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Obeticholic acid raises LDL-cholesterol and reduces HDL-cholesterol in the Diet-Induced NASH (DIN) hamster model

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ABSTRACT

The use of rat and mouse models limits the translation to humans for developing novel drugs targeting non-alcoholic steatohepatitis (NASH). Obeticholic acid (OCA) illustrates this limitation since its dyslipidemic effect in humans cannot be observed in these rodents. Conversely, Golden Syrian hamsters have a lipoprotein metabolism mimicking human dyslipidemia since it does express the cholesteryl ester transfer protein (CETP). We therefore developed a Diet-Induced NASH (DIN) hamster model and evaluated the impact of OCA.

Compared with chow fed controls, hamsters fed for 20 weeks with a free-choice (FC) diet, developed obesity, insulin resistance, dyslipidemia and NASH (microvesicular steatosis, inflammation, hepatocyte ballooning and perisinusoidal to bridging fibrosis). After 20 weeks of diet, FC fed hamsters were treated without or with obeticholic acid (15 mg/kg/day) for 5 weeks. Although a non-significant trend towards higher dietary caloric intake was observed, OCA significantly lowered body weight after 5 weeks of treatment. OCA significantly increased CETP activity and LDL-C levels by 20% and 27%, and reduced HDL-C levels by 20%. OCA blunted hepatic gene expression of Cyp7a1 and Cyp8b1 and reduced fecal bile acids mass excretion by 64% ($P < 0.05$). Hamsters treated with OCA showed a trend towards higher scavenger receptor Class B type I (SR-BI) and lower LDL-receptor hepatic protein expression. OCA reduced NAS score for inflammation ($P < 0.01$) and total NAS score, although not significantly.

Compared to mouse and rat models, the DIN hamster replicates benefits and side effects of OCA as observed in humans, and should be useful for evaluating novel drugs targeting NASH.

1. Introduction

Due to its tight positive correlation with obesity and type 2 diabetes, the prevalence of nonalcoholic fatty liver diseases (NAFLD) is increasing substantially worldwide (Hardy et al., 2015). NAFLD represent a spectrum of disorders, which range from nonalcoholic fatty liver or simple liver steatosis, to nonalcoholic steatohepatitis (NASH), in which inflammatory infiltrates, hepatocyte ballooning and fibrosis can be observed, increasing the risk to reach the most severe stages, i.e. cirrhosis and hepatocellular carcinoma (Wree et al., 2013). Concomitant with the growing urbanization level, physical inactivity and consumption of energy dense foods contributes to raise the prevalence of NASH (Farrell, 2003). Increased dietary fructose intake through processed food and beverages consumption is also suspected as a major contributor to NASH, due to the stimulating effect of fructose on hepatic de novo lipogenesis (Kawasaki et al., 2009; Vos and Lavine, 2013; Softic et al., 2016). Although lifestyle and diet changes remain the primary intervention, novel therapies targeting NASH are needed, but no effective drug for NASH treatment is currently available (Sawangjit et al., 2016). To this aim, preclinical mouse and rat models are extensively used to develop these new drugs. A plethora of NASH models are currently available (see Imajo et al. (2013) and Ibrahim et al. (2016) for review), with various approach based on diet induction (e.g. methionine choline deficient or high fat/cholesterol/fructose diets), chemical induction (carbon tetrachloride or thioacetamide) or genetic modification (ob/ob, db/db mice, etc.). Each experimental approach may be used separately or in combination. While these models are mandatory tools for drug development, none fully replicates the NASH phenotype observed in the clinic (Imajo et al., 2013). Most importantly, interspecies differences may still limit the interpretation of drug evaluation in rodents and may not be relevant to the human disease (Ibrahim et al., 2016). For instance, obeticholic acid (OCA), a farnesoid X receptor (FXR) agonist, has shown to improve NASH features in clinical trials (Neuschwander-Tetri et al., 2015). However, while side-effects, including increased LDL-cholesterol and decreased HDL-cholesterol levels were also observed in clinical trials (Neuschwander-Tetri

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et al., 2015; Pencek et al., 2016), none was previously identifiable in rat and mouse models (Cipriani et al., 2010; Xu et al., 2016). This failure was due to substantial differences in lipid and lipoprotein metabolism, precisely the lack of the cholesteryl ester transfer protein (CETP) in mice and rats, when compared to human. CETP plays an important role in human cholesterol metabolism and alters lipoprotein profile since it transfers cholesterol from high density lipoprotein (HDL) to very low density and low density lipoprotein (VLDL and LDL) particles (Weber et al., 2010). Unlike mouse and rat, the Golden Syrian hamster expresses CETP. Therefore, this animal model better replicates human lipoprotein metabolism and is more predictive for evaluating the effects of drugs on lipoprotein metabolism (Briand, 2010). However, its relevance as a preclinical model for NASH within the context of obesity, has not yet been validated. In the present study, we aimed to develop a Diet-Induced NASH (DIN) hamster model using a free-choice diet approach (la Fleur et al., 2014), mimicking high fat and fructose over consumption in humans. To validate the relevance of such model, we also evaluated the effects of the FXR agonist OCA.

