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Research Paper

Synergistic interaction of fatty acids and oxysterols impairs mitochondrial function and limits liver adaptation during nafld progression

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ABSTRACT

The complete mechanism accounting for the progression from simple steatosis to steatohepatitis in nonalcoholic fatty liver disease (NAFLD) has not been elucidated. Lipotoxicity refers to cellular injury caused by hepatic free fatty acids (FFAs) and cholesterol accumulation. Excess cholesterol autoxidizes to oxysterols during oxidative stress conditions. We hypothesize that interaction of FAs and cholesterol derivatives may primarily impair mitochondrial function and affect biogenesis adaptation during NAFLD progression. We demonstrated that the accumulation of specific non-enzymatic oxysterols in the liver of animals fed high-fat + high-cholesterol diet induces mitochondrial damage and depletion of proteins of the respiratory chain complexes. When tested *in vitro*, 5α-cholestane-3β,5,6β-triol (triol) combined to FFAs was able to reduce respiration in isolated liver mitochondria, induced apoptosis in primary hepatocytes, and down-regulated transcription factors involved in mitochondrial biogenesis. Finally, a lower protein content in the mitochondrial respiratory chain complexes was observed in human non-alcoholic steatohepatitis. In conclusion, hepatic accumulation of FFAs and non-enzymatic oxysterols synergistically facilitates development and progression of NAFLD by impairing mitochondrial function, energy balance and biogenesis adaptation to chronic injury.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), the most common liver pathology in the Western world [1], may clinically present with a heterogeneity of conditions ranging from benign steatosis (NAFL) to steatohepatitis (NASH) – the more progressive form of the disease [2] – and to cirrhosis. The mechanisms underlying the transformation from non inflamed to inflamed fatty liver are not completely elucidated, even though lipid metabolism alterations, mitochondrial dysfunction, inflammation and oxidative stress are suggested to play a significant role [3–6].

Accumulating lipids in hepatocytes may be vulnerable to free radical-induced peroxidation. Lipid peroxides exert toxic effects on the mitochondrial DNA (mtDNA), RNA and proteins of the respiratory chain, leading to mitochondrial dysfunction [7].

Lipotoxicity refers to cellular injury caused by excess of free fatty acids (FFAs) and related-lipid metabolites [8]. Excess cholesterol can lead to dysregulation of cholesterol metabolism, which is considered an underlying pathology in the development of many metabolic diseases [9]. Loading mitochondria with free cholesterol sensitizes hepatic cells to inflammatory mediators such as Tumor Necrosis Factor (TNF) and cell-surface Fas receptor (Fas), which may precipitate steatohepatitis

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; mtDNA, mitochondrial DNA; FFAs, free fatty acids; TNF, tumor necrosis factor; CTRL, control; HF, high-fat; HF + HCh, high-fat + high-cholesterol; 7β-OHC, 7β-hydroxycholesterol; 5β, 6β-epoxy, 5β,6β-epoxycholesterol; 5α, 6α-epoxy, 5α,6α-epoxycholesterol; triol, 5α-cholestane-3β,5,6β-triol; 7KC, 7-ketocholesterol; 6-oxo, 6-oxo-cholestan-3β,5α-diol; PA, palmitic acid; OA, oleic acid; $\Delta\psi_{;}$, mitochondrial membrane potential; CT, threshold cycle; BN-PAGE, Blue Native bidimensional polyacrylamide gel electrophoresis; SDM, standard deviation of the mean; ANOVA, analysis of variance; MMP, mitochondrial membrane potential; UCP2, uncoupling protein 2; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1 α; TFAM, mitochondrial transcription factor A, NRF1, nuclear respiratory factor 1; CYPs, cytochromes P450

