Prevention of liver damages by targeting different physiological mechanisms in two murine NASH models

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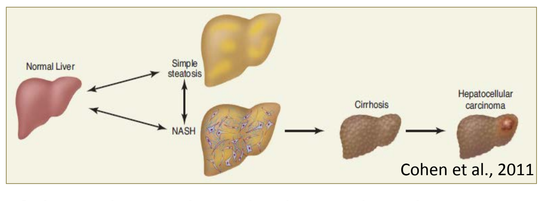
INTRODUCTION

In Western countries, prevalence of NAFLD (non alcoholic fatty liver diseases) has been rising because of its association with metabolic syndrome. Steatosis, the first state of NAFLD, is benign but can progress for 30% to steatohepatitis (NASH), and then to fibrosis, cirrhosis and hepatocellular carcinoma.



Cohen et al., 2011

We investigated the effects of three drugs, described to reduce hepatic steatosis and inflammation through different pathways, in a murine model of NASH associated with obesity and insulin resistance and a murine model of liver fibrosis.

METHODS

- C57Bl/6J mice were fed with a specific diet leading to liver injuries: Diet Induced NASH (DIN : high fat, high cholesterol, high fructose) during 6 or 17 weeks. One group received 9 low-dose CCl_4 i.p. injection during 4 weeks, after 4 weeks of DIN .
- The model was validated by three different pharmacological compounds included in the diet: **ezetimibe** (5mg/kg/day), an inhibitor of intestinal cholesterol absorption, **telmisartan** (5mg/kg/day), an angiotensin receptor blocker and PPAR partial agonist, and **pentoxifylline** (100mg/kg/day), a non-selective phosphodiesterase inhibitor.
- Plasma parameters reflecting liver injury or insulin resistance were followed each two weeks after 4 hours of fasting. As final analysis after the sacrifice, liver lipids, inflammation, fibrosis, and ER/oxidative stress levels were assessed by specific assays, histopathology or gene expression analysis.

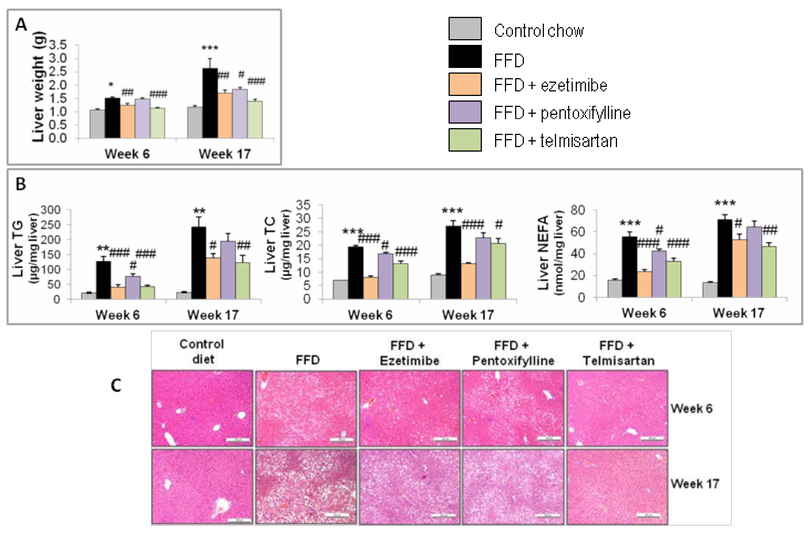
RESULTS

1. Body weight gain and insulin resistance induced by the DIN were mainly improved by telmisartan.

	Week 6			Week 12			Week 17		
	Body weight (g)	HOMA-IR	Glycemia (g/mL)	Body weight (g)	HOMA-IR	Glycemia (g/mL)	Body weight (g)	HOMA-IR	Glycemia (g/mL)
Control diet	29.3±0.5	4.5±0.7	114.4±13.1	31.4±0.5	4.3±0.5	122.9±6.0	31.0±0.6	4.7±0.8	106.1±5.0
FFD	33.1±0.7*	13.5±4.2*	177.4±10.5***	43.3±2.0***	18.3±3.8***	166.1±8.2*	45.4±1.8***	28.7±4.1***	155.4±11.9**
FFD + Ezetimibe	34.2±0.7	10.8±2.7	160.9±12.3	43.7±1.1	31±6.2	173.3±7.5	46.2±1.3	32.9±6.6	144.1±6.6
FFD+Pentoxifylline	33.5±0.8	4.9±1.4#	123.9±7.5##	41.3±1.1	15.0±4.8	171.4±7.5	41.1±0.9#	21.7±3.4	150.5±12.5
FFD + Telmisartan	30.4±0.6	4.7±1.2#	141.0±10.3	36.7±1.5###	7.6±1.8	152.9±11.3	37.8±1.6###	19.7±5.3#q	146.4±9.9