2. Materials and methods

2.1. Animals and diet

All animal protocols were reviewed and approved by the local (Comité régional d'éthique de Midi-Pyrénées) and national (Ministère de l'Enseignement Supérieur et de la Recherche) ethics committees (protocol number CEEA-122–2014-15). Male Golden Syrian hamsters (Elevage Janvier, Le Genest Saint Isle, France, 91–100 g, 6-week old at the beginning of the study) were fed for 20 weeks a control chow (CC) diet (5.1% fat, 19.3% protein, 55.5% carbohydrates, from Safe Diets) with normal drinking water (n = 9) or a free-choice (FC) diet (n = 24), which consists of a choice, within the same cage, between CC diet with normal drinking water or a high fat/high cholesterol (HF/HC) diet (40.8% fat, 14.8% protein, 44.4% carbohydrates and 0.5% cholesterol from Safe Diets, France) with 10% fructose-enriched drinking water. The HF/HC diet was a mixture of 55% CC diet, 20% peanut butter paste (Skippy, Hormel Foods Corporation, Austin, MN, USA) and 25% hazelnut paste (Nustikao, Leclerc, Ivry-sur-Seine, France), with vegetable oils as fat source. A satellite group of 6 additional hamsters fed the FC diet were used to evaluate liver lesions by histology analysis at 12 weeks and 16 weeks of diet (n = 3 hamsters at each time point).

After 20 weeks of diet, hamsters fed the FC diet (n = 24) were allocated into 2 homogenous treatment groups of 12 hamsters based on their HOMA-IR index (calculated from 6-h fasting blood glucose and plasma insulin levels), plasma LDL-cholesterol, total cholesterol, ALT levels and body weight. Hamsters under free-choice diet were then kept on the same diet supplemented without (control FC, n = 12) or with OCA at 15 mg/kg/day (FC + OCA, n = 12) for 5 weeks. OCA (purity > 97%) was purchased from Selleck Chemicals LLC, Houston, TX, USA. The 15 mg/kg/day dose of OCA was selected from a pilot study in a 4-week high fat fed hamster model where significant reduction in liver lipids were observed.

At the end of the treatment, feces were collected over 24 h for determination of fecal total cholesterol and bile acids mass excretion.

A subset of FC (n = 5) and FC + OCA (n = 5) hamsters was used for in vivo intestinal cholesterol absorption assessment.

Other hamsters fasted for 6 h were then bled for plasma isolation, killed and exsanguinated with saline prior to liver collection. Plasma and liver samples were used to perform biochemical and liver histology analysis.

2.2. Biochemical analysis

Blood glucose was determined using a glucometer (Roche, Basel, Switzerland). Plasma insulin was determined using a commercial Elisa kit (Crystal Chem, Downers Grove, IL, USA). Total cholesterol and triglycerides were assayed using commercial kits (Sobioda, Montbonnot-Saint-Martin, France). Plasma CETP activity was measured by fluorescence using a commercial kit (Roarbiomedical, New York, NY, USA).

HDL-cholesterol was determined from supernatant after apolipoprotein B-containing lipoproteins precipitation with phosphotungstate/$MgCl_2$ precipitation method and centrifugation (Austin et al., 1984). Plasma LDL-cholesterol levels were measured using a commercial kit (Wako, Richmond, VA, USA). Plasma Alanine Aminotransferase (ALT) levels were determined using a colorimetric assay kit (Elitech, Puteaux, France) Fast Protein Liquid Chromatography (FPLC) analysis (total cholesterol) was performed as described previously (Briand et al., 2012). Colorimetric assay kits were used to determine hepatic lipids levels from liver homogenate after lipid solubilization with deoxycholate, as described previously (Briand et al., 2012). Colorimetric assay kits were used to measure total cholesterol (Sobioda, Montbonnot-Saint-Martin, France) and bile acids (Bioquant, San Diego, CA, USA) levels from feces homogenate after saponification and chemical extraction of cholesterol and bile acids, as described (Briand et al., 2012).

2.3. Hepatic gene and protein expression

Hepatic gene expression of Cyp7a1 and Cyp8b1, was performed as described previously (Briand et al., 2012). Hepatic protein expression of LDL-receptor and Scavenger Receptor Class B type I (SR-BI) was evaluated by Western Blot analysis with pan-cadherin as a loading control, as described (Briand et al., 2012).

2.4. Histology analysis and NAFLD activity scoring

Histology analysis and blinded NAFLD activity scoring (NAS) was performed by Histalim, Montpellier France. Liver samples were stored in formalin for 24 h then transferred in ethanol 70°, paraffin embedded, prior to Hematoxylin-Eosin (H&E) and Sirius Red staining of liver sections.

After staining, histology slides were used for a blinded histopathological NAS scoring, based on an adapted scoring system of Kleiner et al. (2005). Hepatocellular steatosis, liver inflammation, lobular fibrosis, and hepatocyte ballooning variables were qualitatively assessed and ranked with a score, and an individual NAS total score was then calculated for each animal by summing up the scores.

2.5. In vivo intestinal cholesterol absorption

Intestinal cholesterol absorption was measured after ^{14}C-cholesterol labeled olive oil gavage and poloxamer-407 (a plasma lipase inhibitor) intraperitoneal injection at 1 g/kg body weight, as described (Briand et al., 2012).

2.6. Statistical analysis

Data are presented as mean ± S.E.M. 2-way ANOVA with Bonferroni post-test or unpaired 2-tailed Student *t*-test were used for statistical analysis using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). A $P < 0.05$ was considered significant.

3. Results

3.1. Free-choice diet induces obesity, insulin resistance, dyslipidemia and NASH in hamsters

Hamsters fed the FC diet showed significantly higher caloric intake during the first 12 weeks of diet due to higher consumption of high fat and fructose-enriched drinking water (Fig. 1A–C), which led to significantly higher body weight (Fig. 1D) over 20 weeks of diet, as