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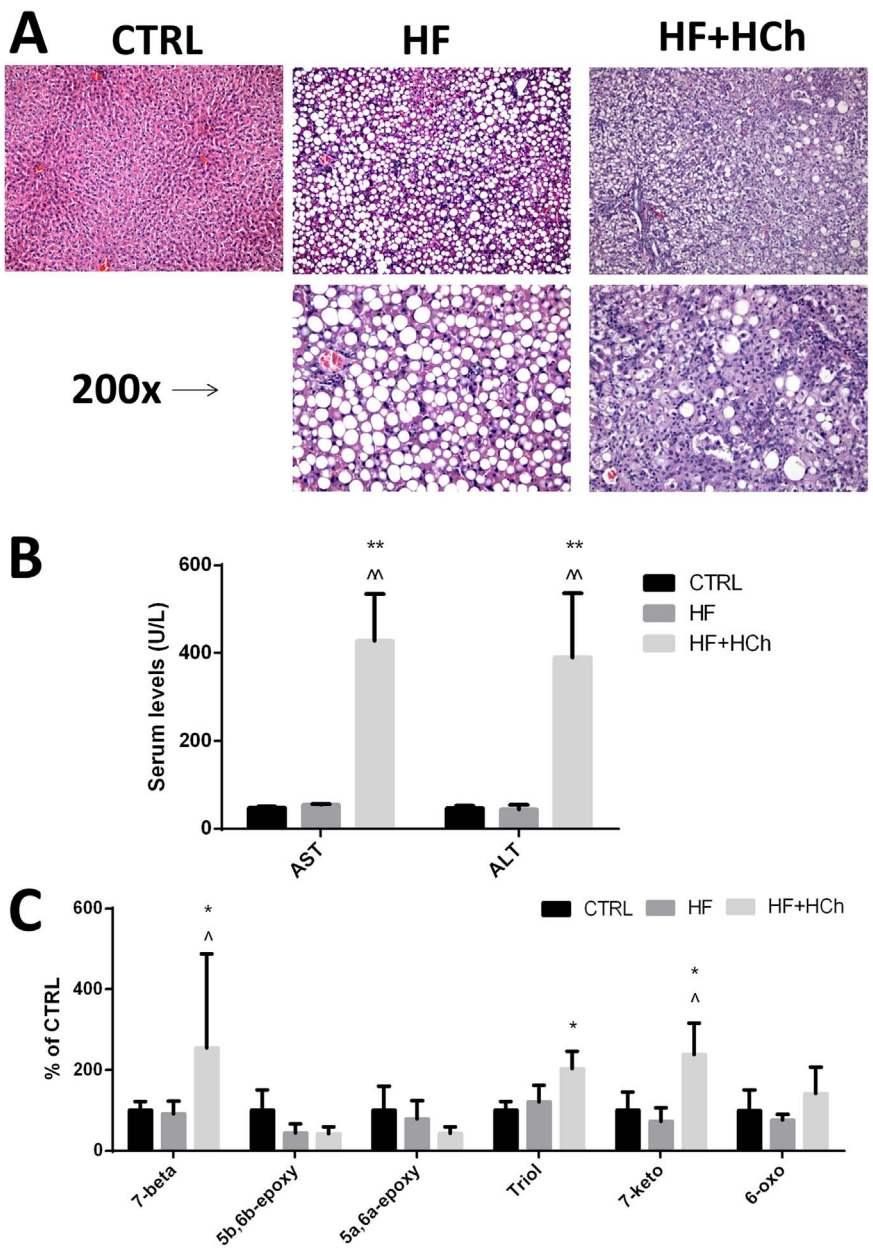

Fig. 1. Increased non-enzymatic oxysterol levels in the liver of HF + HCh-induced NASH. (A) Histological analysis of representative liver samples from rats fed a standard (CTRL), high-fat (HF) or high-fat + high-cholesterol (HF + HCh) diet, stained with Haematoxilin & Eosin (magnification 100x and 200x). (B) Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in all the animal groups studied. (C) Hepatic levels of the non-enzymatic oxysterols measured by mass spectrometry in all the groups studied. Data are expressed as mean ± SDM of five consecutive experiments. Statistical differences were assessed by one-way ANOVA and Tukey-Kramer as post-hoc test (* = $p < 0.05$ vs CTRL; ** = $p < 0.01$ vs CTRL; ^ = $p < 0.05$ vs HF; ^^ = $p < 0.01$ vs HF). 7 beta, 7β-hydroxycholesterol; 5b,6b-epoxy, 5β,6β-epoxycholesterol; 5a,6a-epoxy, 5α,6α-epoxycholesterol; triol, 5α-cholestane-3β,5,6β-triol; 7-keto, 7-ketocholesterol; 6-oxo, 6-oxo-cholestan-3β,5α-diol.

Table 1
Kleiner scoring system applied to liver samples from rats fed a standard (CTRL), high-fat (HF) or high-fat + high-cholesterol (HF + HC) diet.

	Steatosis	Ballooning	Lobular Inflammation	Activity Score	Indication
CTRL	0	0	0	0	Normal
HF	3	0	1	4	NAFL
HF + HCh	2	2	2	6	NASH

[10]. Free cholesterol accumulation in oxidative settings may promote its oxidization with final production of oxysterols that are involved in NAFLD damage [11,12]. Dietary fat and cholesterol induce the metabolic and hepatic features of NASH in mice only when acting synergistically but not when administered alone [13]. We have previously shown that the significant change in fatty acid and oxysterols profile induced by a dietary combination of high fat and high cholesterol accounts for liver injury, allowing the generation of interesting hypotheses on the role of interaction of lipid and cholesterol metabolites in the pathogenesis of NAFLD progression [14].

In the present study, after a lipidomic analysis of hepatic non-enzymatic oxysterols in animals fed high-fat or high-fat + high-cholesterol diet, we analyzed mitochondrial function, biogenesis and mitochondrial respiratory chain proteins, and we demonstrated a significant impairment in the respiratory chain and energy homeostasis secondary to depletion of specific respiratory chain complexes. The analysis of mitochondrial biogenesis signaling revealed that FFAs and non-enzymatic oxysterols synergistically interact and limit the adaptive response of liver cells to chronic lipid accumulation, promoting transition from steatosis to steatohepatitis. The observations were then confirmed in human NASH.

2. Materials and methods

Further details are provided in the Supporting Information.

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