

Statin Effects to Reduce Hepatosteatosi s as Measured by Computed Tomography in Patients With Human Immunodeficiency Virus

Janet Lo,^{1a} Michael T. Lu,^{2a} Elli A. Kim,¹ Eric Nou,¹ Travis R. Hallett,² Jakob Park,² Udo Hoffmann,² and Steven K. Grinspoon¹

¹Department of Medicine, Endocrine Division, Program in Nutritional Metabolism, Massachusetts General Hospital and Harvard Medical School, and ²Department of Radiology, Cardiac PET MR CT Program, Massachusetts General Hospital, and Harvard Medical School, Boston

Hepatosteatosi s is highly prevalent among patients living with human immunodeficiency virus. In a 1-year, randomized, double-blind trial of atorvastatin or placebo, atorvastatin increased liver/spleen ratio among patients with nonalcoholic fatty liver disease, indicating a reduction in hepatosteatosi s. This reduction in hepatosteatosi s is associated with reduction in low-density lipoprotein cholesterol with statin therapy.

Keywords. hepatosteatosi s; human immunodeficiency virus; LDL cholesterol; statin.

Liver disease has emerged as one of the leading causes of death among human immunodeficiency virus (HIV)-infected patients [1]. Nonalcoholic fatty liver disease (NAFLD) is an important cause of liver damage and occurs more commonly in individuals living with HIV (30%–40%) than in the noninfected population (14%–31%) [2, 3]. Antiretroviral therapy (ART) is known to cause adverse changes in body composition including central fat accumulation, insulin, and elevated triglycerides [4, 5]. Such changes and chronic inflammation may lead to increased severity of NAFLD in HIV-infected patients. In a cross-sectional study, patients with HIV-associated NAFLD exhibited higher rates of steatohepatitis and increased severity of liver injury compared with age- and sex-matched non-HIV patients with NAFLD [6].

Given the increased risk of hepatosteatosi s for individuals infected with HIV, effective therapies to limit the progression of

NAFLD in this population are needed. In a randomized, placebo-controlled trial of 13 patients with HIV/hepatitis C virus coinfection, pioglitazone treatment reduced hepatic fat content measured by magnetic resonance spectroscopy [7]. Statins have been proposed to mitigate cardiac disease risk in HIV-infected patients [8], but no studies have yet assessed the effects of statins on NAFLD in the HIV population. Among non-HIV patients, limited studies suggest potential benefit of statins in patients with NAFLD [8–11]. In a Swedish open-label study, statin treatment significantly reduced fatty liver infiltration observed in liver biopsies collected at a 13.8-year follow-up of patients with NAFLD [9]. More recently, lower hepatosteatosi s prevalence was reported in patients who received statins for more than 2 years, using observational data from the Rotterdam Study [11]. These reports highlight the need for data from controlled trials of statins on NAFLD in HIV-infected patients, a population with a particularly high prevalence of both NAFLD and cardiovascular disease.

In a 12-month randomized, placebo-controlled trial, we previously demonstrated significant reductions in coronary plaque in HIV-infected participants treated with atorvastatin versus placebo [8]. Leveraging computed tomography (CT) data obtained in the study, we now investigate the effects of atorvastatin on hepatosteatosi s among HIV participants with NAFLD. We hypothesized that hepatosteatosi s would improve after 1 year with atorvastatin.

METHODS

Study Design

We recruited 40 men and women with HIV on stable ART, no prior history of cardiovascular disease or cardiac symptoms, LDL cholesterol between 70 and 130 mg/dL, and evidence of subclinical coronary atherosclerosis on coronary CT angiography (CCTA). Full exclusion resistance criteria were previously reported [8]. Participants with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 times the upper limit of normal and/or undergoing treatment for active liver disease were excluded. Participants were randomized in 1:1 ratio to either atorvastatin (starting at dose of 20 mg/day and escalating to 40 mg/day at 3-month visit if study drug was well tolerated) or placebo for 12 months. Thirty-seven of the 40 participants completed the study. In the current analysis, we assessed changes in hepatosteatosi s measured by CT in response to atorvastatin. All participants provided written informed consent, and the study was approved by the institutional review board. The trial is registered on ClinicalTrials.gov (NCT00965185).

Noncontrast Computed Tomography (CT) and Contrast Coronary CT Angiography

Cardiac electrocardiogram (ECG)-gated CT was performed at enrollment and 1 year of follow up, using a Somatom Definition

Received 8 December 2015; accepted 18 March 2016.

^aJ. L. and M. T. L. contributed equally to this work.

Correspondence: J. Lo, Massachusetts General Hospital, LON-203, 55 Fruit Street, Boston, MA 02114 (jlo@mgh.harvard.edu).

Open Forum Infectious Diseases®

© The Author 2016. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com. DOI: 10.1093/ofid/ofw062

Flash 128 slice dual source CT scanner (Siemens) according to the guidelines of the Society of Cardiac Computed Tomography [12] as previously described [8]. The protocol included a noncontrast CT that was used to measure the density of liver and spleen.