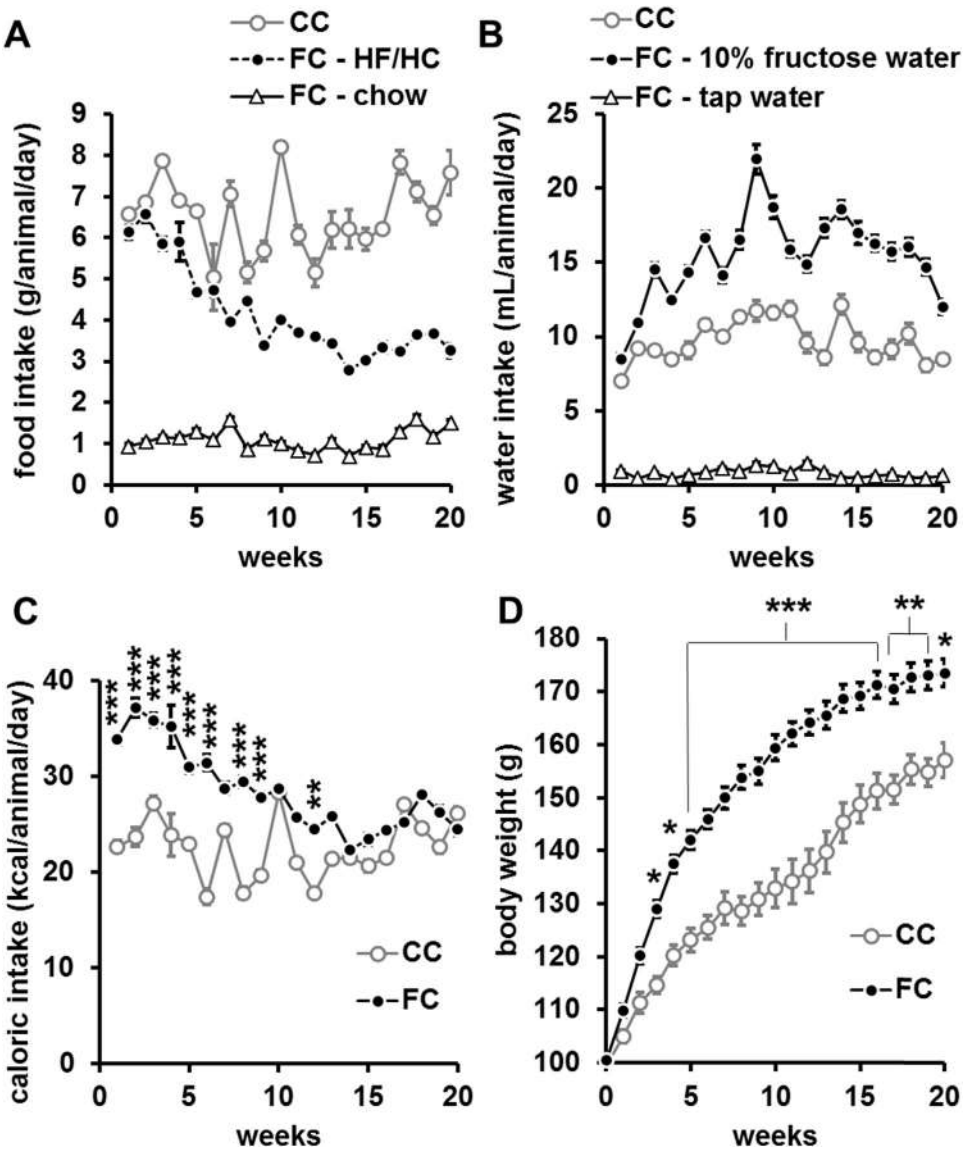

Fig. 1. (A) Food intake in control hamsters having free access to only a chow diet (CC) and in free-choice diet-fed hamsters (FC) having free access in the same cage to both chow (FC – chow) and high fat/high cholesterol (FC - HF/HC) diet; (B) Water intake in chow-fed control hamsters having free access to only normal tap water (CC) and in free-choice diet-fed hamsters (FC) having free access to both normal tap water (FC – tap water) and 10% fructose-enriched water (FC – 10% fructose water); (C) caloric intake and (D) body weight, in control chow-fed hamsters (CC) and free-choice diet-fed hamsters (FC). Data are presented as mean ± S.E.M. *P < 0.05, **P < 0.01 and ***P < 0.001 FC vs. CC hamsters.

Table 1
Plasma biochemical in 6-h fasted hamsters fed a control chow (CC; n = 9) or free-choice (FC; n = 24) diet for 20 weeks.

	CC	FC
Blood glucose (mM)	4.36 ± 0.21	4.29 ± 0.09
Plasma insulin (μU/mL)	8.07 ± 1.41	12.43 ± 1.29[a]
HOMA-IR ([mM x μU/mL]/22.5)	1.50 ± 0.19	2.32 ± 0.22[a]
Plasma ALT (U/L)	76 ± 7	108 ± 9[a]
Plasma triglycerides (mM)	1.38 ± 0.06	2.38 ± 0.13[b]
Plasma total cholesterol (mM)	4.09 ± 0.19	7.31 ± 0.25[b]
Plasma HDL-cholesterol (mM)	1.87 ± 0.15	4.65 ± 0.19[b]
Plasma LDL-cholesterol (mM)	1.29 ± 0.10	2.87 ± 0.17[b]

Data are presented as mean ± S.E.M.
[a] P < 0.05.
[b] P < 0.001 FC vs. CC.

compared with CC hamsters. As shown in Table 1, after 20 weeks of diet, hamsters fed the FC diet did not show altered fasting glycaemia but had significantly higher plasma insulin levels, leading to a 54% higher HOMA-IR index of insulin resistance (P < 0.05 vs. CC). Compared with CC hamsters, plasma ALT levels were significantly higher in FC hamsters by 41%. Hamsters fed the FC diet showed a dyslipidemic profile at 20 weeks of diet, with higher plasma triglycerides and total cholesterol levels by 72% and 79%, respectively (both P < 0.001 vs. CC). Plasma HDL-cholesterol and LDL-cholesterol levels were both increased by 149% and 122%, respectively (both P < 0.001 vs. CC) at the end of the 20-week period.

