: +3150-3638373

E-mail: p.olinga@rug.nl

32 **Orcid number:**

33 Emilia Gore (0000-0001-5553-186X)

34 Emilia Bigaeva (0000-0002-8903-4025)

35 Anouk Oldenburger (0000-0002-0264-2467)

36 Yvette J.M. Jansen (0000-0002-2847-8116)

37 Detlef Schuppan (0000-0002-4972-1293)

38 Miriam Boersema (0000-0001-9356-796X)

39 Jörg F. Rippmann (0000-0002-6666-6222)

40 Andre Broermann (0000-0002-4768-9074)

41 Peter Olinga (0000-0003-4855-8452)

42

43

44 **Abstract**

45 Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, characterized by
46 excess fat accumulation (steatosis). Nonalcoholic steatohepatitis (NASH) develops in 15-20%
47 of NAFLD patients, and frequently progresses to liver fibrosis and cirrhosis. We aimed to
48 develop an *ex vivo* model of inflammation and fibrosis in steatotic murine precision-cut liver
49 slices (PCLS). NASH was induced in C57Bl/6 mice using amylin and choline-deficient L-
50 amino acid-defined (CDAA) diet. PCLS were prepared from steatohepatitic (sPCLS) and
51 control (cPCLS) livers and cultured for 48h with LPS, TGF β 1 or elafibranor. Additionally,
52 C57Bl/6 mice were placed on CDAA diet for 12 weeks, to receive elafibranor or vehicle from
53 week 7-12. Effects were assessed by transcriptome analysis and pro-collagen I α 1 protein
54 production. The diets induced features of human NASH. Upon culture, all PCLS showed an
55 increased gene expression of fibrosis and inflammation related markers, but decreased lipid
56 metabolism markers. LPS and TGF β 1 affected sPCLS more pronouncedly than cPCLS.
57 TGF β 1 increased pro-collagen I α 1 solely in cPCLS. Elafibranor ameliorated fibrosis and
58 inflammation *in vivo*, but not *ex vivo*, where it only increased the expression of genes
59 modulated by PPAR α . sPCLS culture induced inflammation, fibrosis and lipid metabolism
60 related transcripts, explained by spontaneous activation. sPCLS remained responsive to pro-
61 inflammatory and profibrotic stimuli on gene expression. We consider that PCLS represent a
62 useful tool to reproducibly study NASH progression. sPCLS can be used to evaluate potential
63 treatments for NASH, as demonstrated in our elafibranor study, and serves as a model to
64 bridge results from rodent studies to the human system.

65
66 **Keywords:** NASH, precision-cut liver slices, inflammation, fibrosis, elafibranor

67

68 **New & Noteworthy**

69 This study showed that nonalcoholic steatohepatitis can be studied *ex vivo* in precision-cut
70 liver slices obtained from murine diet-induced fatty livers.

71 Liver slices develop a spontaneous inflammatory and fibrogenic response during culture that
72 can be augmented with specific modulators. Additionally, the model can be used to test the
73 efficacy of pharmaceutical compounds (as shown in this investigation with elafibranor) and
74 could be a tool for preclinical assessment of potential therapies.

75

76 Introduction

77 Nonalcoholic fatty liver disease (NAFLD) is the main cause of chronic liver disease in Europe
 78 and USA(71), with increasing prevalence. The pathogenesis of NAFLD is not completely
 79 understood; however, the genetic predisposition, obesity, type 2 diabetes mellitus,
 80 hyperlipidemia and the metabolic syndrome are closely associated(24, 71). NAFLD includes
 81 benign steatosis (fat accumulation) and nonalcoholic steatohepatitis (NASH), which is
 82 characterized by ballooning degeneration and lobular inflammation that can lead to fibrosis,
 83 cirrhosis and hepatocellular carcinoma(47).

84 Currently there are no approved pharmacological therapies to treat NASH. Lifestyle
 85 interventions (e.g. weight loss and exercise) are recommended by the American Association
 86 for the Study of Liver Disease(7), but due to lack of compliance, these cannot be implemented
 87 in the majority of patients. Most drugs in clinical trials that target NASH address upstream
 88 mechanisms related to hepatic steatosis and metabolic stress(45).

89 To advance the scientific understanding of NAFLD and NASH, and to test novel drug
 90 candidates, adequate animal models are essential. The perfect animal model represents the
 91 plethora of pathophysiological changes observed in patients. Conventional mouse models are
 92 based on *ad libitum* feeding of diets enriched in different combinations of fat, fructose,
 93 cholesterol, nutrient deficiencies (e.g., choline and/or methionine), toxic interventions or
 94 genetic manipulation(29). Overnutrition in rodents shows satisfactory results and similarities to
 95 the human pathology of mere obesity and type 2 diabetes(8, 27), although the phenotype is
 96 typically mild NASH with no or minimal fibrosis. Thus, there is a clear need for preclinical
 97 models that reproduce both the disease phenotype and its etiology, to support the mechanistic
 98 and pharmacological studies of NASH in man(13).

99 The amylin liver NASH model (AMLN) is overnutrition-based by incorporates food pellets
 100 that combine fat ($\approx 40\%$) with fructose ($\approx 20\%$), a monosaccharide promoting NAFLD
 101 severity(32). This leads to macro- and microvesicular steatosis, periportal inflammation, portal
 102 and bridging fibrosis after 30 weeks(10). Another option for inducing NASH is a nutrient
 103 deficient diet. The best such model is the choline-deficient L-amino acid-defined (CDAA)

104 diet(13) that causes NASH due to the absence of choline, an essential nutrient, needed for
105 triglyceride packaging and export as very low density lipoprotein, and bile salt excretion from
106 hepatocytes(30, 38). Mice fed with this diet develop steatosis, inflammation and fibrosis(25).
107 However, the grade of inflammation and fibrosis can be variable, depending on the mouse
108 strain and other food components(21).

109 To improve reproducibility of NASH-related inflammation and fibrosis, to permit a
110 standardized test model for potential drugs, such as anti-inflammatory or antifibrotic agents,
111 and to save on experimental animals, we studied the validity of *ex vivo* murine model of
112 precision-cut liver slices (PCLS). PCLS preserve the complex structure of the liver and its
113 cellular interactions, showing a spontaneous profibrotic and pro-inflammatory response during
114 culture(33, 67). Inflammation and fibrosis can be further enhanced by incubating PCLS with
115 TGF β 1 and LPS, respectively(54, 56). Of note, TGF β 1 and LPS are also involved in NAFLD
116 pathology and progression(17, 65). Last, PCLS is a valuable preclinical tool that allows drug
117 testing for efficacy and toxicity(33, 61), while considerably reducing the number of
118 experimental animals. For instance, Ijssennagger et al. successfully tested the effect of
119 obeticholic acid (drug in phase III clinical trials for NASH) in PCLS, providing new insights
120 into the mechanism of action(22).

121 In this study, we aimed to develop and standardize an *ex vivo* model based on steatotic PCLS
122 obtained from livers of mice subjected to two diets that induce NASH, namely AMLN and
123 CDAA diets.

124

125 **Methods**

126 **Chemicals**

127 LPS was purchased at SAS Invivogen (TLRL-3PELPS, Toulouse, France) and human
128 recombinant TGF β 1 was purchased from R&D Systems (240-B-002, Abingdon, UK). They
129 were reconstituted according to the provider's instructions. Elafibranor was purchased from
130 (Sage Chemicals, Johannesburg, South Africa) and dissolved in DMSO. All stocks were stored
131 at -20°C.

132 **Animals for *ex vivo* studies**

133 Adult male C57Bl/6JRj (Bl/6) mice from Janvier were placed on a Choline Deficient L-Amino
134 Acid (CDAA, E15666-94, Ssniff Spezialdiäten GmbH) diet for 12 weeks (10 animals on
135 CDAA diet and 8 on control diet) or amylin liver NASH model diet (AMLN, D09100301,
136 Research Diets, NJ, USA) for 26 weeks (4 animals on AMLN diet and 4 on control diet). Each
137 NAFLD-inducing diet had its matching control diet. The mice were housed on a 12h light/dark
138 cycle, with controlled temperature and humidity. Chow and drinking water were *ad libitum*.
139 The mice were sacrificed under isoflurane/O₂ (Nicholas Piramal, London, UK) anesthesia. The
140 studies were approved by the Animal Review Committee of the German government and were
141 performed according to the German Animal Protection Law.

142 **Animals for *in vivo* studies**

143 Male 8-week-old Bl/6 mice from Janvier were placed on CDAA. After 6 weeks of diet, the
144 animals were treated with 15 mg/kg elafibranor (administered orally twice a day) or vehicle for
145 6 weeks, while continuing the diet (11 mice in each group). The studies were approved by the
146 Animal Review Committee of the German government and were performed according to the
147 German Animal Protection Law.

148 **Preparation of precision-cut liver slices**

149 We excised the mouse livers and collected them in ice-cold University of Wisconsin
150 preservation solution (DuPont Critical Care, Waukegab, IL, USA). The tissue was kept on ice
151 until preparation of PCLS.

