Cholesterol-lowering therapy for the treatment of nonalcoholic fatty liver disease: an update.

UNIVERSITÀ DEGLI STUDI DI TORINO

Cholesterol-lowering therapy for the treatment of non-alcoholic fatty liver disease: an update

RUNNING HEAD: CHOLESTEROL-LOWERING AGENTS IN NAFLD

Giovanni Musso[1], Maurizio Cassader[2], Roberto Gambino[2]

[1]*Gradenigo Hospital, Turin, Italy*

[2]*Department of Internal Medicine, University of Turin, Turin, Italy*

Corresponding author:

Giovanni Musso

Gradenigo Hospital

C.so Regina Margherita 8

10132 Turin, Italy

E-mail: giovanni_musso@yahoo.it

Abstract

Purpose of review. To review recent human trials assessing cholesterol-lowering agents in non-alcoholic fatty liver disease (NAFLD).

Recent findings. Four randomized controlled trials assessed statins in NAFLD. In the only RCT with post-treatment biopsy, simvastatin did not change liver histology. In the remaining RCTs, atorvastatin was safe, significantly improved radiological/biochemical markers of steatosis and plasma lipids, with neutral effects on glucose metabolism; in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) study, atorvastatin reduced incident CVD compared with both untreated

NAFLD patients and with statin-treated patients without NAFLD. Ezetimibe was evaluated in 2 uncontrolled trials and 2 RCTs, consistently improving liver histology and plasma lipids, while glucose metabolism was generally unaffected; however, HbA1c increased with ezetimibe in one RCT.

Summary. From the analysis of available trials it emerges that cholesterol-lowering agents may considerably benefit NAFLD patients. Statins are safe, and atorvastatin improved surrogate markers of liver disease, while their effect on liver histology is unknown. Furthermore, the GREACE study was the first trial to show clinical benefit from the use of a pharmacological agent in NAFLD. Ezetimibe improved liver histology. The benefit of combination therapy, as well as the safety on glucose metabolism, need further evaluation

Key words: NAFLD,cholesterol-lowering drugs, hyperlipidemia, ezetimibe, statins

Introduction

Nonalcoholic fatty liver disease (NAFLD) can be encountered in up to 30% of the general adult population and is considered the hepatic manifestation of the metabolic syndrome, affecting up to 60-70% of diabetic and obese patients [1]. NAFLD encompasses a histological spectrum ranging from simple steatosis (SS) to steatosis plus necro-inflammation (non-alcoholic steatohepatitis, NASH), with or without fibrosis, that can only be differentiated by liver biopsy; NASH affects 3-5% of the general adult population and up to 20-40% of obese and diabetic subjects [1].

Patients with NAFLD have an increased risk of liver-related complications (largely confined to NASH), of cardiovascular disease (CVD) and of type 2 diabetes [2].

NAFLD frequently coexists with overt dyslipidaemia (hypercholesterolemia or atherogenic dyslipidemia, i.e. elevated triacylglycerol (TG) and/or low HDL-cholesterol levels) and is constantly associated with altered lipoprotein metabolism, as liver TG excess drives hepatic large very low density lipoprotein(VLDL)-1 subfraction oversecretion, which accumulate in the blood, compete with chylomicrons for lipoprotein lipase, exchange their TG with LDL and HDL particles, eventually leading to the atherogenic phenotype of small, dense LDLs, and low HDL-C(prevalently represented by HDL-3) [3]. Beyond these associations, growing animal and human evidence connects altered hepatocyte cholesterol metabolism and hepatic free cholesterol accumulation to the pathogenesis of liver injury in NAFLD. Mari et al first showed mitochondrial free cholesterol accumulation sensitizes the liver to inflammatory stimuli in rats through mitochondrial glutathione depletion [4**]. Other authors. demonstrated that cholesterol intake is essential for determining hepatic inflammation and NASH in hyperlipidemic mouse models fed a Western diet, likely through direct Kupffer cell activation upon scavenging of remnant cholesterol-rich lipoproteins; consistently, omitting cholesterol from the high-fat diet prevented hepatic free cholesterol accumulation, hepatocyte injury or apoptosis, macrophage recruitment, and the development of diet-induced NASH[5**,6**]. A recent study in *Alms1* mutant (*foz/foz*) mice elegantly elucidated the mechanisms underlying hepatocyte free cholesterol accumulation: up-regulation of low density lipoprotein receptor (LDLR) via activation of sterol

regulatory element binding protein-2 (SREBP-2), reduced biotransformation to bile acids, and suppression of canalicular pathways for cholesterol and bile acid excretion in bile [6**].