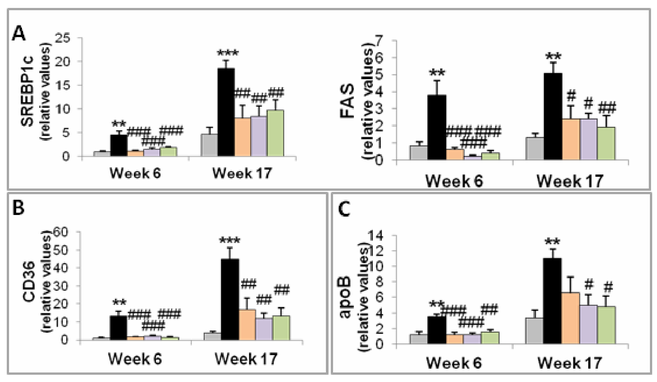
HOMA-IR and glycemia were assessed after 4 hours of fasting. Data are expressed as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control diet. (t-test); #p<0.05, ##p<0.01, ###p<0.001 vs. DIN (One way anova with Dunett's post test); q p<0.05 vs DIN in one-way anova without ezetimibe treated mice.

2. DIN led to liver steatosis development, which was prevented by the three drugs.



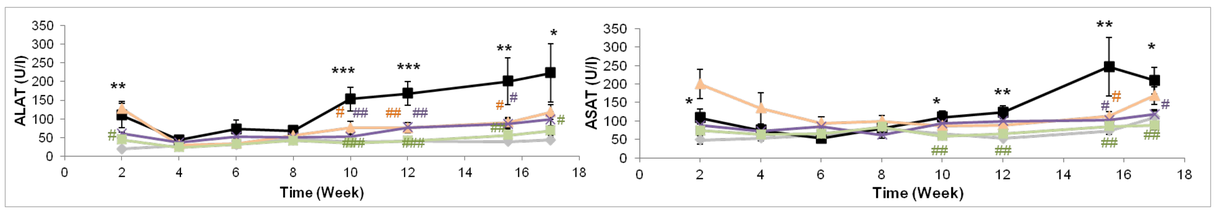
A: Liver weight; B: Liver lipid accumulation was measured by triglycerides (TG), total cholesterol (TC) and non-esterified fatty acid (NEFA) contents. C : Representative H/E staining of liver sections. Parameters were assessed after 4 hours of fasting. Data are expressed as the mean ± SEM. *p<0.05, **p<0.01 ***p<0.001 vs. control diet. (t-test); #p<0.05, ##p<0.01, ###p<0.001 vs. DIN (One way anova with Dunett's post test).

3. Liver lipid metabolic gene expression was induced by DIN and decreased by all treatments.



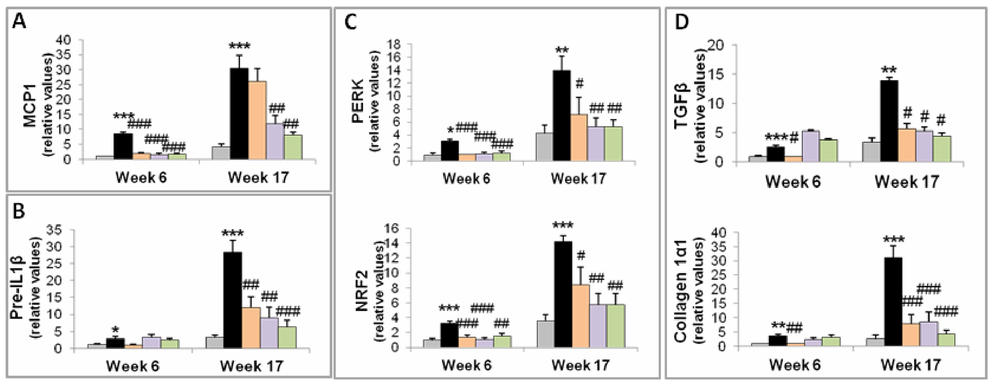
Gene expression level of A: lipogenic transcription factor and enzyme (SREBP1c: sterol regulatory element binding protein 1c; FAS: fatty acid synthase) B: fatty acid transporter; C: apolipoprotein B, major VLDL lipoprotein. Parameters were assessed after 4 hours of fasting. Data are expressed as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. control diet. (t-test); #p<0.05, ##p<0.01, ###p<0.001 vs. DIN (One way anova with Dunett's post test).