PCLS were prepared as previously described(18) from the whole liver, with a Krumdieck tissue slicer (Alabama Research and Development, USA). PCLS had the following characteristics: diameter – 5 mm, thickness – 250-300 µm, weight – 4-5 mg. We incubated the PCLS individually in 12-well plates in 1.3 ml of Williams Medium E (with L glutamine, Invitrogen, Paisly, Scotland) supplemented with 25 mM glucose and 50 µg/ml gentamycin (Invitrogen). PCLS were exposed to 1 µg/ml LPS, 5 ng/ml TGFβ or elafibranor 0.2 or 1 µM. Culture medium was changed after 24h. Culture lasted 48h. PCLS were cultured in an incubator (Binder, Tuttlingen, Germany) with 37°C, 90% O₂ and 5% CO₂, horizontally shaken at 90 rpm. An outline of the sample preparation is presented in Fig. 1.

PCLS viability

PCLS viability was assessed by adenosine triphosphate (ATP) content with a bioluminescence kit (Roche Diagnostics, Mannheim, Germany). The obtained ATP content (pmol) was corrected for the total protein content (µg), determined with the Lowry method (RC DC Protein Assay, Bio Rad, Veenendaal, The Netherlands).

Gene expression analysis

We used quantitative reverse transcription polymerase chain reaction (qRT-PCR) as a method to evaluate the gene expression of markers related to fibrosis, inflammation and fat metabolism. Three PCLS were pooled, snap-frozen and RNA was extracted with FavorPrep™ Tissue Total RNA Mini Kit (Favorgen, Vienna, Austria). We determined RNA quantity and quality with BioTek Synergy HT (BioTek Instruments, Vermont, USA). 1 µg total RNA was reverse transcribed to cDNA using the Reverse Transcription System (Promega, Leiden, The Netherlands). qRT-PCR was performed using ViiA 7 Real-Time PCR System (Applied Biosystems, California, USA) and SYBR Green (Roche) based detection. We assessed the gene expression of the selected markers (Supplementary Information Table 1) with the Double Delta Ct analysis ($2^{-\Delta\Delta C_t}$), using Hydroxymethylbilane Synthase (*Hmbs*) as a reference gene.

Hydroxyproline analysis

Hepatic hydroxyproline (hyp) was determined from 250-350 mg tissue, which was hydrolyzed in 5 ml of HCl 6N overnight at 110°C. The samples were diluted in citric-acetate buffer and

180 treated with Chloramine T (Sigma-Aldrich, Zwijndrecht, Netherlands) and 4-
181 (dimethyl)aminobenzaldehyde (Sigma-Aldrich). The absorbance of the samples was measured
182 at 550 nm. The results show the μg of hepatic hyp per mg tissue.

183 **Histopathological analysis**

184 Formalin-fixed, paraffin embedded PCLS were sectioned at 4 μm and stained with
185 hematoxylin and eosin (H&E) to assess hepatic steatosis, sirius red (SR) and Masson's
186 trichrome for collagen deposition. The images were acquired with NanoZoomer S360
187 (Hamamatsu, Hamamatsu, Japan) and the quantification of the SR staining was performed
188 using the Aperio ImageScope software (Leica Biosystems, IL, USA).

189 **Serum triglyceride**

190 Serum triglyceride content was assessed in a COBAS Integra 400 plus (Roche Diagnostics,
191 Mannheim, Germany) using the provided protocol.

192 **Pro-collagen I α 1**

193 We measured the content of murine pro-collagen I α 1 in the culture media of PCLS using an
194 ELISA kit (ab210579, Cambridge, UK). The determination was performed on media from the
195 last 24h of culture and pooled from three slices of the same group. The assay was done
196 according to the manufacturer's protocol.

197 **Data and statistical analysis**

198 We used 4 to 10 different livers per diet, using slices in triplicates from each liver. The results
199 are presented as mean $\pm$ standard error of the mean (SEM). Significance was established using
200 non-parametric tests: Mann-Whitney test (unpaired and two-tailed p value) when comparing
201 two groups and Kruskal-Wallis test (exact p value) when comparing three groups. The
202 difference was considered significant when $p < 0.05$.

203

204 Results

205 AMLN and CDAA diets induce NASH-associated changes

206 We initially evaluated the presence of liver steatosis and fibrosis. To this end, we assessed the
 207 differences in liver to body weight (LBW) ratio, hydroxyproline (hyp) content, histology and
 208 transcriptional levels of fibrosis, inflammation and fat metabolism markers (Fig. 2). First, the
 209 LBW ratio (Fig. 2A) showed a marked difference between the NASH diets and their controls,
 210 indicating liver enlargement mainly due to steatosis. Second, the hyp content (Fig. 2B)
 211 revealed the presence of fibrosis in AMLN and CDAA livers, where the concentration of
 212 hyp/mg liver increased by 100% and 500%, respectively. Third, the morphological analysis
 213 showed that the NASH diets led to liver steatosis, characterized by macrovesicular steatosis in
 214 CDAA-fed mice and macro- and microvesicular steatosis in AMLN fed animals (Fig. 2C).
 215 Additionally, we observed infiltrating immune cells in sections from both diets. The Sirius Red
 216 (Fig. 2C) and Masson's trichrome (SI Fig. 1) stainings revealed the presence of fibrosis in both
 217 models, with the mice on the CDAA diet having more ECM deposition. Last, we investigated
 218 the differences in gene expression of several markers related to fibrosis, inflammation and fat
 219 metabolism in PCLS prior culture (Fig. 2D). Fibrosis markers (*Colla1*, *Serpinh1*, *Acta2*) were
 220 increased in the diets compared to control. *Fnl* showed an increase only for the CDAA diet.
 221 We next evaluated inflammation by measuring gene transcription of cytokines: IL-1b, IL-6 and
 222 TNF α . Increased gene expression of *Il1b* and *Tnf α* was observed for both diets. To assess the
 223 transcriptional changes associated with fat metabolism, we tested the gene expression of two
 224 anabolism markers involved in fatty acid synthesis (*Fasn*, *Acaca*) and three markers related to
 225 fatty acid catabolism (*Acox*, *Cpt1a*, *Ppara*). All tested lipid metabolism markers were
 226 downregulated by the CDAA diet, while no difference was observed for the AMLN diet. These
 227 PCR results were obtained by comparing each NAFLD diet to its respective control diet;
 228 however, there were certain differences at baseline between the two control diets (SI Fig. 2),
 229 which are not the focus of this study and were not taken into consideration for the next

analyses. These results show major diet-induced changes related to hepatic fat accumulation, fibrosis and inflammation, which mimic pathological characteristics of human NASH.

232

233 **Culture of steatotic PCLS induces fibrosis and inflammation and reduces fat metabolism**

234 Tissue slicing and culture induces a pro-inflammatory and profibrotic response, most probably
235 due to the mechanical stress and cold ischemia(33, 55, 66). Therefore, we assessed the effect of
236 culture on all PCLS. Slices maintained viability during the 48h of culture (SI Fig. 3A). Next,
237 we analyzed transcriptional changes for fibrosis, inflammation and fat metabolism related
238 markers. To facilitate comparison, we divided the slices into two groups: steatotic PCLS –
239 sPCLS (from the livers of mice on AMLN and CDAA diets) and control PCLS – cPCLS from
240 the corresponding control diets.

241 PCLS culture increased gene expression of fibrosis markers (*Colla1*, *Serpinh1* and *Fnl1*) in all
242 groups (Fig. 3A). Moreover, the expression levels reached in sPCLS were higher than cPCLS.
243 We also observed that the gene expression levels of these three markers were higher in sPCLS
244 from mice livers of CDAA than AMLN. The expression of the myofibroblast activation
245 marker *Acta2* was increased only in sPCLS. The results show that incubation triggers a
246 profibrotic response in healthy and steatotic PCLS.

247 Next, the inflammation status was evaluated through the gene expression of *Il1b*, *Il6* and *Tnfa*
248 (Fig. 3B). Culture-induced changes for *Il1b* were represented by a small gene expression
249 increase in AMLN cPCLS. The gene expression of *Il6* was strongly upregulated during
250 incubation and we observed differences between sPCLS (100-200 times fold induction
251 compared to cPCLS prior incubation) and cPCLS (30-40 times fold induction). Similarly, *Tnfa*
252 gene expression was increased in all groups, with sPCLS reaching a higher expression level
253 than their corresponding cPCLS. Altogether, this shows that the presence of steatosis in PCLS
254 has a synergistic effect on the induction of transcripts of inflammation during culture.

255 Further, we evaluated transcriptional changes related to lipid anabolism by measuring the
256 expression of *Fasn* and *Acaca* (Fig. 3C). Culture decreased the expression of *Fasn* in all
257 groups except CDAA sPCLS. Similarly, *Acaca* was downregulated in AMLN sPCLS and

CDAA cPCLS. Of note, the expression levels of *Fasn* and *Acaca* in CDAA sPCLS compared to CDAA cPCLS were already decreased prior to the culture. Regarding the transcription of lipid catabolism markers (Fig. 3C), culture led to a decrease in the gene expression of *Acox*, *Cpt1a* and *Ppara* in most of the groups. Thus, culture of steatotic and control PCLS for 48h reduces the gene expression of lipid metabolism related markers.