Additionally, epidemiological studies linked excessive dietary cholesterol intake to the development and severity of NAFLD, independently of caloric and macronutrient intake, even in the absence of overt hypercholesterolemia [7*, 8], and lipidomic analysis of human livers has shown a progressive increase of hepatic free cholesterol during different stages of NASH [9*, 10].

Altogether, these features make cholesterol-lowering drugs an attractive therapeutic tool in NAFLD: we will review recent advances in cholesterol-lowering agents in NAFLD.

Statins

A recent Liver Expert Panel stated that patients with NAFLD are not at increased risk of statin hepatotoxicity and routine transaminase monitoring is not warranted in these subjects [11]. Even their feared potential for worsening glucose tolerance seems largely outweighed by their well-established cardiovascular benefit[12].

To date, safety and efficacy of statins in NAFLD have been evaluated in 4 randomized controlled trials (RCTs) (Table 1).

In the only, small RCT with post-treatment histology, 1 year of simvastatin did not change liver histology in 16 patients with NASH [13].

The Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) study was a 3-year prospective, randomised, open label survival study enrolling 1600 patients (78% male, 20% diabetic, 45% with metabolic syndrome) with established coronary heart disease (CHD), serum LDL cholesterol >2.6 mmol/L and triglyceride <4.5 mmol/L, who were randomized to receive statins or usual care (lifestyle changes, such as a low-fat diet, weight loss, and exercise, and all necessary drug treatment, including statin) [14]. Primary end-point was the first occurrence of any cardiovascular event.

In a post-hoc analysis of the GREACE study, safety and efficacy of statins were evaluated in 437 patients (80% male, 51% diabetic, 91% with metabolic syndrome) with abnormal (< 3 x ULN) baseline

transaminases, presumably due to NAFLD, based on ultrasonography and exclusion of other common causes of abnormal liver function tests.

The risk reduction in NAFLD patients was compared with the relative risk reduction seen between patients with normal liver tests who were treated with a statin and those not treated with a statin.

Overall, statins (mainly atorvastatin 24 mg/d) were safe, with <1% patients discontinuing statin because of liver-related adverse effects (transaminase elevation >three-times the ULN). Importantly, diabetes incidence was low (4%) in statin-treated NAFLD patients and similar to that of patients not on statin (4.3%).

Statins improved liver function tests in NAFLD patients, normalizing AST, ALT and GGT in 89% of patients after 3 years, while liver tests worsened in NAFLD patients not receiving statins;

Beside a reduction in LDL-C (-44% from baseline), statin-treated NAFLD patients also improved also plasma triglyceride (-32%), HDL-C (+8%) and estimated glomerular filtration rate (EGFR) (from 59 ml/min to 70 ml/min/1.73 m^2, $p<0.0001$). Most importantly, statin treatment was associated with a significant (-68%) risk reduction of CVD events compared with both NAFLD patients not on statin and with statin-treated patients with normal transaminases

With all the limitation of a post-hoc analysis and the methods used to detect NAFLD, some considerations can be drawn from this important study:

1)statins appear safe in NAFLD and improve biochemical markers of liver disease. The actual steatosis regression rate with statins is however unknown, as a substantial number of NAFLD patients still have significant liver fat infiltration despite normal liver enzymes.

1)the GREACE is the first RCT showing therapeutic benefit on clinical end-points in NAFLD. Statin-related CVD risk reduction was greater in patients with presumed NAFLD than in those with normal liver tests, with a number needed to treat of 5 NAFLD patients to save 1 CVD event. Therefore, the risk-to-benefit ratio of long-term treatment with statins (mainly atorvastatin in this study) seems to favour statin administration for patients with NAFLD-related moderately abnormal liver tests.

2)consistently with the results of a recent meta-analysis, statins are safe and do not seem to affect the risk of incident diabetes[12], although their impact on insulin resistance and glucose metabolism in NAFLD requires further evaluation.

3)the benefit of statins in NAFLD appear to extend to renal function. Emerging evidence connects NAFLD, and more consistently NASH, to an increased risk of developing chronic kidney disease, defined as either an estimated glomerular filtration rate of <60 mL/min/1.73 m^2 of body surface area or abnormal proteinuria, independently of traditional risk factors, metabolic syndrome, and insulin resistance[15, 16, 17]. Mechanisms potentially linking obesity, NAFLD and kidney disease include altered fetuin-A, adiponectin and tissue 5'-AMP activated protein kinase (AMPK) expression [18**] . Mechanisms responsible for the beneficial effects of statins on renal function are still unclear, but may involve transcriptional regulation of organic anion transporters (OATs) localized in the basolateral side of proximal tubular cells, like SLCO4C1, which are involved in the renal elimination of uremic toxins and kidney inflammation[19,20**].

