FEBS Letters 584 (2010) 1001–1005

journal homepage: www.FEBSLetters.org

Intrahepatic cholesterol influences progression, inhibition and reversal of non-alcoholic steatohepatitis in hyperlipidemic mice

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ARTICLE INFO

Article history:
Received 12 January 2010
Accepted 21 January 2010
Available online 29 January 2010

Edited by Veli-Pekka Lehto

Keywords:
Inflammation
Liver
Cholesterol
Mouse
Liver X receptor
Peroxisome proliferator-activated receptor

ABSTRACT

Hepatic inflammation is the key factor in non-alcoholic steatohepatitis (NASH) and promotes progression to liver damage. We recently identified dietary cholesterol as the cause of hepatic inflammation in hyperlipidemic mice. We now show that hepatic transcriptome responses are strongly dependent on cholesterol metabolism during diet-induced NASH and its inhibition by fenofibrate. Furthermore, we show that, despite doubling hepatic steatosis, pharmacological LXR activation reverses hepatic inflammation, in parallel with reversing hepatic cholesterol levels. Together, the results indicate a prominent role of cholesterol during the development, inhibition and reversal of hepatic inflammation in NASH and reveal potential new therapeutic strategies against NASH.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by lipid accumulation in the liver (steatosis) and has become a major health problem. NAFLD may progress towards a more harmful condition, i.e. non-alcoholic steatohepatitis (NASH), in which inflammation is present. Ultimately, NASH can lead to further liver damage such as fibrosis, cirrhosis and liver failure [1,2].

We have previously shown that hyperlipidemic mice, like the humanized apolipoprotein E2 knock-in (APOE2ki) mice and the low density lipoprotein (LDL) receptor-deficient mice, develop NASH after only a few days of high fat, high cholesterol (HFC) feeding [3,4]. In APOE2ki mice, the human APOE2 allele replaces the murine variant and is expressed under the control of endogenous promoter sequences in a tissue specific manner and at physiological levels. APOE2 has a markedly reduced affinity for the LDL receptor, leading to a plasma lipoprotein profile resembling human type III hyperlipoproteinemia [5]. NASH development in APOE2ki mice could be inhibited by co-administrating fenofibrate (FF), a synthetic ligand of peroxisome proliferator-activated receptor alpha (PPARα) [3]. PPARα is a nuclear receptor with fatty acids as its natural ligands [6,7]. Upon activation, it initiates transcription of genes involved in lipid metabolism resulting in increased fatty acid oxidation [8].

Abbreviations: NASH, non-alcoholic steatohepatitis; LXR, liver X receptor; APOE2ki, humanized apolipoprotein E2 knock-in; NAFLD, non-alcoholic fatty liver disease; LDL, low density lipoprotein; HFC, high fat, high cholesterol; FF, fenofibrate; PPARα, peroxisome proliferator-activated receptor alpha; T09, T0901317; TG, triglyceride; Acox1, acyl-CoA oxidase 1; WT, wild type; SREBP, sterol regulatory element-binding protein ½; IPA, ingenuity pathway analysis; Cept1, choline/ethanolaminephosphotransferase 1; FFA, free fatty acid; ABC, ATP binding cassette; Acaa, acetyl-CoA acyltransferase; Acta1, actin alpha; Arg, arginase; Acl, argininosuccinate lyase; Cyp7a1, cytochrome P450 7a1; Cyp8b1, cytochrome P450 8b1; Fdft1, farnesyldiphosphatefarnesyltransferase 1; Fdps, farnesyldiphosphate synthase; Gpdh, glyceraldehyde-3-phosphate dehydrogenase; Hmgcs, HMG-CoA synthase; Insig2, insulin stimulated gene 2; Lpl, lipoprotein lipase; SAT, spermidine/spermine n(1)-acyltransferase 1; Sc5dl, sterol 55-desaturase-like; Scd, stearoyl-CoA desaturase

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.01.046

Table 1
Activation of hepatic transcription factors. Table shows the regulation of genes according to IPA®. Regulation determined by literature mining regulation is shown as ↑ (up-regulated by the transcription factor), or ↓ (down-regulated by the transcription factor). In numbers, the actual regulation of these genes in our dataset is shown by either diet only (HFC) or the diet supplemented by fenofibrate (HFCff); non-significant regulation ($P > 10^{-6}$) is shown by "ns".