Fibrosis and inflammation can be further enhanced in PCLS with activating mediators

LPS is a bacterial endotoxin that generates an immune response characterized by the induction of proinflammatory cytokines(62). TGF β 1 is a multifunctional cytokine and is one of the main promoters of fibrosis(37). Both molecules are extensively used in *in vitro* research, due to their well-characterized and reproducible responses. We treated the PCLS with LPS or TGF β 1 for 48h to assess if inflammation and fibrosis could be further enhanced. All PCLS remained viable during culture (SI Fig. 3B), but TGF β 1 reduced the ATP content by 20% in AMLN cPCLS and CDAA sPCLS. No significant differences were observed between the fibrotic areas of AMLN, CDAA s/cPCLS treated with TGF β 1 and untreated PCLS (SI Fig. 4).

Next, we analyzed LPS and TGF β 1 induced gene expression changes. LPS had almost no effect on the expression of fibrosis markers (Fig. 4A), with the exception of a small increase in *Serpinh1* and *Acta2* expression in AMLN sPCLS. Additionally, no effect was observed on the content of pro-collagen Ia1 released in culture media (Fig. 4B). As expected, the main effect of LPS was observed in the expression of inflammation markers (Fig. 4C). In all groups (except *Il1b* in CDAA cPCLS), LPS increased the gene expression of inflammatory markers. Moreover, in both diets, the treatment with LPS led to a higher gene expression level of *Il1b*, *Il6* and *Tnfa* in sPCLS than cPCLS. With regard to lipid metabolism markers (Fig. 4D), LPS reduced exclusively the expression of catabolism markers: *Acox* (AMLN sPCLS), *Cpt1a* (AMLN sPCLS) and *Ppara* (AMLN c/sPCLS and CDAA cPCLS). These results show that LPS induces an additional inflammatory effect and can also affect lipid catabolism.

In all groups, TGF β 1 increased the gene expression of all studied fibrosis markers (Fig. 5A), except AMLN cPCLS, which showed an increase, but was not statistically significant. After

286 TGF β 1 treatment, the gene expression level of sPCLS was higher than in cPCLS (for *Colla1*,
 287 *Acta2*, *Serpinh1*). With regards to the secretion of pro-collagen I α 1, TGF β 1 increased the
 288 production of this protein solely in the control diets (Fig. 5B). Beside fibrosis, TGF β 1 also
 289 influenced transcripts of inflammation (Fig. 5C) and lipid metabolism in certain groups (Fig.
 290 5D). Hence, PCLS treated with TGF β 1 displayed transcriptional changes related to fibrosis
 291 (increase), inflammation (slight increase) and lipid metabolism (decrease), especially in the
 292 presence of steatosis.

293 **PPAR α / δ agonist increases lipid metabolism in the *ex vivo* CDAA model**

294 Elafibranor, a PPAR α / δ agonist, is a potential treatment for NASH, which is now investigated
 295 in a phase 3 clinical trial (<https://clinicaltrials.gov/ct2/show/NCT02704403>). Our *ex vivo*
 296 NASH model has the potential of becoming a drug-testing system that can help evaluate the
 297 efficacy of drugs to reduce steatosis, inflammation and/or fibrosis. A critical validation step for
 298 this *ex vivo* model is to provide evidence of target engagement and pharmacological effects of
 299 the drugs that have been proven effective in *in vivo* studies. Therefore, we investigated the
 300 effect of elafibranor in PCLS from CDAA-induced NASH. We selected the CDAA model due
 301 to the higher amount of hepatic fibrosis compared to AMLN model and the possibility of direct
 302 comparison to *in vivo* results(63). We tested two concentrations of elafibranor, 0.2 μ M and 1
 303 μ M, based on the half maximal effective concentration of the drug(31). Elafibranor was well
 304 tolerated in PCLS, and a decrease in ATP content (25%) was observed only in cPCLS when
 305 treated with the 1 μ M concentration (SI Fig. 3C). After 48h treatment, there were no changes
 306 regarding the gene expression of fibrosis and inflammation markers and pro-collagen I α 1
 307 production in PCLS treated with elafibranor compared to untreated PCLS of the same diet
 308 (Fig. 6A, B, C).

309 Treatment of PCLS with elafibranor had no effect on the gene expression of fat anabolism
 310 markers, *Acaca* and *Fasn* (Fig. 6D). Regarding fat catabolism, the gene expression of *Acox*
 311 was increased by elafibranor 1 μ M in sPCLS; additionally, we observed a trend of increased
 312 gene expression for *Acox* and *Ppara* in cPCLS. Considering that the increased expression of
 313 *Acox* is a direct effect of PPAR α stimulation(43), we further tested several other markers that

are regulated by PPAR α/δ in mice(3, 11, 36, 42, 43). These include genes involved in: fatty acid oxidation and ketogenesis (*Cyp4a*, *Acadm*, *Hmgcs2*), fatty acid transport (*Cd36*, *Fabp1*), production of fatty acids and very low density lipoproteins (*Me1*, *Scd1*), apolipoproteins (*Apoa2*, *Apoa5*), triglyceride clearance (*Angptl4*), glucose metabolism (*Pdk4*) and peroxisome proliferation (*Pex11a*). The differences regarding these genes between CDAA diet and its control, prior incubation, are presented in SI Fig. 5. After 12 weeks of diet, the gene expression of *Fabp1*, *Scd1*, *Me1*, *Apoa5* and *Pex11a* were significantly decreased compared to control diet. Moreover, a trend for decreased gene expression is observed for *Cyp4a* (p=0.06) and *Apoa2* (p=0.06). The effects of elafibranor on these genes in PCLS are presented in Fig. 6D. Elafibranor 1 μ M increased the gene expression of *Cyp4a* in both cPCLS and sPCLS. The sPCLS responded more pronouncedly than the cPCLS; moreover, sPCLS treated with elafibranor 1 μ M showed a gene expression level that was 2-fold higher than cPCLS at 0h. Elafibranor increased in PCLS the gene expression of enzymes involved in microsomal (*Cyp4a*) and peroxisomal (*Acox*) fatty acid oxidation, but not mitochondrial (*Acadm*, *Hmgcs2*) (SI Fig. 6A). The transcripts for fatty acid transport were influenced in cPCLS by elafibranor, as shown by the increased expression of *Fabp1*; for sPCLS only an increasing trend is observed for this gene. However, the gene expression level of *Fabp1* in sPCLS was higher than in cPCLS. The gene expression of *Scd1* and *Pdk4* were increased by elafibranor in both groups, but the expression levels in sPCLS were lower than in cPCLS. Nonetheless, the fold induction due to the treatment was higher in sPCLS compared to cPCLS. Additionally, elafibranor 1 μ M increased the expression of *Angptl4* and *Pex11a* only in cPCLS. No differences were observed for the following genes: *Cd36*, *Me1*, *Apoa2*, *Apoa5* (SI Fig. 6A). The effects of elafibranor were observed on transcriptional level of fat metabolism markers, while no significant change was observed on the fibrosis area (SI Fig. 6B). These results show that elafibranor can activate PPAR α/δ signaling in murine PCLS, triggering the modulation of lipid and carbohydrate metabolism, whereas fibrosis and inflammation were not affected in PCLS during 48h culture.

341

Elafibranor improves the metabolic profile and ameliorates fibrosis *in vivo* in CDAA diet

We next asked if the results obtained with elafibranor *ex vivo* were predictive for *in vivo*. To compare the results between the *ex vivo* and *in vivo* systems for the markers regulated by PPAR α/δ , BL/6 mice were placed on the CDAA diet for 6 weeks, followed by 6 weeks of diet and elafibranor treatment (15 mg/kg administered orally twice a day). Elafibranor improved the metabolic profile with a reduction of liver triglycerides by 70% (Fig. 7A), but increased liver weight compared to untreated mice (Fig. 7B). Regarding fibrosis, elafibranor reduced total liver collagen (hyp) by 30% (Fig. 7C). In the same line, elafibranor reduced fibrosis (*Colla1*, *Acta2*) and inflammation (*Tnfa*) related transcripts (Fig. 7D). Treatment with elafibranor beneficially modulated the transcripts of fat metabolism markers (Fig. 7E and SI Fig. 7). After 6 weeks of treatment, elafibranor increased the mRNA expression of *Acox*, the first enzyme involved in peroxisomal fatty acid β -oxidation. The drug also increased the gene expression of enzymes involved in microsomal (*Cyp4a*) and mitochondrial (*Acadm*, *Hmgcs2*) fatty acid oxidation. Elafibranor can increase fat metabolism in the liver by promoting: fatty acid transport (*Cd36*, *Fabp1*), lipoprotein production (*Me1*, *Scd1*), trygliceride clearance (*Angptl4*) and glucose metabolism inhibition (*Pdk4*). The gene expression of apolipoproteins was differentially regulated by elafibranor, with *Apoa2* being increased and *Apoa5* being decreased by the treatment. Lastly, elafibranor increased the expression of *Pex11a*, indicating peroxisome proliferation.

362 Discussion

363 Our goal was to develop an *ex vivo* NASH model that closely mimics the changes associated
 364 with this condition and is relevant for testing therapeutic options. The model is based on
 365 steatotic murine livers as a source for PCLS, maintaining the original organ architecture and
 366 cellular composition.