Compared with CC-fed hamsters, H&E and Sirius Red staining indicated that FC-fed hamsters showed progressive hepatic disorders that started to appear at 12 weeks, and were firmly established from 16 weeks of diet (Fig. 2A). Animals presented a marked pan-lobular microvesicular steatosis, accompanied by hepatocellular ballooning and degeneration, which was associated with a discrete to mild mixed inflammatory reaction surrounding degenerated hepatocytes (Fig. 2A, bottom left panel). Peri-sinusoidal, peri-portal and bridging fibrosis was also observed (Fig. 2A, bottom right panel). Of note, these progressive hepatic disorders were concomitant with significantly elevated plasma ALT (Fig. 2B), triglycerides (Fig. 2C) and total cholesterol levels (Fig. 2D) at 12 and 16 weeks.

Altogether, these data indicate that hamsters fed the FC diet for 20 weeks develop obesity, insulin resistance, dyslipidemia and NASH.

3.2. Obeticholic acid promotes body weight loss but induces dyslipidemic side effects

To test the relevance of the DIN hamster model, the effects of the

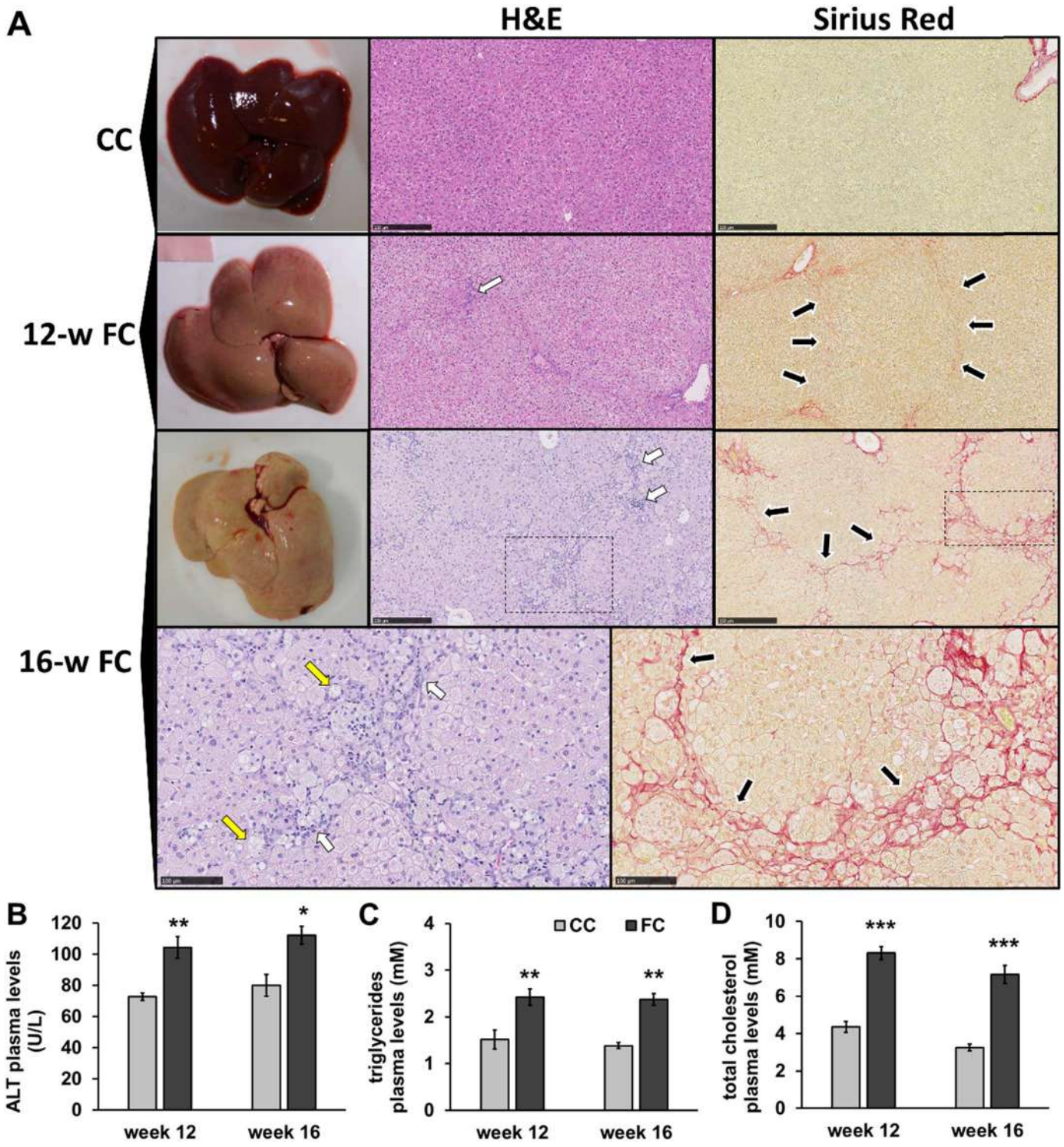

Fig. 2. (A) Representative pictures of macroscopic liver aspect, hematoxylin and eosin (H&E) and Sirius Red staining (X10) in chow fed hamsters (CC), and in free-choice (FC) diet-fed hamsters at 12 (12-w FC) or 16 (16-w FC) weeks of diet. White arrows indicate mononuclear cells infiltrate (inflammation), black arrows indicate fibrosis, yellow arrows indicate hepatocyte ballooning. Square with dashed line refers to the bottom pictures of H&E and Sirius red staining at 16 weeks of diet at higher magnification (X20); (B) Plasma ALT, (C) triglycerides, (D) total cholesterol levels at 12 and 16 weeks of diet in CC and FC hamsters. Data are presented as mean ± S.E.M. *P < 0.05, **P < 0.01 and ***P < 0.001 FC vs. CC hamsters.

FXR agonist OCA at 15 mg/kg/day for 5 weeks were evaluated.