3)atorvastatin was the most widely used statin in the GREACE study. It is currently unknown whether different statins have different effectiveness in NAFLD: atorvastatin was assessed in all but 1 RCT in NAFLD and constantly improved biochemical and radiological surrogates of steatosis, while simvastatin did not change liver histology in the only RCT with post-treatment histology [13]. These differences may be related to the varying lipophilicity and related extrahepatic effectsof different statins, which need to be further addressed by future research[21**] (Table 2; Table 3).

4) the median daily dose of statins (atorvastatin 24 mg, simvastatin 22 mg, pravastatin 31 mg, and fluvastatin 40 mg) in NAFLD patients who normalized liver tests was similar to that used in patients who did not normalize liver enzymes at the end of the study. Of the measured variables, only reduction in plasma triglyceride correlated with ALT improvement in the GREACE population (r=0.59, p=0.002). Therefore, a major issue for future RCTs would be the individuate predictors of response to statin in NAFLD patients. Patients with NAFLD are an heterogeneous population, with different mechanisms contributing to liver injury in the single patient. A major challenge for research will be to

individuate those genetic or environmental factors associated with a favourable response to statin treatment, to tailor treatment to individual patient characteristics.

In a subgroup of 80 NAFLD patients from the St Francis Heart Study Randomized Clinical Trial, the combination of atorvastatin 20 mg+vitamin C 1 g/d + vitamin E 1000 IU/d was safe and improved computed tomography(CT)-assessed steatosis after 3.6 years[22]. The St. Francis RCT was originally designed to evaluate the effect of this treatment on the risk of developing CVD in healthy individuals deemed high risk by coronary calcium score. Several observations can be made on this study.

1)The exclusion of individuals with baseline transaminases > 1.5 x ULN likely excluded patients with moderate-to-severe NASH; furthermore, alcohol intake was not assessed, and some participants might actually have had alcoholic rather than nonalcoholic fatty liver.

2)Most importantly, it is unclear whether the post-treatment 2.2-fold reduction in NAFLD prevalence in the treatment arm compared to placebo was the effect of the combination of atorvastatin + antioxidants or of either agent alone, given that vitamin E showed significant benefit on liver disease in the 2 large PIVENS and TONIC trials[23, 24].

3)Significant reduction in NAFLD prevalence in the treatment group was still observed in patients with normal cholesterol and triglyceride levels at baseline and after controlling for age, sex, baseline BMI and blood pressure, and follow-up lipid values, suggesting that hyperlipidemia is not required for this treatment to be effective. Although the antioxidant effect of vitamins C and E may have contributed to the observed improvement, the documented antioxidant, anti-lipogenic, anti-inflammatory and antifibrogenic effects of statins may have played a relevant role (Table 2)[25**, 26**,27*, 28*, 29, 30*, 31, 32, 33*, 34, 35]. Consistent with this view, in a small RCT enrolling 29 normocholesterolemic overweight subjects, atorvastatin 80 mg/d reduced liver TG content by 40% compared with placebo; this effect was coupled with pervasive changes in hepatic expression of genes involved in lipogenesis, while fasting glucose, NEFA and insulin sensitivity were unchanged [26**] (Table 1).

Ezetimibe

Ezetimibe inhibits the Niemann-Pick C1 like 1 (NPC1L1) protein, which catalyzes absorption of cholesterol by enterocytes and hepatocytes in the gut and the liver, and showed promising results in animal models of NASH [36, 37] .

In 2 nonrandomized trials, ezetimibe improved histological steatosis and necroinflammatory grade, but not fibrosis in NAFLD [38, 39]. These improvements were associated with an improvement in insulin resistance and with a favourable redistribution in plasma VLDL/LDL lipoprotein subfractions, in unsaturated/saturated fatty acid profile and in estimated desaturase activity index [38, 40] (Table 1).

Two RCTs assessed ezetimibe in NAFLD. In abdominally obese NAFLD patients, ezetimibe plus a low-fat diet significantly reduced magnetic resonance (MR)-assessed liver fat content and plasma inflammatory markers compared to placebo, despite similar weight, waist and HOMA reduction [41] (Table 1). In the first RCT with post-treatment histology, ezetimibe significantly improved histological ballooning and fibrosis, but worsened HbA1c, suggesting the impact of this agent on glucose metabolism needs further evaluation [42*] .