367 The first part of the study focused on the viability of steatotic liver slices and the effects of
 368 culture. All slices remained viable, but for the overnutrition model (AMLN) we observed
 369 lower absolute values in ATP content compared to cPCLS, showing that steatosis etiology can
 370 influence PCLS viability. This difference might arise from the types of lipids accumulated in
 371 hepatocytes during NASH development in these livers. High carbohydrate and fructose feeding
 372 increases free fatty acids levels, especially due to *de novo* lipogenesis(49). The free fatty acids
 373 have a lipotoxic effect that leads to mitochondrial dysfunction(15), reduced ATP content and
 374 apoptosis via the death receptor Fas and TRAIL receptor 5(14, 35). Lack of choline was also
 375 associated with mitochondrial dysfunction(20), but the choline present in the culture media (14
 376 μ M) might have had a beneficial effect on CDAA slices, allowing them to recover and to have
 377 a similar ATP level to their cPCLS. The beneficial effect of choline in culture media (28 μ M)
 378 was previously shown, when similar amounts of triacylglycerol were secreted by hepatocytes
 379 derived from mice on choline deficient and supplemented diets(28).

380 PCLS can be advantageous for NASH research, as culture spontaneously triggers key
 381 inflammation and fibrotic genes(33, 54). This could be beneficial especially for currently used
 382 *in vivo* steatotic murine models that show only mild inflammation and fibrosis. We expected an
 383 inflammatory and fibrotic response during incubation, together with higher gene expression
 384 levels in the sPCLS than cPCLS, since steatosis can trigger both inflammation(53) and
 385 fibrosis(40). Spontaneous fibrosis was observed in all PCLS during culture, with sPCLS
 386 surpassing cPCLS in regards to gene expression levels. Although the AMLN and CDAA diets
 387 induce steatosis through different mechanisms, the increase in fibrosis markers during culture
 388 was similar between the two diets. The results showed also a pro-inflammatory response

389 during culture in all PCLS; from the three analyzed markers, *Il6* was the most sensitive, having
390 higher fold induction and attained expression levels in sPCLS. Increased levels of hepatic and
391 circulating IL-6 were reported in animal models of NAFLD and patients(51, 68, 69). Long-
392 term IL-6 stimulation aggravates NAFLD by inhibiting hepatic insulin receptor signaling,
393 hence causing insulin resistance(48). Inflammation plays a role in NAFLD pathophysiology
394 and prognosis; therefore, the pro-inflammatory effect induced by culture could help identify
395 the roles of different cytokines and chemokines in NAFLD/NASH and their potential as
396 therapeutic targets.

397 To our knowledge, this is the first study to assess gene expression related to lipid metabolism
398 during culture of murine sPCLS. CDAA sPCLS showed less changes than AMLN sPCLS; this
399 might be due to the decrease of fat metabolism related gene expression observed in the CDAA
400 PCLS prior to culture (Fig. 2D₃). The reduction of fat metabolism markers gene expression
401 after culture can be caused by the absence of fructose, fatty acids and insulin in the culture
402 media. Further investigations should be conducted to optimize the culture media in order to
403 ensure the conservation and functionality of the lipid metabolism.

404 The versatility of the PCLS model is reflected by the possibility of enhancing biological
405 processes in order to answer specific research questions. Therefore, in the second part of our
406 study, we focused on further induction of inflammation and fibrosis to mimic *ex vivo* the
407 pathology observed in NASH. This would allow mechanistic studies and drug testing in a
408 variety of settings. For this reason, we tested if sPCLS can still respond to the effects of
409 powerful modulators of inflammation (LPS) and fibrosis (TGFβ1), which are also associated
410 with NASH in patients(6, 12, 34). The results showed that LPS can accentuate inflammation
411 and the transcriptional levels reached were higher in steatotic slices than the controls.
412 Interestingly, LPS activated PCLS from the AMLN model more intensively than CDAA diet.
413 This could be caused by the presence of fructose in the AMLN, a nutrient that leads to the
414 increased hepatic LPS levels and activation of toll-like receptor 4 signaling(50, 58). Although
415 pre-exposure to LPS can lead to LPS tolerance(60), this can be different in NASH due to
416 impaired LPS clearance and enhanced Kupffer cells activation(1). Additionally, the

composition of lipids stored in hepatocytes may modulate the activity of Kupffer cells(1). Marked inflammation could have a negative effect on fat catabolism, as the increased inflammation caused by LPS decreased the gene expression of the studied fat catabolism markers, especially in sPCLS.

Regarding fibrosis, TGF β 1 showed a clear profibrotic effect. sPCLS reached higher expression levels for fibrosis markers than cPCLS, confirming that we can accentuate fibrosis *ex vivo*, especially in the presence of steatosis and fibrosis. This is in accordance with human data, where an overexpression of the *TGFBI* gene was found in NASH patients with fibrosis compared to NASH patients without fibrosis(5). An interesting result was that TGF β 1 could increase the production of pro-collagen I α 1 only in healthy slices. The lack of response from sPCLS could be due to the fact that a maximum production of **pro-collagen I α 1 is induced solely by culture. Moreover**, the high secretion of this protein in sPCLS when compared to cPCLS could be explained by more ECM-secreting cells in steatotic slices and a more susceptible response to the profibrotic effect of culture. Additionally, TGF β 1 reduced the gene expression of fat metabolism markers, especially for sPCLS, showing that an ongoing fibrotic process may contribute to lipid metabolism compromise. The detrimental effect of TGF β 1 in NAFLD was reported in murine hepatocytes, where TGF β 1 had a synergistic effect on palmitate, increasing lipogenesis and decreasing catabolism markers(70). Altogether, we showed that sPCLS are still responsive to further induction of fibrosis or inflammation, processes that also impact fat metabolism. This shows that the model is not limited to the effects triggered by culture and we can accentuate pathological conditions with activators or inhibitors, generating various stages of disease.

Development and efficacy assessment of drugs is an expensive and time-consuming process. More relevant *in vitro* methods are needed to prevent unnecessary *in vivo* animal studies. Therefore, the goal of the last part of this study was to determine if the *ex vivo* steatotic PCLS model could be used for testing anti-NAFLD compounds. An advantage of this model is that several compounds and concentrations can be studied in slices from the same animal. We chose to evaluate the effects of elafibranor, since it is a promising candidate for treating

445 NASH, with good results in clinical trials(44). In addition, we aimed to investigate if this drug
 446 had a direct effect on fibrosis and inflammation in PCLS, since elafibranor can reduce
 447 inflammation and fibrosis in mice *in vivo*(59). Elafibranor activates lipid catabolism as a result
 448 of PPAR α / δ activation. Transcriptional markers of fatty acid oxidation were increased by
 449 elafibranor in healthy control and CDAA sPCLS and in our *in vivo* experiment; however, the
 450 gene expression of mitochondrial oxidation markers was induced only *in vivo*. This may
 451 indicate that mitochondrial oxidation needs more than 48h (PCLS incubation time) to be
 452 induced by elafibranor, while the activation of PPAR α triggers initial microsomal and
 453 peroxisomal oxidation. Elafibranor had similar effects in PCLS and *in vivo* for fatty acid
 454 transport transcripts, where it increased *Fabp1* expression. FABP1, has an antioxidant and
 455 detoxifying role(64, 65) in hepatocytes due to its function in intracellular storage and transport
 456 of fatty acids. Moreover, a reduced level of FABP1 was reported in NASH patients and might
 457 predict NASH susceptibility in NAFLD patients(9). By increasing *Fabp1* gene expression,
 458 elafibranor shows a protective role against oxidative stress and NAFLD progression. Another
 459 positive effect of elafibranor on lipid metabolism regulation was the increase of *Scd1* gene
 460 expression, which was achieved in PCLS and *in vivo*. This gene was reported to be
 461 downregulated in animal models of NAFLD(16) and the hepatic protein activity was
 462 negatively correlated with liver fat in obese patients(52). Moreover, elafibranor influences
 463 glucose metabolism by inducing *Pdk4*, *ex vivo* as well as *in vivo*. Increased *Pdk4* expression
 464 shows that glucose metabolism is inhibited and fatty acids are used instead to provide energy
 465 for the cell(46). A characteristic effect of PPAR α agonists in the liver of rodents is hepatocyte
 466 peroxisome proliferation, which causes liver enlargement through hyperplasia and
 467 hypertrophy(2). Interestingly, the activation of PPAR α in man does not lead to cell
 468 proliferation and therefore, the agonists of this receptor do not have a hepatocarcinogenic
 469 potential(57). Peroxisome proliferation in rodents was reported *in vivo* and *in vitro*(2). This
 470 process was observed in our study from the increased gene expression of *Pex11a* (*ex vivo* and
 471 *in vivo*) and liver weight increase *in vivo*. These results might indicate that the efficacy of
 472 elafibranor in increasing fat oxidation in mice is achieved through peroxisome proliferation. *Ex*

473 *vivo*, elafibranor showed clear effects on promoting fatty acids catabolism, but it does not
 474 ameliorate fibrosis and inflammation. *In vivo*, six weeks of elafibranor treatment had positive
 475 effects on fibrosis, inflammation and fat metabolism. We believe that in the *in vivo*
 476 experiments elafibranor improved lipid metabolism due to its mechanism of action, whereas
 477 amelioration of fibrosis and inflammation are indirect effects due to the reduction of fat and
 478 oxidative stress. Since fibrosis is triggered by inflammation, a reduction of inflammation
 479 would have a beneficial effect on fibrosis. The effects on inflammation and fibrosis are not
 480 observed in sPCLS probably due to the short culture time, but the similar effects on genes
 481 modulated by PPAR α/δ are a confirmation that PCLS can correctly predict the efficacy of a
 482 drug on certain targets (receptors/pathways). Mouse results cannot be directly translated to
 483 patients, especially since the two species show different sensitivity to peroxisome proliferation,
 484 which might indicate faster steatosis resolution in mice than humans. Nevertheless, the phase
 485 two clinical trial of elafibranor showed that after one year, NASH patients had substantial
 486 histological improvement and resolution of steatohepatitis, without fibrosis worsening(44).
 487 Given these points, we consider that PCLS might have high predictive value for evaluating the
 488 efficacy of anti-NAFLD compounds.