As shown in Fig. 3A, OCA treatment for 5 weeks in FC fed hamsters resulted in significant body weight loss (− 11 g vs. FC). Body weight loss was not related to lower dietary caloric intake, since this parameter rather increased, although not significantly (Fig. 3B). Compared with FC hamsters, no significant change in fasting blood glucose, plasma insulin and HOMA-IR index of insulin resistance was observed in FC fed hamsters treated with OCA (data not shown). We next evaluated the effects of OCA on fecal steroids mass excretion after 5 weeks of treatment (Fig. 3C). A significant reduction in fecal cholesterol mass excretion was observed (44% lower vs. FC) in FC fed hamsters treated with OCA. OCA significantly reduced the mass of bile acids excreted in

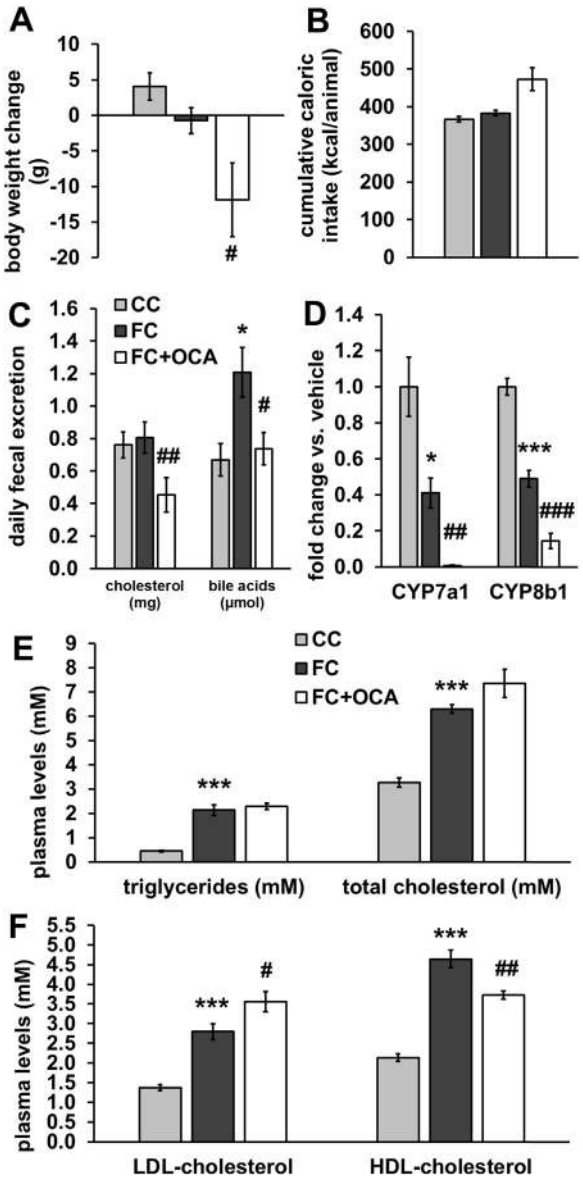

Fig. 3. (A) Body weight change, (B) cumulative caloric intake, (C) daily total cholesterol and bile acids fecal mass excretion, (D) hepatic gene expression of CYP7a1 and CYP8b1, (E) plasma triglycerides and total cholesterol levels, (F) plasma LDL-cholesterol and HDL-cholesterol levels in control chow-fed hamsters (CC) and free-choice diet-fed hamsters without (FC) or with obeticholic acid (FC + OCA). Data are presented as mean ± S.E.M. *P < 0.05 and ***P < 0.001 FC vs. CC hamsters; #P < 0.05, ##P < 0.01 and ###P < 0.001 FC + OCA vs. FC.

feces (39% lower, P < 0.05 vs. FC hamsters), an effect expected with a FXR agonist. To confirm that the FXR agonist OCA repress the key enzymes regulating bile acids synthesis from cholesterol in FC fed hamsters, the hepatic gene expression of FXR target genes CYP7a1 and CYP8b1, was measured (Fig. 3D). Compared to CC hamsters, expression of both genes was significantly reduced in FC hamsters, and was further blunted by OCA (P < 0.01 and P < 0.001 vs. FC hamsters, respectively), as expected.

After 5 weeks of treatment, OCA did not change fasting plasma triglycerides and total cholesterol levels (Fig. 3E). However, FC fed hamsters treated with OCA showed 27% higher plasma LDL-cholesterol levels (Fig. 3F), as compared with FC (P < 0.05). Additionally, OCA significantly reduced HDL-cholesterol levels by 20% as compared with FC hamsters (Fig. 3F), further indicating dyslipidemic side effects with OCA treatment.