Combination therapy of ezetimibe with other agents may also have synergistic effects in NAFLD. To further reduce hepatotoxic free cholesterol accumulation, inhibition of cholesterol absorption by ezetimibe and synthesis by statins may offer additive benefits over either agent alone. Ezetimibe/simvastatin (10/20mg/d) significantly improved liver enzymes and atherogenic dyslipidemia in 19 diabetic patients with NAFLD [43].

The combination of ezetimibe with alpha-glucosidase inhibitors significantly improved liver histology compared with either drug alone, an effect at least in part explained by synergistic effects on intestinal incretin glucagon-like peptide-1 secretion and hepatic microsomal triglyceride transfer protein (MTP) and peroxisome proliferators-activated receptor-α1 (PPAR-α1) expression [44**, 45, 46]. Consistent with these experimental findings, Nagai et al. reported dramatic improvement in liver enzymes and insulin resistance with ezetimibe+voglibose in a patients with NASH, refractory to ezetimibe+ursodeoxycholic acid [47]..

Conclusions. On the basis of mounting evidence, strategies aiming at reducing hepatic free cholesterol content show promise in NAFLD. Inhibition of cholesterol synthesis with statins and/or absorption by

ezetimibe warrants assessment in larger RCTs; the benefits of cholesterol-lowering agents appear to extend beyond liver disease, and the combination of ezetimibe/statins with each other and with other agents acting on different steps involved in lipid (dys)metabolism and adipose tissue inflammation may also offer synergistic benefits over either agent alone.

Key points

-in the light of increasing evidence connecting increased hepatic free cholesterol content to liver disease in NAFLD, cholesterol-lowering agents are receiving much attention for the treatment of NAFLD

-among statins, atorvastatin improved biochemical/radiological markers of steatosis in NAFLD, and reduced also CVD events in the GREACE study. The benefit of statins on CVD risk seem to be even greater in patients with NAFLD than in patients with normal liver enzymes. The impact of statins on liver histology is unknown.

-ezetimibe improved liver histology in the few trials available, but its glucose-related safety needs further evaluation

-these drugs may offer synergistic benefit when used in combination with each other or with other drugs targeting different steps of lipid/glucose metabolism.

Acknowledgements: this work was supported in part by grants from the Piedmont Regional Funds Comitato Interministeriale per la Programmazione Economica 2008. No author has any conflict of interest to disclose.

Table 1: recent trials with cholesterol-lowering drugs in NAFLD

Statins								
Study population	Design	Agent (daily	Duratio	Response				Comments
				Liver enzymes	Steatosis (imaging)	Histology	Cardio-metabolic parameters	
16 NASH BMI 36 44% DM	RCT	Simvastatin 40 mg	12 mo	↔	NA	↔	↔ plasma lipids	First RCT to assess the effect 9of statins on liver histology in NASH
437 NAFLD BMI 29 51% DM	RCT 8	Atorvastatin 24 mg or other statins	36 mo	↓	NA	NA	↓↓C, TG	First trial reporting effectiveness of an afent on clinical outcomes in NAFLD
80 NAFLD BMI 33 9%DM	RCT 8	Atorvastatin 20mg +vit C 1 g+vit E 1000 IU	3.6 yr	↔	↓(CT)	NA	↓ C, LDL	The association of statin with antioxidants is safe and effective in NAFLD
19 Normocholestero	RCT	Atorvastatin 80 mg	4 wk	NA	NA	↓ St	↓ VLDL TG	Atorvastatin induced substantial changes in hepatic

Ezetimibe							
Design	Agent (daily	Duration	Response				Comments
			Liver enzymes	Steatosis (imaging)	Histology	Cardio-metabolic parameters	Inflammatory markers BP
UNT	EZE10 mg+LI	6 mo	↓	+(CT)	↓St	↓ BMI visceral fat ↓ TG. LDL-C, , ↓ CRP/Type IV collagen 7s,	First trial reporting effectiveness of ezetimibe in NAFLD
RCT	EZE10 mg+diet	22 wk	↔	+(MRS)	NA	↓ C, LDL, HDL, TG ↓ IL-6, CRP	First RCT assessing eezetimibe in NAFLD
UNT	EZE 10 mg+diet	24 mo	↓	NA	↓St Ne	↓ visceral fat and HOMA ↓ TG/ total and LDL-C,↓VLDL-1, ↓ small LDL, ↓	Favourable impact on VLDL and LDL subfraction distribution and on unsaturated/saturated
RCT	EZE 10 mg	24 wk	↔	NA	↓ Ne Fi	↓ total cholesterol ↑ HbA1c	The impact of ezetimibe on glucose metabolism needs