489 An important aspect of animal experiments is the relevance for human disease. NAFLD has a
 490 complex and heterogeneous pathogenesis, characterized by numerous interrelated processes
 491 that occur in different organs (liver, intestine, adipose tissue)(4). Although the methods used to
 492 induce NAFLD in animals are derived from human studies (overnutrition, diets rich in fat and
 493 carbohydrates, choline deprivation), the animal models of NAFLD may not recapitulate all
 494 characteristics of the condition(13). The overnutrition models show similar metabolic features
 495 to patients; however, the outcome is not severe and requires more time to develop(23). The
 496 choline deficient diet needs less time to show steatohepatitis features and fibrosis similar to
 497 patients with rapid NASH progression(26). However, in CDAA-fed mice the metabolic profile
 498 is opposite to the human condition, as they do not gain body weight, nor do they display
 499 hepatic insulin resistance(19). The animal model choice for preparing sPCLS depends mostly
 500 on the scientific question that needs to be answered. The chosen animal model for obtaining

PCLS should take into consideration the drug's mechanism of action. The overnutrition model of AMLN can elucidate questions regarding steatosis, while CDAA is more indicated for later NAFLD stages, where increased inflammation and fibrosis can be investigated. We consider both models relevant for preclinical drug development, as they displayed increased inflammation and fibrosis during culture, and responded to pro-inflammatory and profibrotic stimuli. Additionally, modulators of inflammation and fibrosis can create more severe phenotypes to inquire drug efficacy. The model cannot replace *in vivo* experiments, but can reduce the number of animals by providing more relevant outcomes regarding safety and efficacy.

Based on our data, we suggest that sPCLS is a promising tool to study NASH pathogenesis and test pharmaceutical compounds. Beside murine PCLS, this model could be used for (fatty) human livers from surgical procedures, in order to exclude murine-human translation. Nevertheless, there are drawbacks of the PCLS model, such as absence of communication with other organs involved in NAFLD, such as adipose tissue, or circulating immune cells and adipokines. However, it is still possible to study the effect of the adipose tissue on liver *in vitro*, by co-culturing sPCLS with adipocytes. Another option is the addition of adipokines to the sPCLS incubation media. An alternative to sPCLS would be inducing fat accumulation *in vitro* in healthy murine/human PCLS by adding fatty acids, sugars and insulin to the culture media(41). Although we observed that the transcripts of fat metabolism related markers are decreased during PCLS incubation, this might change in the presence of fatty acids, as observed *in vitro* in hepatocytes(39). Therefore, we consider that murine steatotic PCLS are fundamental for paving the way for studies in human liver slices (culture conditions optimization).

In conclusion, PCLS appear to be a valuable preclinical model that preserves liver cellular structure and reduces significantly the number of animals used for research. Steatotic PCLS can be obtained from various animal models with different degrees of steatosis and fibrosis. As an *ex vivo* model, sPCLS shows fibrosis, inflammation and fat metabolism transcriptional changes during culture. Fibrosis and inflammation can be further induced with specific

Investigating an *ex vivo* NASH model

529 molecules and drugs can be evaluated for their anti-NAFLD effect. The selection of the animal
530 model should be done according to the research question. Future studies should be conducted
531 to optimize culture conditions, especially for the lipid metabolism, and to obtain the proof of
532 clinical translation of new NAFLD therapies, as a critical step for sPCLS validation.
533

534 **Acknowledgments:**

535 This study was supported by ZonMw (the Netherlands Organisation for Health
536 Research and Development) – grant number 114025003.

537 DS receives project related support by the EU Horizon 2020 under grant agreement n.
538 634413 (EPoS, European Project on Steatohepatitis) and 777377 (LITMUS, Liver
539 Investigation on Marker Utility in Steatohepatitis), and by the German Research
540 Foundation collaborative research project grants DFG CRC 1066/B3 and CRC
541 1292/08.

542 We would like to thank Anke Voigt (Boehringer Ingelheim) for excellent technical
543 support with *in vivo* experiments.

544

545 **Author contributions**

546 EG designed the experiments in collaboration with PO and MB. AB provided the
547 murine tissue for experiments. EG, EB, and AO performed the experiments, processed
548 the experimental data and performed the analysis. EG designed the figures. EG wrote
549 the manuscript with critical review from EB, AO, DS, MB, JFR, AB. and PO. All
550 authors discussed the results and contributed to the final manuscript.

551

552 **Conflict of interest:**

553 A. Oldenburger, J.F. Rippmann and A. Broermann are employees at Boehringer
554 Ingelheim Pharma GmbH & Co. KG.

555

556

557 **References**

- 558 1. **Baffy G.** Kupffer cells in non-alcoholic fatty liver disease: The emerging view.
559 *J Hepatol* 51: 212–223, 2009.
- 560 2. **Bentley P, Calder I, Elcombe C, Grasso P, Stringer D, Wiegand HJ.** Hepatic
561 peroxisome proliferation in rodents and its significance for humans. *Food Chem*
562 *Toxicol* 31: 857–907, 1993.
- 563 3. **Bojic LA, Sawyez CG, Telford DE, Edwards JY, Hegele RA, Huff MW.**
564 Activation of peroxisome proliferator-activated receptor δ inhibits human
565 macrophage foam cell formation and the inflammatory response induced by
566 very low-density lipoprotein. *Arterioscler Thromb Vasc Biol* 32: 2919–2928,
567 2012.
- 568 4. **Byrne CD, Targher G.** Review NAFLD : A multisystem disease. *J Hepatol* 62:
569 S47–S64, 2015.
- 570 5. **Cayón A, Crespo J, Mayorga M, Guerra A, Pons-Romero F.** Increased
571 expression of Ob-Rb and its relationship with the overexpression of TGF- β 1
572 and the stage of fibrosis in patients with nonalcoholic steatohepatitis. *Liver Int* :
573 1065–1071, 2006.
- 574 6. **Ceccarelli S, Panera N, Mina M, Gnani D, De Stefanis C, Crudele A,**
575 **Rychlicki C, Petrini S, Bruscalupi G, Agostinelli L, Stronati L, Cucchiara**
576 **S, Musso G, Furlanello C, Svegliati-Baroni G, Nobili V, Alisi A.** LPS-
577 induced TNF- α factor mediates pro-inflammatory and pro-fibrogenic pattern in
578 non-alcoholic fatty liver disease. *Oncotarget* 6, 2015.
- 579 7. **Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M,**
580 **Harrison SA, Brunt EM, Sanyal AJ.** The diagnosis and management of
581 nonalcoholic fatty liver disease: Practice guidance from the American
582 Association for the Study of Liver Diseases. *Hepatology* 67: 328–357, 2018.
- 583 8. **Charlton M, Krishnan A, Viker K, Sanderson S, Cazanave S, McConico A,**
584 **Masuoko H, Gores G.** Fast food diet mouse: novel small animal model of
585 NASH with ballooning, progressive fibrosis, and high physiological fidelity to
586 the human condition. *AJP Gastrointest Liver Physiol* 301: G825–G834, 2011.
- 587 9. **Charlton M, Viker K, Krishnan A, Sanderson S, Kaalsbeek AJ, Kendrick**
588 **M, Thompson G, Que F, Sarr M.** Differential Expression of Lumican and
589 Fatty Acid Binding Protein-1 – New Insights into the Histologic Spectrum of
590 Non- Alcoholic Fatty Liver Disease. *Hepatology* 49: 1375–1384, 2009.
- 591 10. **Clapper JR, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J,**
592 **Athanacio J, Villescaz C, Ghosh SS, Heilig JS, Lowe C, Roth JD.** Diet-
593 induced mouse model of fatty liver disease and nonalcoholic steatohepatitis
594 reflecting clinical disease progression and methods of assessment. *Am J Physiol*
595 *Liver Physiol* 305: G483–G495, 2013.
- 596 11. **Desvergne B, Wahli W.** Peroxisome Proliferator-Activated Receptors: Nuclear
597 Control of Metabolism. *Endocr Rev* 20: 649–688, 1999.
- 598 12. **Farhadi A, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny**
599 **MM, Keshavarzian A.** Susceptibility to gut leakiness: a possible mechanism
600 for endotoxaemia in non-alcoholic steatohepatitis. *Liver Int* 28: 1026–1033,
601 2008.
- 602 13. **Farrell G, Schattenberg JM, Leclercq I, Yeh MM, Goldin R, Teoh N,**
603 **Schuppan D.** Mouse models of nonalcoholic steatohepatitis Towards
604 optimization of their relevance to human NASH. *Hepatology* 2: 0–2, 2018.
- 605 14. **Feldstein AE, Canbay A, Guicciardi ME, Higuchi H, Bronk SF, Gores GJ.**