Gene	Reported effect transcription factors			Measured response			
	LXR	PPARa	SREBP	HFC 2 days	HFC 7 days	HFCff 2 days	HFCff 7 days
ABCA1	↑			ns	1.9	ns	ns
ABCC3	↑			−1.7	−1.5	2.7	2.3
ABCG5	↑			ns	1.5	1.7	ns
ACAA1		↑		1.4	ns	3.1	4.1
ACAA2		↑	↑	−1.8	−3.2	−2.7	ns
acta1		↑		ns	−9.3	ns	24
arg		↓		−1.5	−1.6	ns	1.4
asl		↓		−1.8	−2	ns	ns
Cyp7a1	↑	↓	↓	ns	−2.4	ns	ns
CYP8B1		↑	↑	−1.9	−2.5	ns	ns
fdft1	↑		↑	ns	−3.1	ns	1.9
fdps			↑	ns	−2.8	−2.3	ns
gpdh		↑		ns	1.8	ns	ns
HMGCS			↑	−1,5	ns	ns	2,9
Insig2				−1,5	ns	1,8	1,6
LpL	↑	↑	↑	ns	2.8	2.2	3.3
SAT		↓		ns	2	ns	ns
Sc5dl		↑	↑	ns	−1.4	ns	ns
scd	↑		↑	1.2	ns	ns	1.9
scd2	↑		↑	ns	2.6	ns	ns
SREBP	↑			ns	2.1	ns	ns

ATP binding cassette (ABC), acetyl-coa acyltransferase (Acaa), actin alpha (Acta1), arginase (Arg), argininosuccinate lyase (Acl), cytochrome P450 7a1 (Cyp7a1), cytochrome P450 8b1 (Cyp8b1), farnesyldiphosphatefarnesyltransferase 1 (Fdft1), farnesyldiphosphate synthase (Fdps), glyceraldehyde-3-phosphate dehydrogenase (Gpdh), HMGCoA synthase (Hmgcs), insulin stimulated gene 2 (Insig2), lipoprotein lipase (Lpl), spermidine/spermine n(1)-acyltransferase 1 (SAT), sterol 55-desaturase-like (Sc5dl), stearoyl-CoA desaturase (Scd), sterol regulatory element-binding protein ½ (SREBP).

In the current study, the hepatic transcriptional responses induced by feeding APOE2ki mice a HFC diet were investigated in detail. We identified the cholesterol metabolism pathway to be of major importance during the early development of NASH. Subsequently, we aimed to identify how cholesterol metabolism influences NASH development and to investigate the role of intrahepatic cholesterol and triglycerides during inhibition and reversal of NASH by using pharmacological ligands of PPARα and liver X receptor (LXR).

2. Materials and methods

2.1. Mice and diet

*APOE2*ki mice were housed under standard conditions. Experiments were performed according to Dutch and French laws, approved by the Committee for Animal Welfare of Maastricht University, Maastricht, The Netherlands, and the Pasteur Institute review board, Lille, France. Groups of 10 homozygote female mice were fed either chow or HFC [3] (diet 1635, SAFE, Villemoisson-sur-orge, France) for 2 or 7 days with or without FF 0.2% (F6020, Sigma–Aldrich, Zwijndrecht, the Netherlands). Additionally, mice were given HFC for 2 months, followed by 5 days of gavage treatment with an LXR agonist, T0901317 (T09), (30 mg/kg) or vehicle. Mice were sacrificed by cervical dislocation. Tissues were isolated, snap-frozen in liquid nitrogen and stored at −80 °C.

2.2. RNA analysis

Hepatic mRNA extraction, cDNA synthesis, and microarray data generation has been described previously [3]. In short, microarray analysis was performed by selecting genes according to their *P*-value of the diet/treatment effect for each delay coefficient (four *P*-values per probe set). For the multi-testing problem, Bonferroni correction was used. Genes with a *P*-value lower than 10^{-6} were selected as significantly regulated. Biological pathway analysis was done with ingenuity pathway analysis (IPA®) (Ingenuity Systems, Redwood City, USA) software. Microarray data were

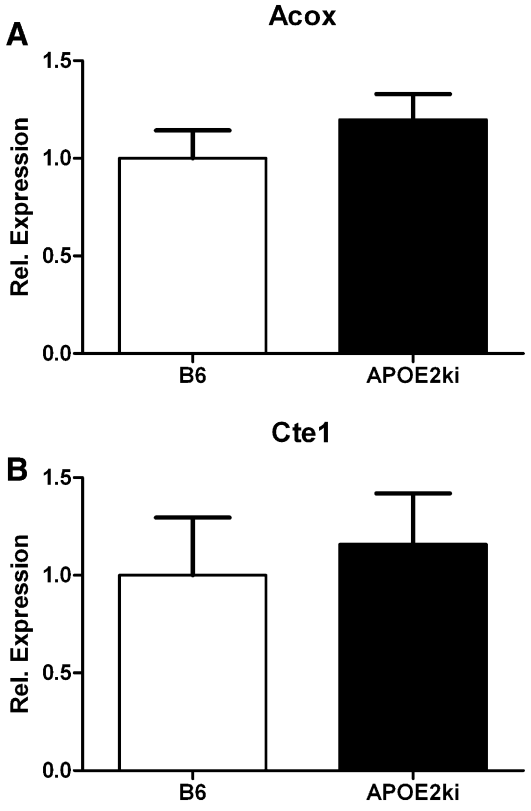

Fig. 1. Acox1 and Cte1 gene expression. Relative gene expression of Acox1 (A) and Cte1 (B) in hepatic mRNA of APOE2ki female mice (black bar) and C57Bl/6 (B6) female mice (white bar) on a chow diet.