Author
Nelson 2009 [13]
Athyros 2010 [14]
Foster 2011 [22]
Pramfalk 2011 [26]

	Study population		10 hyperlipidemic NASH	25 NAFLD BMI 32	45 hyperlipidemic NAFLD	29 NAFLD BMI 29
	Author		Yoneda 2010[39]	Chan 2010[41]	Park 2011[40]	Takeshita 2011[42]

Abbreviations: UNT: uncontrolled trial; RCT: randomized controlled trial; HbA1c: glycated Haemoglobin A1c; M%: % males; DM: type 2 diabetes mellitus; St: steatosis; Ne: necroinflammation; Fi: fibrosis; NA: not available; CT: computed tomography; MRS: magnetic resonance spectroscopy; C: total cholesterol; LDL: low density lipoprotein; TG: triacylglycerolTNF: tumor necrosis factor, CRP: C-reactive protein; IL-6: interleukin-6; LI: lifestyle intervention; oxLDL: oxidized LDL; SCD-1: stearoyl-CoA desaturase-1; CE: cholesterol esters; EZE: ezetimibe; ANGPTL3: angiopoietin-like protein 3

Table 2 Pharmacological properties of statins which may favourably affect liver disease in NAFLD

Mechanism	**Functional consequences**	**Biological effect**	**Ref**
↓ SREBP-1c	↓ hepatic *de novo* lipogenesis	↓ hepatic TG ↓ plasma VLDL TG	26
↓ hepatic ANGPTL3 (inhibitor of LPL)	↑ hepatic lipolysis of plasma VLDL	↓ plasma VLDL TG	**26**
↑ apolipoprotein C III clearance from the blood	↑ VLDL TG clearance	↓ plasma VLDL TG ↓ hepatic TG uptake	**34**
↓ hepatic glucokinase	↓ hepatic glycolysis, glycogen synthesis and pentosephosphate pathway	↓ insulin resistance ↓ hepatic TG	**26**
↑ hepatic glucose-6-phosphatase	↓ hepatic gluconeogenesis	↓ insulin resistance	**26**
↑ hepatic acetyl-coenzyme A carboxylase 1	↑ hepatic *de novo* lipogenesis	↑ hepatic TG	**26**
↑ ACSS2	↑ acetyl-CoA→↑ hepatic *de novo* lipogenesis	↑ hepatic TG	**26**
↓ hepatic ChREBP	↓ hepatic *de novo* lipogenesis	↓ hepatic TG	**25**

↓ hepatic fructokinase activity in fructose-fed rats	↓ fructose-induced lipogenesis and gluconeogenesis	↓ hepatic TG	**25**
↓ phosphorylated inhibitor of κB (p-IκB)	↓ nuclear factor-κB (NF-κB) activation	↓ hepatic necro-inflammation	**25** **29** **30**
↑ hepatic ATP-binding cassette transporter A1 (ABCA1) expression by enhancing SREBP2 and PPARα-expression	↑ apolipoprotein A-I lipidation and high-density lipoprotein (HDL) production	↑ plasma HDL-C levels	**31**
↓ hepatic angiotensin II-induced inflammation	↓ HSC proliferation, ↓NF-κB activation, ↓ICAM-1 expression, ↓interleukin-8 secretion, ↓ procollagen-$\alpha 1_{(1)}$ and TGF-β1. expression	↓ hepatic inflammation and fibrogenesis	**30**
↓ glyceraldehyde-derived AGE production	↓ AGE-RAGE axis activation	↓ AGE-induced oxidative stress, inflammation and insulin resistance	**28**
↓ hepatic nitrotyrosine production ↓ HSC expression of TNF-α,TGF-β1 TIMP-1, TIMP-2, MMP-2 and type I procollagen	↓ hepatic oxidative stress ↓ HSC activation and fibrogenesis	↓ hepatic inflammation and fibrogenesis	**33**

↑ HSC expression of PPAR-γ			
↓ TLR4 activation by LPS in liver, muscle and adipose tissue	↓ downstream signalling pathways c-JNK and NF-kB activation ↓ unfolded protein response	↓ hepatic, adipose tissue and muscle inflammation ↓ cellular apoptosis	**35**
↑ adiponectin secretion (hydrophilic statins)	↓ hepatic lipogenesis, inflammation and fibrogenesis	↓ hepatic steatosis, necroinflammation and fibrosis	**21**