- 606 Diet associated hepatic steatosis sensitizes to Fas mediated liver injury in mice.
607 *J Hepatol* 39: 978–983, 2003.
- 608 15. **Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF,**
609 **Rydzewski R, Burgart LJ, Gores GJ.** Free fatty acids promote hepatic
610 lipotoxicity by stimulating TNF- α expression via a lysosomal pathway.
611 *Hepatology* 40: 185–194, 2004.
- 612 16. **Fernández Gianotti T, Burgueño A, Gonzales Mansilla N, Pirola CJ,**
613 **Sookoian S.** Fatty Liver Is Associated with Transcriptional Downregulation of
614 Stearoyl-CoA Desaturase and Impaired Protein Dimerization. *PLoS One* 8: 1–
615 11, 2013.
- 616 17. **Fukunishi S, Sujishi T, Takeshita A, Ohama H, Tsuchimoto Y, Asai A,**
617 **Tsuda Y, Higuchi K.** Lipopolysaccharides accelerate hepatic steatosis in the
618 development of nonalcoholic fatty liver disease in Zucker rats. *J Clin Biochem*
619 *Nutr* 54: 39–44, 2014.
- 620 18. **de Graaf IAM, Olinga P, de Jager MH, Merema MT, de Kanter R, van de**
621 **Kerkhof EG, Groothuis GMM.** Preparation and incubation of precision-cut
622 liver and intestinal slices for application in drug metabolism and toxicity studies.
623 *Nat Protoc* 5: 1540–1551, 2010.
- 624 19. **Hebbard L, George J.** Animal models of nonalcoholic fatty liver disease
625 [Online]. *Nat Rev Gastroenterol & Hepatol* 8: 35, 2010.
626 <https://doi.org/10.1038/nrgastro.2010.191>.
- 627 20. **Hensley K.** Dietary choline restriction causes complex I dysfunction and
628 increased H₂O₂ generation in liver mitochondria. *Carcinogenesis* 21: 983–989,
629 2000.
- 630 21. **Van Herck M, Vonghia L, Francque S.** Animal Models of Nonalcoholic Fatty
631 Liver Disease—A Starter’s Guide. *Nutrients* 9: 1072, 2017.
- 632 22. **Ijssennagger N, Janssen AWF, Milona A, Ramos Pittol JM, Hollman DAA,**
633 **Mokry M, Betzel B, Berends FJ, Janssen IM, Van Mil SWC, Kersten S.**
634 Gene expression profiling in human precision cut liver slices in response to the
635 FXR agonist obeticholic acid. *J Hepatol* 64: 1158–1166, 2016.
- 636 23. **Ito M, Suzuki J, Tsujioka S, Sasaki M, Gomori A, Shirakura T, Hirose H,**
637 **Ito M, Ishihara A, Iwaasa H, Kanatani A.** Longitudinal analysis of murine
638 steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res*
639 37: 50–57, 2007.
- 640 24. **Kalia HS, Gaglio PJ.** The Prevalence and Pathobiology of Nonalcoholic Fatty
641 Liver Disease in Patients of Different Races or Ethnicities. *Clin Liver Dis* 20:
642 215–224, 2016.
- 643 25. **Kodama Y, Kisseleva T, Iwaisako K, Miura K, Taura K, De S.** NIH Public
644 Access. *October* 137: 1467–1477, 2010.
- 645 26. **Kodama Y, Kisseleva T, Iwaisako K, Miura K, Taura K, De Minicis S,**
646 **Österreicher CH, Schnabl B, Seki E, Brenner DA.** c-Jun N-terminal Kinase-1
647 From Hematopoietic Cells Mediates Progression From Hepatic Steatosis to
648 Steatohepatitis and Fibrosis in Mice. *Gastroenterology* 137: 1467–1477.e5,
649 2009.
- 650 27. **Kohli R, Kirby M, Xanthakos SA, Softic S, Feldstein AE, Tang PH, Miles**
651 **L, Miles M V, Balistreri WF, Stephen C, Seeley RJ.** High-Fructose Medium-
652 Chain-Trans-Fat Diet Induces Liver Fibrosis & Elevates Plasma Coenzyme Q9
653 in a Novel Murine Model of Obesity and NASH. *Hepatology* 52: 934–944,
654 2010.
- 655 28. **Kulinski A, Vance DE, Vance JE.** A Choline-deficient Diet in Mice Inhibits

- neither the CDP-choline Pathway for Phosphatidylcholine Synthesis in Hepatocytes nor Apolipoprotein B Secretion. *J Biol Chem* 279: 23916–23924, 2004.
29. **Lau JKC, Zhang X, Yu J.** Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. *J Pathol* 241: 36–44, 2017.
 30. **Li Z, Agellon LB, Vance DE.** Phosphatidylcholine homeostasis and liver failure. *J Biol Chem* 280: 37798–37802, 2005.
 31. **Liu Z-M, Hu M, Chan P, Tomlinson B.** Early investigational drugs targeting PPAR- α for the treatment of metabolic disease. *Expert Opin Investig Drugs* 24: 611–621, 2015.
 32. **Longato L.** Non-alcoholic fatty liver disease (NAFLD): a tale of fat and sugar? *Fibrogenesis Tissue Repair* 6: 14, 2013.
 33. **Luangmonkong T, Suriguga S, Bigaeva E, Boersema M, Oosterhuis D, de Jong KP, Schuppan D, Mutsaers HAM, Olinga P.** Evaluating the antifibrotic potency of galunisertib in a human *ex vivo* model of liver fibrosis. *Br J Pharmacol* 174: 3107–3117, 2017.
 34. **Mahmoud AA, Bakir AS, Shabana SS.** Serum TGF- β , Serum MMP-1, and HOMA-IR as non-invasive predictors of fibrosis in Egyptian patients with NAFLD. *Saudi J Gastroenterol* 18: 327–33, 2012.
 35. **Malhi H, Barreyro FJ, Isomoto H, Bronk SF, Gores GJ.** Free fatty acids sensitise hepatocytes to TRAIL mediated cytotoxicity. *Gut* 56: 1124–1131, 2007.
 36. **Mandard S, Müller M, Kersten S.** Peroxisome proliferator-activated receptor a target genes. *Cell Mol Life Sci* 61: 393–416, 2004.
 37. **Meng XM, Nikolic-Paterson DJ, Lan HY.** TGF- β : The master regulator of fibrosis. *Nat Rev Nephrol* 12: 325–338, 2016.
 38. **Noga AA, Vance DE.** A gender-specific role for phosphatidylethanolamine N-methyltransferase-derived phosphatidylcholine in the regulation of plasma high density and very low density lipoproteins in mice. *J Biol Chem* 278: 21851–21859, 2003.
 39. **Park MJ, Kim D II, Choi JH, Heo YR, Park SH.** New role of irisin in hepatocytes: The protective effect of hepatic steatosis *in vitro*. *Cell Signal* 27: 1831–1839, 2015.
 40. **Peverill W, Powell LW, Skoien R.** Evolving concepts in the pathogenesis of NASH: Beyond steatosis and inflammation. *Int J Mol Sci* 15: 8591–8638, 2014.
 41. **Prins G, Luangmonkong T, Oosterhuis D, Mutsaers H, Dekker F, Olinga P.** A Pathophysiological Model of Non-Alcoholic Fatty Liver Disease Using Precision-Cut Liver Slices. *Nutrients* 11: 507, 2019.
 42. **Qi C, Zhu Y, Reddy JK.** Peroxisome Proliferator-Activated Receptors, Coactivators, and Downstream Targets. *Cell Biochem Biophys* 32: 187–204, 2000.
 43. **Rakhshandehroo M, Knoch B, Müller M, Kersten S.** Peroxisome Proliferator-Activated Receptor Alpha Target Genes. *PPAR Res* 2010: 1–20, 2010.
 44. **Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, Romero-Gomez M, Boursier J, Abdelmalek M, Caldwell S, Drenth J, Anstee QM, Hum D, Hanf R, Roudot A, Megnien S, Staels B, Sanyal A, Mathurin P, Gournay J, Nguyen-Khac E, De Ledinghen V, Larrey D, Tran A, Bourliere M, Maynard-Muet M, Asselah T, Henrion J, Nevens F, Cassiman D, Geerts A, Moreno C, Beuers UH, Galle PR, Spengler U,**