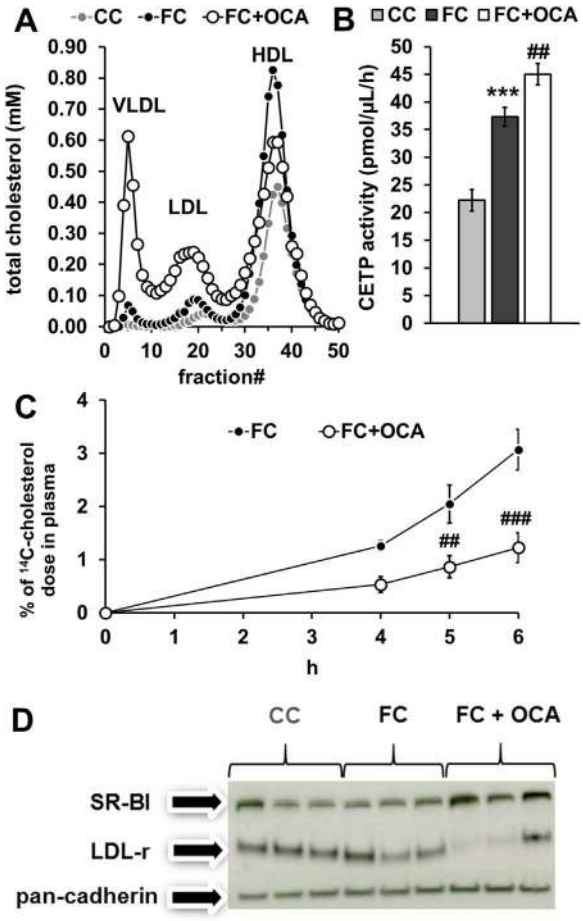

Fig. 4. (A) Fast Protein Liquid Chromatography profile for total cholesterol, (B) plasma cholesteryl ester transfer protein (CETP) activity, (C) ^{14}C-tracer appearance in plasma after ^{14}C-cholesterol gavage and poloxamer-407 intraperitoneal injection, (D) hepatic protein expression of SR-BI and LDL-receptor in control chow-fed hamsters (CC) and free-choice diet-fed hamsters without (FC) or with obeticholic acid (FC + OCA). Data are presented as mean ± S.E.M. ***P < 0.001 FC vs. CC hamsters; ##P < 0.01 and ###P < 0.001 FC + OCA vs. FC.

3.3. Obeticholic acid induces a dyslipidemic profile by altering CETP activity and hepatic protein expression of LDL-receptor and SR-BI

The dyslipidemic profile induced by OCA was confirmed by FPLC profile analysis (Fig. 4A), with higher levels of total cholesterol in fractions corresponding to VLDL and LDL, and lower levels in fractions corresponding to HDL particles. To understand the mechanism by which OCA alters the lipoprotein profile, CETP activity was measured, since higher activity may favour higher non-HDL cholesterol levels (Fig. 4B). As compared with CC fed hamsters, FC diet significantly increased CETP activity by 68% (P < 0.001 vs. CC). Hamsters treated with OCA showed a further 20% increase in CETP activity, when compared to FC fed hamsters (P < 0.01). Since a higher intestinal cholesterol absorption could have also contributed to higher LDL-cholesterol levels, a set of FC and FC + OCA hamsters were administrated orally with ^{14}C-cholesterol to assess plasma appearance of the radiotracer over time (Fig. 4C). Hamsters treated with OCA showed a significant reduction in the ^{14}C-tracer appearance at 5 and 6 h after radiolabelled cholesterol gavage. This result indicates a lower intestinal cholesterol absorption under OCA treatment, which excludes a possible contribution in the OCA-induced LDL-cholesterol increase. We next evaluated the hepatic protein expression of LDL-receptor and SR-BI by western blot analysis (Fig. 4D). Hepatic protein expression of SR-BI tended to be increased, while the expression of LDL-r tended to be reduced, indicating that the decrease in HDL-cholesterol and the raise in