Abbreviations: SREBP-1c: sterol regulatory element-binding protein-1c;

ANGPTL3: angiopoietin-like protein 3; LPL: lipoprotein lipase; ACSS2: acyl-CoA synthetase short-chain family member 2; ChREBP: Carbohydrate Response Element Binding Protein; AGE: advanced glycation end-products; RAGE: receptor for AGE; HSC: hepatic stellate cells; ICAM-1:

ntercellular adhesion molecule-1; TGF: transforming growth factor;

TIMP: Tissue inhibitor of metalloproteinase; MMP-2: matrix metalloproteinase-2; TNF-α: tumor necrosis factor-α; TLR-4: toll-like receptor-4; LPS: lipopolysaccharide

JNK: Jun N-terminal kinase

Table 3 Open issues in the use of statins for the treatment of NAFLD

The impact of statins on different histological features (steatosis, necroinflammation and fibrosis) in NASH is unknown.			
The effect of different statins on liver histology and cardio-metabolic profile in NASH may vary widely and is currently unknown. The new statin pitavastatin seems more potent an inhibitor of cells involved in fibrogenesis than older statins			
The predictors of response to statins, as well as their effectiveness in normolipidemic patients with NAFLD, are unknown.			
The potential for combination therapies targeting multiple pathways involved in liver, metabolic and cardiovascular disease in largely unexplored (i.e. statins+ezetimibe; cholesterol-lowering drugs+insulin sensitizers; cholesterol-lowering drugs+angiotensin receptor blockers)			
The impact of statins on glucose metabolism and risk of diabetes in NAFLD patients requires further investigation			
The benefits of statins on renal function, and their efficacy in preventing/slowing the development of end-stage renal disease, as well as underlying mechanisms, needs further confirmation			

REFERENCES

1 Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol. 2011;9: 524-530.

**2Musso G, Gambino R, Cassader M, Pagano G. Prognosis and non-invasive methods to assess liver disease severity in Non-alcoholic Fatty Liver Disease (NAFLD): systematic review and meta-analysis. Ann Med 2010 Ann Med. 2010 Nov 2. [Epub ahead of print] PMID: 21039302

A comprehensive meta-analysis addressing the risk of NAFLD and its histological subtypes of developing liver-related, cardiovascular disease and type 2 diabetes.

3Fon Tacer K, Rozman D. Nonalcoholic Fatty liver disease: focus on lipoprotein and lipid deregulation. J Lipids. 2011;2011:783976. Epub 2011 Jul 2. PMID: 21773052

**4Marí M, Caballero F, Colell A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. Cell Metab. 2006; 4:185-98.

This landmark study proposex a critical role for mitochondrial free cholesterol loading in precipitating liver injury in NASH, by sensitizing hepatocytes to TNF and Fas through mitochondrial glutathione depletion. Atorvastatin administration prevented free cholesterol accumulation and related alteration

5Wouters K, van Bilsen M, van Gorp PJ, et al. Intrahepatic cholesterol influences progression, inhibition and reversal of non-alcoholic steatohepatitis in hyperlipidemic mice. FEBS Letters 2010; 584: 1001–1005

6 Van Rooyen DM, Larter CZ, Haigh WG, et al. Hepatic Free Cholesterol Accumulates in Obese, Diabetic Mice and Causes Non-Alcoholic Steatohepatitis, Gastroenterology (2011), doi: 10.1053/j.gastro.2011.06.040.

*7 Yasutake K, Nakamuta M, Shima Y, et al. Nutritional investigation of non-obese patients with non

alcoholic fatty liver disease: the significance of dietary cholesterol. Scand J Gastroenterol. 2009;44: 471-7.

This paper shows dietary cholesterol intake is a risk factor for NAFLD in non-obese subjects

8Enjoji M, Nakamuta M. Is the control of dietary cholesterol intake sufficiently effective to ameliorate nonalcoholic fatty liver disease? World J Gastroenterol. 2010;16: 800-3.

*9 Puri P, Wiest MM, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology 2007; 46: 1081–90.

First study to link hepatic free cholesterol accumulation to the development of NASH in humans

10 Caballero F, Fernández A, De Lacy AM, et al. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. J Hepatology 2009; 50:789-796

11Cohen DE, Anania FA, Chalasani N. An assessment of statin safety by hepatologists. Am J Cardiol 2006; 97(8A): 77C-81C.