- 706 Bugianesi E, Craxi A, Angelico M, Fargion S, Voiculescu M, Gheorghe L,
 707 Preotescu L, Caballeria J, Andrade RJ, Crespo J, Callera JL, Ala A, Aithal
 708 G, Abouda G, Luketic V, Huang MA, Gordon S, Pockros P, Poordad F,
 709 Shores N, Moehlen MW, Bambha K, Clark V, Satapathy S, Parekh S,
 710 Reddy RK, Sheikh MY, Szabo G, Vierling J, Foster T, Umpierrez G, Chang
 711 C, Box T, Gallegos-Orozco J. Elafibranor, an Agonist of the Peroxisome
 712 Proliferator–Activated Receptor– α and – δ , Induces Resolution of Nonalcoholic
 713 Steatohepatitis Without Fibrosis Worsening. *Gastroenterology* 150: 1147–
 714 1159.e5, 2016.
- 715 45. Rotman Y, Sanyal AJ. Current and upcoming pharmacotherapy for non-
 716 alcoholic fatty liver disease. *Gut* 66: 180–190, 2017.
- 717 46. Savkur RS, Bramlett KS, Michael LF, Burris TP. Regulation of pyruvate
 718 dehydrogenase kinase expression by the farnesoid X receptor. *Biochem Biophys*
 719 *Res Commun* 329: 391–396, 2005.
- 720 47. Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression
 721 and regression in NASH. *J Hepatol* 68: 238–250, 2018.
- 722 48. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW,
 723 Mooney RA. Suppressor of Cytokine Signaling-3 (SOCS-3), a Potential
 724 Mediator of Interleukin-6-dependent Insulin Resistance in Hepatocytes *. 278:
 725 13740–13746, 2003.
- 726 49. Softic S, Cohen DE, Kahn CR. Role of Dietary Fructose and Hepatic De Novo
 727 Lipogenesis in Fatty Liver Disease. *Dig Dis Sci* 61: 1282–1293, 2016.
- 728 50. Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I.
 729 Toll-like receptor 4 is involved in the development of fructose-induced hepatic
 730 steatosis in mice. *Hepatology* 50: 1094–1104, 2009.
- 731 51. Steatohepatitis N, Se B, Mas E, Danjoux M, Garcia V. IL-6 Deficiency
 732 Attenuates Murine Diet-Induced. 4: 1–10, 2009.
- 733 52. Stefan N, Peter A, Cegan A, Staiger H, Machann J, Schick F, Claussen CD,
 734 Fritsche A, Häring HU, Schleicher E. Low hepatic stearyl-CoA desaturase 1
 735 activity is associated with fatty liver and insulin resistance in obese humans.
 736 *Diabetologia* 51: 648–656, 2008.
- 737 53. Stojšavljević S, Gomerčić Palčić M, Jukić, Virović L, Smirčić Duvnjak L,
 738 Duvnjak M. Adipokines and proinflammatory cytokines, the key mediators in
 739 the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 20:
 740 18070–18091, 2014.
- 741 54. Stribos EGD, Luangmonkong T, Leliveld AM, de Jong IJ, van Son WJ,
 742 Hillebrands J-L, Seelen MA, van Goor H, Olinga P, Mutsaers HAM.
 743 Precision-cut human kidney slices as a model to elucidate the process of renal
 744 fibrosis. *Transl Res* 170: 8-16.e1, 2016.
- 745 55. Stribos EGD, Seelen MA, van Goor H, Olinga P, Mutsaers HAM. Murine
 746 Precision-Cut Kidney Slices as an ex vivo Model to Evaluate the Role of
 747 Transforming Growth Factor- β 1 Signaling in the Onset of Renal Fibrosis. *Front*
 748 *Physiol* 8: 1–9, 2017.
- 749 56. Suriguga S, Luangmonkong T, Bigaeva E, Oosterhuis D, Mutsaers HAM,
 750 Groothuis GMM, Olinga P. LPS aggravates fibrosis only in the early onset but
 751 not in the end stage of liver fibrosis. *Hepatology* 64: 840A, 2016.
- 752 57. Thomas M, Bayha C, Klein K, Müller S, Weiss TS, Schwab M, Zanger UM.
 753 The truncated splice variant of peroxisome proliferator-activated receptor alpha,
 754 PPAR α -tr, autonomously regulates proliferative and pro-inflammatory genes.
 755 *BMC Cancer* 15: 488, 2015.

- 756 58. **Thuy S, Ladurner R, Volynets V, Wagner S, Strahl S, Königsrainer A,**
 757 **Maier K-P, Bischoff SC, Bergheim I.** Nonalcoholic Fatty Liver Disease in
 758 Humans Is Associated with Increased Plasma Endotoxin and Plasminogen
 759 Activator Inhibitor 1 Concentrations and with Fructose Intake. *J Nutr* 138:
 760 1452–1455, 2008.
- 761 59. **Tølbøl KS, Kristiansen MNB, Hansen HH, Veidal SS, Rigbolt KTG, Gillum**
 762 **MP, Jelsing J, Vrang N, Feigh M.** Metabolic and hepatic effects of liraglutide,
 763 obeticholic acid and elafibranor in diet-induced obese mouse models of biopsy-
 764 confirmed nonalcoholic steatohepatitis. *World J Gastroenterol* 24: 179–194,
 765 2018.
- 766 60. **Uhrig A, Banafsche R, Kremer M, Hegenbarth S, Hamann A, Neurath M,**
 767 **Gerken G, Limmer A, Knolle PA.** Development and functional consequences
 768 of LPS tolerance in sinusoidal endothelial cells of the liver thelial cells (LSEC)
 769 clear portal venous blood from TLR4 surface expression of LSEC is not altered
 770 by Isolation of murine LSEC. (2005). doi: 10.1189/jlb.0604332.
- 771 61. **Vatakuti S, Pennings JLA, Gore E, Olinga P, Groothuis GMM.**
 772 Classification of Cholestatic and Necrotic Hepatotoxicants Using
 773 Transcriptomics on Human Precision-Cut Liver Slices. *Chem Res Toxicol* 29,
 774 2016.
- 775 62. **de Waal Malefyt R.** Interleukin 10(IL-10) inhibits cytokine synthesis by human
 776 monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med*
 777 174: 1209–1220, 1991.
- 778 63. **Walczak R, Carole B, Benoit N, Descamps E, Nathalie D, Megnien S, Hum**
 779 **D, Staels B, Friedman S, Loomba R, Harrison S.** Elafibranor and
 780 nitazoxanide synergize to reduce fibrosis in a NASH model. *J Hepatol* 68:
 781 S352–S353, 2018.
- 782 64. **Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski**
 783 **FJ.** Antioxidative function of L-FABP in L-FABP stably transfected Chang
 784 liver cells. *Hepatology* 42: 871–879, 2005.
- 785 65. **Wanninger J, Neumeier M, Hellerbrand C, Schacherer D, Bauer S, Weiss**
 786 **TS, Huber H, Schäffler A, Aslanidis C, Schölmerich J, Buechler C.** Lipid
 787 accumulation impairs adiponectin-mediated induction of activin A by increasing
 788 TGFbeta in primary human hepatocytes. *Biochim Biophys Acta - Mol Cell Biol*
 789 *Lipids* 1811: 626–633, 2011.
- 790 66. **Westra IM, Mutsaers HAM, Luangmonkong T, Hadi M, Oosterhuis D, de**
 791 **Jong KP, Groothuis GMM, Olinga P.** Human precision-cut liver slices as a
 792 model to test antifibrotic drugs in the early onset of liver fibrosis. *Toxicol Vitro*
 793 35: 77–85, 2016.
- 794 67. **Westra IM, Pham BT, Groothuis GMM, Olinga P.** Evaluation of fibrosis in
 795 precision-cut tissue slices. *Xenobiotica* 43: 98–112, 2013.
- 796 68. **Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE.**
 797 Liver and Biliary Tract Increased Hepatic and Circulating Interleukin-6 Levels
 798 in Human Nonalcoholic Steatohepatitis. 6: 1372–1379, 2008.
- 799 69. **Yamaguchi K, Itoh Y, Yokomizo C, Nishimura T, Niimi T, Fujii H,**
 800 **Okanoue T, Yoshikawa T.** Blockade of interleukin-6 signaling enhances
 801 hepatic steatosis but improves liver injury in methionine choline-deficient diet-
 802 fed mice. *Lab Invest* 90: 1169–1178, 2010.
- 803 70. **Yang L, Roh YS, Song J, Zhang B, Liu C, Loomba R, Seki E.** Transforming
 804 growth factor beta signaling in hepatocytes participates in steatohepatitis
 805 through regulation of cell death and lipid metabolism in mice. *Hepatology* 59:

- 806 483–495, 2014.
- 807 71. **Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George**
- 808 **J, Bugianesi E.** Global burden of NAFLD and NASH: trends, predictions, risk
- 809 factors and prevention. *Nat Rev Gastroenterol Hepatol* 15: 11–20, 2017.
- 810

Figure legends

Fig. 1 – Schematic representation of the NAFLD induction in Bl/6 mice and precision-cut liver preparation and culture.

Fig. 2 – The effects of AMLN and CDAA on the livers of Bl/6 mice. (A) Liver to body weight ratio; (B) Hydroxyproline (hyp) content; (C) H&E and Sirius Red staining of representative mouse liver section; Note the presence of macrosteatosis (black arrow), microsteatosis (white arrow), inflammatory infiltrated cells (dashed arrow), ballooning degeneration (double head black arrow) and collagen I and III fibers (black arrow head); (D) mRNA expression levels of (D1) fibrosis, (D2) inflammation and (D3) fat metabolism related markers in PCLS prior culture. *p*-value assessed by Mann-Whitney test; **p* < 0.05, ***p* < 0.01, ****p* < 0.001 significantly different from livers of the corresponding control diet. Data are expressed as means (± SEM), n=4 for AMLN, n=3 for hyp AMLN, n=4 for CTR AMLN, n=10 for CDAA diet and n=8 for CTR CDAA diet.