4. Liver injury occurred after 10 weeks of DIN, and was prevented by the three drugs.



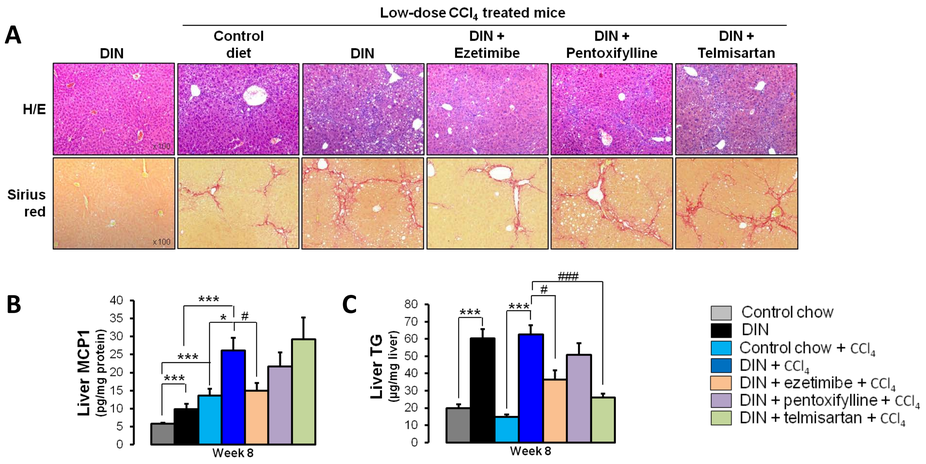
Plasma parameters follow-up after 4 hours of fasting. Data are expressed as the mean ± SEM. *p<0.05, **p<0.01 ***p<0.001 vs. control diet. (t-test); #p<0.05, ##p<0.01, ###p<0.001 vs. DIN (One way anova with Dunett's post test).

5. The three treatments prevented the DIN-increased gene expression, related to inflammation, cellular stress and fibrosis in the liver.



Gene expression level of A: inflammation marker (MCP1: monocyte chemoattractant protein 1) B: inflammasome complex member; C: ER-stress marker (PERK: PKR-like endoplasmic-reticulum localized kinase) and oxidative stress marker (NRF2: Nuclear factor-erythroid-2-related factor 2); D: profibrotic cytokine (TGFβ: transforming growth factor β), stellate cell activation marker (collagen). Data are expressed as the mean ± SEM. *p<0.05, **p<0.01 ***p<0.001 vs. control diet. (t-test); #p<0.05, ##p<0.01, ###p<0.001 vs. DIN (One way anova with Dunett's post test).

6. Ezetimibe prevented fibrosis development induced by DIN and low-dose CCl_4.



A: Representative H/E and sirius red staining of liver sections. B: Liver MCP1 (ELISA). C: Liver triglycerides (TG). Parameters were assessed after 4 hours of fasting. Data are expressed as the mean ± SEM. *p<0.05, ***p<0.001 vs. control diet. (t-test); #p<0.05, ###p<0.001 vs. DIN (One way anova with Dunett's post test).

CONCLUSION AND PERSPECTIVES

- A new Physiogenex-established high fat, high cholesterol and high fructose diet leads to liver steatosis development in 6 weeks and early NASH in 17 weeks associated with insulin resistance and obesity. Moreover, the diet sensitizes liver to fibrosis development induced by low-dose CCl_4.

	Obesity and insulin resistance	Liver lipid content TG NEFA		TC	Cellular stress markers	Inflammation/ fibrosis markers	Fibrosis induced by low dose CCl_4
Ezetimibe	-	+	++	+	+	+	+
Pentoxifylline	+/-	+/-	+/-	+/-	++	++	-
Telmisartan	+	++	+	+	++	++	---

+ : improvement of disease compared to DIN group.
- : no improvement compared to DIN group.

- This preclinical model is a new tool to validate the drugs targeting early grades of NAFLD, obesity and/or insulin resistance. The three different pharmacological compounds prevent liver injury induced by DIN. Finally, telmisartan strongly improves liver injury through a reduction of the main molecular pathways involved in NASH as well as metabolic parameters. This result underlines the multi-factorial component of NAFLD development.
- The DIN+CCl_4 model develops fibrosis associated to steatosis and inflammation. However, this chemical induction is to strict to prove drug efficacy. Only ezetimibe presents a protective effect on fibrosis development because of its function on cholesterol absorption from the diet.
- Physiogenex is currently developing innovative models of NASH and liver fibrosis, in a context of insulin resistance and obesity like in human physiopathology.

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