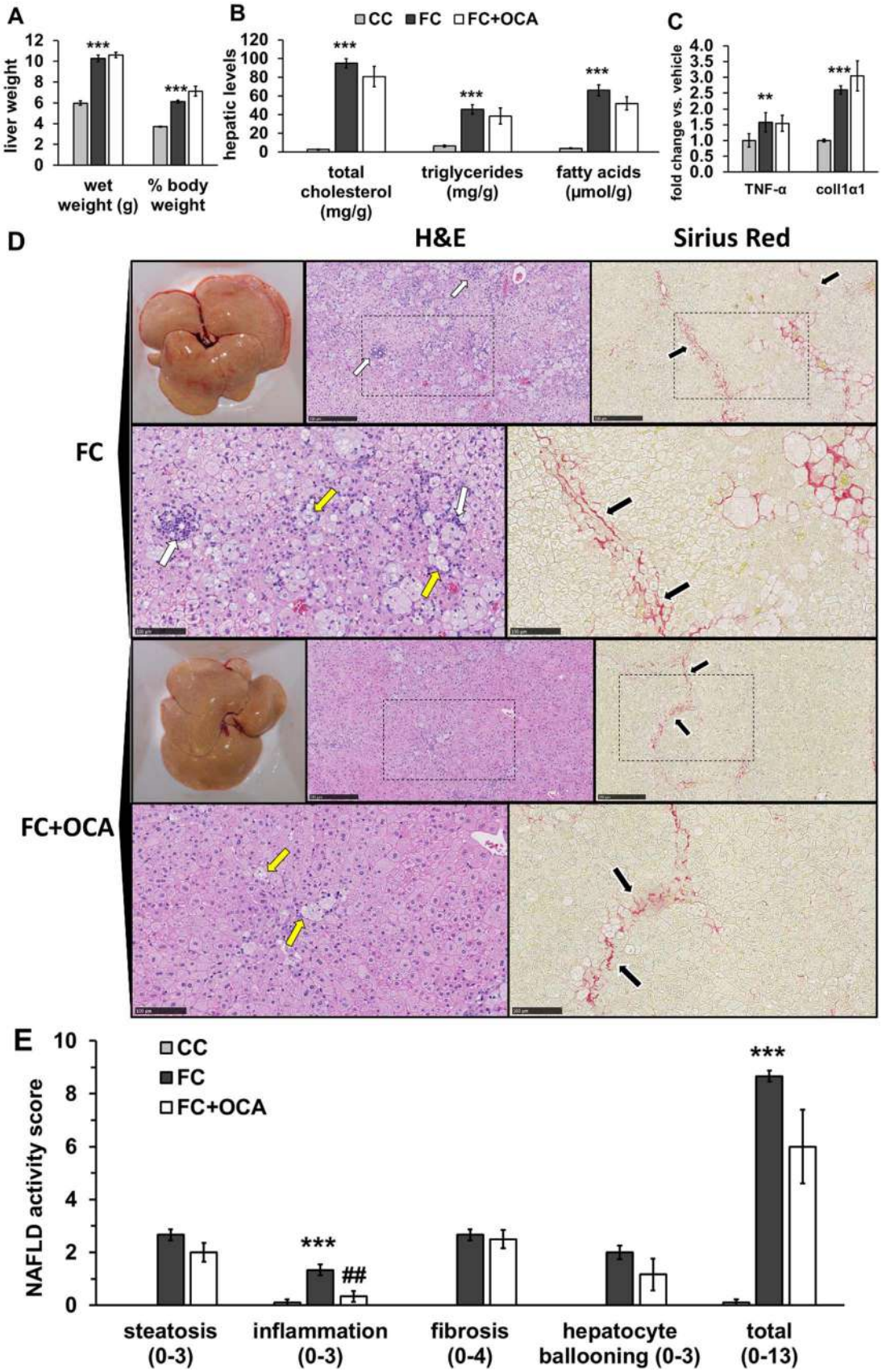

Fig. 5. (A) Liver weight, (B) hepatic total cholesterol, triglycerides and fatty acids levels, (C) hepatic gene expression of TNF-α and coll1α1, (D) representative pictures of macroscopic liver aspect, hematoxylin and eosin (H&E) and Sirius Red staining (X10). White arrows indicate mononuclear cells infiltrate (inflammation), black arrows indicate fibrosis, yellow arrows indicate hepatocyte ballooning. Square with dashed line refers to the bottom pictures of H&E and Sirius red staining for FC and FC + OCA at higher magnification (X20). (E) NAFLD activity score in control chow-fed hamsters (CC) and free-choice diet-fed hamsters without (FC) or with obeticholic acid (FC + OCA). Data are presented as mean ± S.E.M. **P < 0.01 and ***P < 0.001 FC vs. CC hamsters; ##P < 0.01 FC + OCA vs. FC.

LDL-cholesterol may be also related to the altered hepatic expression of both proteins.

3.4. Obeticholic acid markedly reduces liver inflammation and tends to improve NAS score

Compared with CC hamsters, hamsters fed the FC diet showed a 72% higher liver weight (Fig. 5A), which was not changed by OCA treatment. However, given OCA induced body weight loss, liver weight tended to be higher by 16% when expressed as % body weight (Fig. 5B), although this did not reach statistical significance ($P = 0.06$ vs. FC). Compared with CC fed hamsters, hepatic total cholesterol, triglycerides and fatty acids levels were 33-, 6- and 15-fold higher in FC fed hamsters (all $P < 0.001$), but were not changed with OCA treatment (Fig. 5B). Although hepatic gene expression of both TNF-α (involved in inflammation) and collagen1α1 (involved in fibrosis) was significantly higher in FC hamsters as compared with CC, OCA did not alter the expression of both genes (Fig. 5C).

As shown in Fig. 5D, histology analysis was performed using H&E and Sirius Red staining (hamsters fed the CC had similar histological features illustrated in Fig. 2 and pictures are not shown for clarity). Hamsters fed the FC diet showed micro-vesicular steatosis, accompanied by hepatocellular ballooning and degeneration associated, with inflammatory reaction surrounding degenerated hepatocytes. Peri-sinusoidal, peri-portal and bridging fibrosis was also observed in FC fed hamsters. Hamsters fed the FC + OCA diet presented mild to marked micro-vesicular steatosis, which was matching the extent of hepatocyte ballooning. OCA treatment profoundly reduced inflammation while fibrosis remained present in the majority of hamsters, and only a limited number of hamsters showed fibrosis limited to peri-sinusoidal and peri-portal spaces. In line with the histological observations, NAFLD activity scoring showed a trend towards lower steatosis score in FC + OCA hamsters, but not significantly (Fig. 5E). The inflammation score was markedly reduced by OCA ($P < 0.01$ vs. FC), while fibrosis score was not changed significantly. As for steatosis score, hepatocyte ballooning score tended to be reduced, but not significantly. Overall, OCA treatment resulted in a non-significant decrease in total NAS score.

4. Discussion

Using a nutritional approach that reflects overconsumption of fat and fructose observed in humans, we have developed a DIN hamster model combining obesity, insulin resistance, dyslipidemia and NASH. Despite its similarities with human lipoprotein metabolism, and the rapid induction of dyslipidemia and liver steatosis under high fat/cholesterol diets (Briand, 2010), the use of this species for the study of NASH has been very limited. To our knowledge, only one study has previously highlighted its potential as a NASH model by feeding hamsters with a high fat/high cholesterol diet (Lai et al., 2016), leading to similar liver complications (i.e. inflammation, hepatocyte ballooning and fibrosis) observed in our study. However, the model of Lai et al. was fed with a 2-fold higher percentage of cholesterol in the diet as in our study, and was not associated with obesity and insulin resistance, which does not fully replicate the context of human NASH. Indeed, feeding hamsters with diets supplemented with fat, cholesterol and also fructose, induces several metabolic disorders (i.e. insulin resistance, dyslipidemia and liver steatosis), but not obesity (Briand et al., 2012; Basciano et al., 2009). The lack of obesity induction is probably related to an unchanged eating behaviour of the hamsters when fed such diets. Therefore, no marked increase in energy intake is expected. In contrast, our study indicates that the free-choice diet approach avoids this drawback by favouring fat, cholesterol and fructose overconsumption, resulting in substantial weight gain and obesity in hamsters. Besides induction of insulin resistance and dyslipidemia, free-choice diet progressively induced liver disorders during the diet period (liver steatosis, inflammation, hepatocellular ballooning and fibrosis). Compared to our DIN hamster model, a large number of NAFLD/NASH rat and mouse models have already been developed (reviewed by Imajo et al. (2013) and Ibrahim et al. (2016)). While chemically-induced and genetic models may not reproduce human NAFLD/NASH robustly, high fat/cholesterol/fructose fed models may better reflect the context of human NASH (i.e. obesity and insulin resistance). However, these dietary models seem to develop less inflammation, hepatocyte ballooning and fibrosis upon high fat diet, although the addition of fructose seems to compensate this weaker induction (Ibrahim et al., 2016). These variable diet-induction of NASH also depends on the strain itself: for instance, C57BL6/J mice are known to be resistant to hepatic fibrosis (Walkin et al., 2013). When fed the same high cholesterol, high fat diet, C57BL6/J mice develop mild steatosis and little inflammatory cell infiltration, while hamsters developed advanced steatosis, inflammation, ballooning and fibrosis (Lai et al., 2016). Additionally, a clear difference in bile acids metabolism and composition is also observed. Mouse and rat mainly synthesize β-muricholic acid, which is not synthesized in hamsters and humans (Matsuzaki et al., 2002). Hence, compared to rats and mice, the hamster seems to respond better to diet promoting NASH, with evident inflammation, hepatocyte ballooning and fibrosis, and has the advantage to present a closer lipoprotein and bile acids metabolism to humans. While it further confirms this potential, the present study also indicates that our DIN hamster model may also recapitulate human NASH in the context of obesity, insulin resistance and dyslipidemia, and should be helpful to evaluate the therapeutic potential of drugs targeting NASH.