12Sattar N, Preiss D, Murray HM, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. Lancet. 2010; 375:735-42.

13 Nelson A, Torres DM, Morgan AE, et al. A Pilot Study Using Simvastatin in the Treatment of Nonalcoholic Steatohepatitis: A Randomized Placebo-controlled Trial. J Clin Gastroenterol. 2009; 43: 990-4.

14 Athyros VG, Tziomalos K, Gossios TD, et al. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. Lancet. 2010; 376:1916-22.

**15 Targher G, Bertolini L, Rodella S, et al. Relationship between kidney function and liver histology in subjects with nonalcoholic steatohepatitis. Clin J Am Soc Nephrol. 2010 ;5: 2166-71

Cross-sectional study connecting the severity of liver histology in NASH to reduced kidney function, independently of traditional risk factors, metabolic syndrome and insulin resistance

16 Yasui K, Sumida Y, Mori Y, et al Nonalcoholic steatohepatitis and increased risk of chronic kidney disease. Metabolism. 2011;60: 735-9.

17 Arase Y, Suzuki F, Kobayashi M, et al The development of chronic kidney disease in Japanese patients with non-alcoholic fatty liver disease. Intern Med. 2011;50:1081-7.

**18 Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. J Am Soc Nephrol. 2010;21: 406-12.

Comprehensive review addressing mechanisms connecting adipose tissue escess to non-alcoholic fatty liver and chronic kidney disease

19 Suzuki T, Toyohara T, Akiyama Y, et al. Transcriptional regulation of organic anion transporting polypeptide SLCO4C1 as a new therapeutic modality to prevent chronic kidney disease. J Pharm Sci. 2011; 100: 3696-707

**20 Krane V, Wanner C. Statins, inflammation and kidney disease. Nat Rev Nephrol. 2011;7: 385-97

Comprehensive review addressing the role of anti-inflammatory properties of statins in their renal-protective effect

**21 Wanders D, Plaisance EP, Judd RL. Pharmacological effects of lipid-lowering drugs on circulating adipokines. World J Diabetes 2010; 1: 116-128

Comprehensive review on the impact of different statins on circulating adipokines

22 Foster T, Budoff MJ, Saab S, et al. Atorvastatin and antioxidants for the treatment of nonalcoholic fatty liver disease: the St Francis Heart Study randomized clinical trial. Am J Gastroenterol. 2011; 106:71-7.

23 Sanyal AJ , Chalasani N , Kowdley KV et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis . N Engl J Med 2010; 362 : 1675 – 85

24 Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial.

JAMA. 2011 ;305:1 659-68.

**25Vilà L, Rebollo A, Aðalsteisson GS, et al. Reduction of liver fructokinase expression and improved hepatic inflammation and metabolism in liquid fructose-fed rats after atorvastatin treatment. Toxicol Appl Pharmacol. 2011; 251: 32-40.

**26 Pramfalk C, Parini P, Gustafsson U, et al. Effects of high-dose statin on the human

hepatic expression of genes involved in carbohydrate and triglyceride metabolism. J Intern Med. 2011;

269:333-9

This small randomized trial shows atorvastatin 80 mg/d for 4 weeks significantly reduces hepatic and plasma triglyceride in humans. This effect is associated with an altered expression of key regulatory transcription factors involved in hepatic lipogenesis and fatty acid oxidation

*27 Quist-Paulsen P . Statins and infl ammation: an update . Curr Opin Cardiol 2010 ; 25 : 399 – 405

Comprehensive and update review on the anti-inflammatory effects of statins

*28 Kimura Y, Hyogo H, Yamagishi S, et al. Atorvastatin decreases serum levels of advanced glycation endproducts (AGEs) in nonalcoholic steatohepatitis (NASH) patients with dyslipidemia: clinical usefulness of AGEs as a biomarker for the attenuation of NASH. J Gastroenterol. 2010; 45: 750-7.