Fig. 3 – The effects of 48h culture on PCLS regarding (A) fibrosis, (B) inflammation and (C) fat metabolism related markers. mRNA expression levels of markers after 48h culture; fold induction is relative to cPCLS prior culture, using the corresponding control diet for each NAFLD-inducing diet; Data are expressed as means (± SEM); *p*-value assessed by Mann-Whitney test; **p* < 0.05, ***p* < 0.01, ****p* < 0.001 significantly different from PCLS of the corresponding diet prior incubation (0h); n=4 for AMLN and CTR AMLN, n=10 for CDAA diet and n=8 for CTR CDAA.

Fig. 4 – The effects of LPS on PCLS regarding (A) fibrosis markers, (B) pro-collagen Iα1 secretion, (C) inflammation and (D) fat metabolism related markers. mRNA expression levels of markers after 48h LPS treatment; fold induction is relative to cPCLS prior culture (0h), using the corresponding control diet for each NAFLD-inducing diet; Pro-collagen Iα1 released in the culture media in the last 24h of incubation of control and steatotics PCLS treated with LPS; Data are expressed as means (± SEM); *p*-value assessed by Mann-Whitney test; **p* < 0.05, ***p* < 0.01, ****p* < 0.001 significantly different from PCLS of the corresponding diet; n=4 for AMLN and CTR AMLN, n=10 for CDAA diet, n=8 for CTR CDAA diet, n=5 for pro-collagen Iα1 CDAA and CTR CDAA.

Fig. 5 – The effects of TGFβ1 on PCLS regarding (A) fibrosis markers, (B) pro-collagen Iα1 secretion, (C) inflammation and (D) fat metabolism related markers. mRNA expression levels of markers after 48h TGFβ1 treatment; fold induction is relative to cPCLS prior culture (0h), using the corresponding control diet for each NAFLD-inducing diet; Pro-collagen Iα1 released in the culture media in the last 24h of incubation of control and steatotics PCLS treated with TGFβ1; Data are expressed as means (± SEM); *p*-value assessed by Mann-Whitney test; **p* < 0.05, ***p* < 0.01, ****p* < 0.001 significantly different from PCLS of the corresponding diet; n=4 for AMLN, n=3 for CTR AMLN, n=9 for CDAA, n=8 for CTR CDAA, pro-collagen Iα1: n=5 for CDAA and CTR CDAA and n=4 for AMLN and CTR AMLN.

Fig. 6 – The effect of elafibranor in steatotic and control CDAA PCLS. mRNA expression levels of (A) fibrosis, (B) inflammation, (C) fat metabolism related markers after 48h elafibranor treatment (0.2 or 1 μM); fold induction is relative to cPCLS prior culture (0h); Data are expressed as means (± SEM); *p*-value assessed by Kruskal-Wallis test (exact *p* value); **p* < 0.05, ***p* < 0.01, ****p* < 0.001 significantly different from untreated PCLS of the corresponding diet; n=4 for CTR CDAA and n=5 for CDAA PCLS.

864 **Fig. 7 – The effect of elafibranor on mice on CDAA diet.** (A) Serum triglycerides; (B) Liver
 865 to body weight ratios; (C) Hepatic hyp content expressed as $\mu\text{g hyp/mg liver tissue}$; mRNA
 866 expression levels of (D) fibrosis, inflammation, and (E) fat metabolism related markers; fold
 867 induction is relative to mice on CDAA treated with vehicle; Data are expressed as means ($\pm$
 868 SEM), p -value assessed by Mann-Whitney test; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly
 869 different from CDAA vehicle; $n=11$.
 870

871 **Supplementary figures**

872 **S1 –Masson’s trichrome staining of representative mouse liver sections from mice on**
 873 **AMLN and CDAA and their corresponding control diets.**

874 Supplemental Material available at

875 URL: <https://figshare.com/s/19c5e641d85460ee0b20>

876 DOI: 10.6084/m9.figshare.11310515

877

878 **S2 – The mRNA expression levels of fibrosis, inflammation and fat metabolism markers**
 879 **in mouse PCLS obtained from mice on the control diets for AMLN and CDAA diets.**

880 Supplemental Material available at

881 URL: <https://figshare.com/s/3d60c81eeb44731879f7>

882 DOI: 10.6084/m9.figshare.8966267

883

884 **S3 – Viability of murine PCLS from mice on AMLN and CDAA and their corresponding**
 885 **control diets.**

886 Supplemental Material available at

887 URL: <https://figshare.com/s/460965ae3d8f30d95da1>

888 DOI: 10.6084/m9.figshare.8966327

889

890 **S4 - The effect of TGFβ1 on the fibrotic area of AMLN and CDAA sPCLS and cPCLS.**

891 Supplemental Material available at

892 URL: <https://figshare.com/s/04dc920a89b309cdc54a>

893 DOI: 10.6084/m9.figshare.8966330

894

895 **S5 - The effect of CDAA diet on fat metabolism in murine livers.**

896 Supplemental Material available at

897 URL: <https://figshare.com/s/628e714cc0b605875371>

898 DOI: 10.6084/m9.figshare.8966333

899

900 **S6 - The effect of elafibranor *ex vivo* in CDAA s/cPCLS.**

901 Supplemental Material available at

902 URL: <https://figshare.com/s/e77fc1cd8dec825531c5>

903 DOI: 10.6084/m9.figshare.8966336

904

905 **S7 - The effect of elafibranor *in vivo* in CDAA-fed mice.**

906 Supplemental Material available at

907 URL: <https://figshare.com/s/23f5c930b9409ff68c15>

908 DOI: 10.6084/m9.figshare.8966339

909

Table 1 – Overview of Sybr Green primers

Name	Forward	Reverse
<i>Hmbs</i>	ATGAGGGTGATTCGAGTGGG	TTGTCTCCCGTGGTGGACATA
<i>Col1a1</i>	TGACTGGAAGAGCGGCGAGT	ATCCATCGGTCATGCTCTCT
<i>Serpinh1</i>	AGGTCACCAAGGATGTGGAG	CAGCTTCTCCTTCTCGTCGT
<i>Acta2</i>	ACTACTGCCGAGCGTGAGAT	CCAATGAAAGATGGCTGGAA
<i>Fn1</i>	CGGAGAGAGTGCCCCCTACTA	CGATATTGGTGAATCGCAGA
<i>Il1b</i>	GCCAAGACAGGTCGCTCAGGG	CCCCCACACGTTGACAGCTAGG
<i>Il6</i>	TGATGCTGGTGACAACCACGGC	TAAGCCTCCGACTTGTGAAGTGGTA
<i>Tnf</i>	CTGTAGCCACGTCGTAGC	TTGAGATCCATGCCGTTG
<i>Fasn</i>	CTGCGGAACTTCAGGAAATG	GGTTCGGAATGCTATCCAGG
<i>Acaca</i>	GCGTCGGGTAGATCCAGTT	CTCAGTGGGGCTTAGCTCTG
<i>Acox</i>	ATGCCTTTGTTGTCCCTATC	CCATCTTCAGGTAGCCATTATC
<i>Cpt1a</i>	TCCACCCTGAGGCATCTATT	ATGACCTCCTGGCATTCTCC
<i>Ppara</i>	CACGCATGTGAAGGCTGTAA	GCTCCGATCACACTTGTGCG
<i>Cyp4a</i>	GCTAGCTCCTTGATTGGGTA	AGGGTTTCAGAATGTCATAGTGG
<i>Acadm</i>	AGTACCCTGTGGAGAAGCTGAT	TCAATGTGCTCACGAGCTATG
<i>Hmgcs2</i>	CTGTGGCAATGCTGATCG	TCCATGTGAGTTCCCCCTCA
<i>Cd36</i>	TTGAAAAGTCTCGGACATTGAG	TCAGATCCGAACACAGCGTA
<i>Fabp1</i>	CCATGACTGGGGAAAAAGTC	GCCTTTGAAAGTTGTCACCAT
<i>Me1</i>	CAGAGGCCCTGAGTATGACG	CCGATTGGCAAAATCTTCAA
<i>Scd1</i>	TTCCCTCCTGCAAGCTCTAC	CAGAGCGCTGGTCATGTAGT
<i>Apoa2</i>	CAGCACAGAATCGCACTGTT	TCCGTCTGCCTGTCTCTTAAC
<i>Apoa5</i>	GCCAAAACAGTTGGAGCAA	GAAGCTGCCTTTCAGGTTCTC
<i>Angptl4</i>	GGGACCTTAAGTGTGCAAG	GAATGGCTACAGGTACCAAACC
<i>Pdk4</i>	CGCTTAGTGAACACTCCTTCG	CTTCTGGGCTCTTCTCATGG
<i>Pex11a</i>	TTCATCCGAGTCGCCAAC	CATGCATGCGTGCTGAGT

A.

B.

C.

A.

B.

C.

D.

Fatty acid oxidation and ketogenesis

Fatty acid and very low density lipoproteins production

Fatty acid transport

Triglyceride clearance

Glucose metabolism

Peroxisome proliferation

A. Serum triglycerides

B. Liver weight

C. Hyp

D. *Col1a1*

Acta2

Tnfa

E. *Acox1*

Cyp4a10

Scd1

Fabp1

Angptl4

Pdk4

Pex11a

A. Ex vivo

	Fibrosis	Inflammation	Lipid metabolism
Control	↑	↑	↓
LPS	-	↑↑	-
TGFβ	↑↑	-	↓
Elafibranor	-	-	↑

B. In vivo

	Fibrosis	Inflammation	Lipid metabolism
Elafibranor	↓	↓	↑