To validate the relevance of our present animal model, the effects of OCA, a FXR agonist evaluated in a phase 3 clinical trial, were investigated. We here show that in the DIN hamster model, the impact of OCA on lipoprotein profile was similar to that observed in humans during clinical trials [3,4]. LDL-cholesterol levels were higher and HDL-cholesterol levels were lower in the former model. This dyslipidemic lipoprotein profile was linked to higher activity of CETP and expression of hepatic SR-BI, both known to alter HDL-cholesterol levels (Weber et al., 2010; Hoekstra, 2017), and a trend towards lower expression of LDL-receptor expression, which regulates plasma LDL-cholesterol levels. In line with another preclinical study (Xu et al., 2016), FXR activation with OCA led to lower intestinal cholesterol absorption, which excludes a possible intestinal contribution to higher LDL-cholesterol levels. Despite the reduced intestinal cholesterol absorption, a significantly lower fecal cholesterol mass excretion was observed in hamsters treated with OCA. Although this was not investigated in the present study, it is indeed possible that inhibition of bile acids synthesis under OCA treatment would lead to impaired biliary cholesterol secretion (Li and Chiang, 2009), which in turn would limit fecal cholesterol mass excretion.

A reduction of HDL-cholesterol levels in hamsters treated with OCA was also observed, in agreement with previously published mouse (Xu et al., 2016), rat (Cipriani et al., 2010) and hamster studies (Dong et al., 2017). However, these studies did not detect an increase in LDL-cholesterol, due to the absence of CETP in the mouse and rat models (Cipriani et al., 2010; Xu et al., 2016). In contrast with human trials and our present study, Dong et al. measured a reduction in plasma LDL-cholesterol and hepatic total cholesterol with OCA in their hyperlipidemic hamster model (Dong et al., 2017). The shorter diet duration to induce hyperlipidemia may explain the different effect of OCA on LDL-cholesterol levels and suggests that a minimum duration of diet induction is required to evaluate the therapeutic efficacy of drugs targeting NASH in hamster models.

Overall, some reports indicate that hamsters fed a high fat/high cholesterol diet can develop marked dyslipidemia and liver steatosis in a very short period of time, i.e. within 10 days (Wang et al., 2001; Briand, 2010; Srivastava and He, 2010; Briand et al., 2012; Chen et al., 2014), which would correspond to the first stage of NAFLD, namely simple steatosis. However, a longer period of diet intervention is required to reach more advanced liver lesions with the characteristics of

NASH. In the present study, a minimum of 16-week diet duration was needed to reach advanced complications, which is a required experimental condition to evaluate the benefits and side effects of a drug targeting NASH, such as OCA.

Besides weight loss, treatment with OCA led to significant improvement in liver inflammation in the DIN hamster, in agreement with rodent (Zhang et al., 2017) and human (Neuschwander-Tetri et al., 2015) studies. More variability was observed for steatosis and hepatocyte ballooning, suggesting that NASH resolution with OCA may be limited to a number of individuals, as observed in the FLINT trial (Neuschwander-Tetri et al., 2015). Unlike humans, fibrosis score was not changed in DIN hamster treated with OCA. We acknowledge that unchanged fibrosis score would represent a drawback of our model for translating the present data to humans. Meanwhile, it is possible that an earlier and longer treatment with OCA, and/or a different dose than 15 mg/kg, may be required to impact the score of fibrosis in our model. Alternatively, the 20-week diet-induced fibrosis could also induce irreversible crosslinking of the extracellular matrix, leading to uncleavable collagen fibers (Xu et al., 2014; Ikenaga et al., 2017). Such mechanism would compromise the resolution of fibrosis with OCA in the DIN hamster.

5. Conclusion

The present study suggests that the DIN hamster described here represents a good alternative to overcome the drawbacks characterizing other rodent models, therefore allowing a proper evaluation of the therapeutic efficacy of novel compounds targeting NASH. Furthermore, this animal model allows detecting dyslipidemic side effects. Further investigations are required to dissect the molecular mechanisms behind the hepatic benefits and the dyslipidemic side effects observed with OCA in our model. Meanwhile, this preclinical model should already be useful to detect potential side effects and better predict the benefits of future NASH therapies in humans.

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Authors contribution

F.B., R.B. and T.S. designed research, F.B., E.B., and M.Q. conducted research, F.B. analysed data, F.B. and R.B. wrote the manuscript.

Conflict of interest

F.B., T.S., E.B., M.Q. are employees of Physiogenex.
R.B. has shares in Physiogenex.

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