This paper connects altered shows atorvastatin decreases AGE-triggered inflammation and improves liver histology in NASH patients

29 Zhang N, Huan Y, Huang H, et al, Atorvastatin improves insulin sensitivity in mice with obesity induced by monosodium glutamate. Acta Pharmacologica Sinica (2010) 31: 35–42

*30 Moreno M, Ramalo LN, Sancho-Bru P, et al. Atorvastatin attenuates angiotensin II-induced inflammatory actions in the liver. Am J Physiol Gastrointest Liver Physiol 2009; 296: G147–156. Landmark study showing the angiotensin II axis inhibition mediates the favourable effects of atorvastatin on liver disease in experimental NASH

31 Maejima1 T,, Sugano1T, Yamazaki H, et al. Pitavastatin Increases ABCA1 Expression by Dual Mechanisms: SREBP2-Driven Transcriptional Activation and PPARα-Dependent Protein Stabilization but Without Activating LXR in Rat Hepatoma McARH7777 Cells. J Pharmacol Sci 2011; 116, 107–15

32 Kobayashi M, Gouda K, Chisaki I, et al. Regulation mechanism of ABCA1 expression by statins in hepatocytes. Eur J Pharmacol. 2011 Jul 15;662(1-3):9-14

*33 Miyaki T, Nojiri S, Shinkai N, et al. Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. Hepatol Res. 2011;41:375-85.
This study shows pitavastatin significantly attenuates production of pro-inflammatory and pro-fibrogenic cytokines

34 Chan DC, Nguyen MN, Watts GF, et al. Effects of atorvastatin and n-3 fatty acid supplementation on VLDL apolipoprotein C-III kinetics in men with abdominal obesity. Am J Clin Nutr. 2010; 91:900-6

35 Calisto KL, Carvalho Bde M, Ropelle ER, et al. Atorvastatin improves survival in septic rats: effect on tissue inflammatory pathway and on insulin signaling. PLoS One. 2010;5: :e14232.

36 Zheng S, Hoos L, Cook J, et al. Ezetimibe improves high fat and cholesterol diet-induced non alcoholic fatty liver disease in mice. Eur J Pharmacol. 2008; 584: 118-24.

37 Nozaki Y, Fujita K, Yoneda M, et al. Long-term combination therapy of ezetimibe and acarbose for non-alcoholic fatty liver disease. J Hepatol. 2009 Sep;51:548-56.

38 Park H, Shima T, Yamaguchi K, et al. Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease. J Gastroenterol. 2011; 46: 101-7

39 Yoneda M, Fujita K, Nozaki Y, et al. Efficacy of ezetimibe for the treatment of non-alcoholic steatohepatitis: An open-label, pilot study. Hepatol Res. 2010; 40: 613-21

40Park H, Hasegawa G, Shima T, et al. The fatty acid composition of plasma cholesteryl esters and estimated desaturase activities in patients with nonalcoholic fatty liver disease and the effect of long-term ezetimibe therapy on these levels. Clin Chim Acta. 2010; 411: 21-22

41Chan DC, Watts GF, Gan SK, Ooi EM, Barrett PH Effect of ezetimibe on hepatic fat, inflammatory markers and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. Diabetes Care. 2010; 33: 1134-9

*42 Takeshita Y, Takamura T, Kita Y, et al. Efficacy of ezetimibe for the treatment of non-alcoholic fatty liver disease: a randomized controlled trial. J Hep 2011; 54: S346

Firs RCT with ezetimibe in NASH, showing improvement in hepatocyte ballooning and fibrosis score and an increase in HbA1c

43 Abel T, Fehér J, Dinya E, et al. Efficacy and safety of ezetimibe/simvastatin combination therapy in patients with type 2 diabetes and nonalcoholic fatty liver disease. Orv Hetil. 2009;150: 989-93.

**44 Nozaki Y, Fujita K, Yoneda M, et al. Long-term combination therapy of ezetimibe and acarbose for non-alcoholic fatty liver disease. J Hepatol. 2009;51: :548-56.

This study in C57BL/61 mice demonstrates the additive benefits of ezetimibe+acarbose on liver histology over either agents alone, and individuates in increased hepatic expression of microsomal triglyceride transfer protein (MTP) and peroxisome proliferators-activated receptor-alpha1 (PPAR alpha1) the potential mechanisms underlying this synergy.

45 Rudovich NN, Weickert MO, Machann J, Pfeiffer AF. Combination of acarbose and ezetimibe prevents non-alcoholic fatty liver disease: a break of intestinal insulin resistance? J Hepatol. 2010;52:952-3.

46 Moritoh Y, Takeuchi K, Hazama M. Combination treatment with alogliptin and voglibose increases active GLP-1 circulation, prevents the development of diabetes and preserves pancreatic beta-cells in prediabetic db/db mice. Diabetes Obes Metab. 2010;12:224-33.

47 Nagai K, Matsumaru K, Takahashi Y, Nakamura N. Effective therapy using voglibose for nonalcoholic steatohepatitis in a patient with insufficient dietary and exercise therapy: exploring other treatment possibilities. Case Rep Gastroenterol. 2011; 5:336-43.