Measurement of Liver and Spleen Density

Noncontrast CT allows for the noninvasive measurement of liver density (attenuation) in Hounsfield Units (HUs). Liver density is inversely correlated to fat content and has been validated as an accurate, reproducible means to characterize hepato steatosis [13–16]. Liver density was measured on noncontrast ECG-gated CT images originally obtained for coronary artery calcium. A reader blinded to participant allocation, CCTA, and biomarker results measured the hepatic and splenic CT attenuation using a dedicated workstation (AQi; TeraRecon) [17, 18]. Hepatic density was measured by selecting 3 circular regions of interest with an area of at least 2 cm² on 3 axial CT slices, taking care to avoid the hepatic vessels and bile ducts. Likewise, 3 density measurements were taken in the spleen

(Figure 1A). The spleen is visible on most liver CT slices, it is normally less dense than the liver, and it is used as an internal control for assessing liver density. The ratio of the mean liver measurements divided by the mean spleen measurements was calculated, with a liver/spleen ratio <1.0 defined as NAFLD [14, 16]. This definition is commonly used with good accuracy compared with liver biopsy and minimal intra- and interobserver variability [14, 16]. Reproducibility was assessed with the intraclass correlation coefficient (ICC) of mean liver attenuation, spleen attenuation, and liver/spleen ratio measurements used in the analysis compared with 20 randomly selected CTs remeasured by the same reader more than 6 months after the original measurements. The ICCs of mean liver and spleen attenuation were 0.977 and 0.986, respectively. The ICC for the liver/spleen ratio was 0.982.

Lipid Parameters

Direct LDL was measured by homogeneous enzymatic colorimetric assay (COBAS INTEGRA; Roche Diagnostics). Other

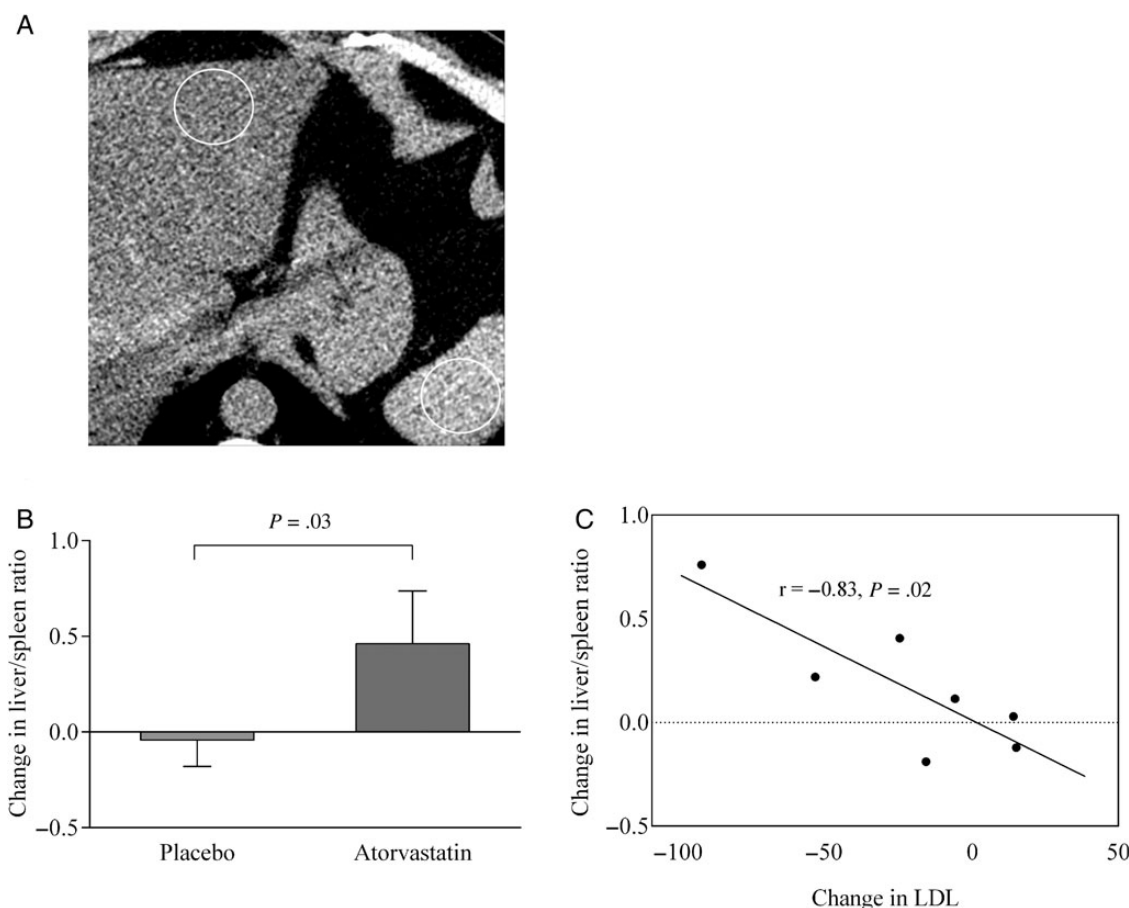


Figure 1. (A) Entry noncontrast computed tomography with regions of interest (ROI) placed over the liver (white circle, top left) and spleen (bottom right). The liver is visibly darker than the spleen, indicating that it is less dense. The liver/spleen ratio averaged over 3 ROIs was 0.38, compatible with hepatosteatosis. (B) Comparison of change in liver/spleen attenuation ratio between atorvastatin and placebo groups in patients with nonalcoholic fatty liver disease at baseline. Bar denotes mean and error bar denotes standard deviation. (C) Linear regression between change in liver/spleen ratio and direct low-density lipoprotein (LDL). Pearson correlation coefficient = -0.83 ($P = .02$).

lipid levels were determined using standard techniques. Blood was drawn after a 12-hour fast.

Inflammatory, Metabolic, Biochemical, and Immunologic Assessments
Monocyte chemoattractant protein-1 (MCP-1) was measured by ELISA (R&D Systems). Glucose and hemoglobin A1c were determined using standard techniques after 12-hour overnight fast. CD4⁺ T-cell counts were assessed by flow cytometry. Human immunodeficiency virus ribonucleic acid (RNA) was measured in real time using clinically available ultrasensitive reverse transcription-polymerase chain reaction.

Body Composition and Dietary Assessment

Abdominal visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue areas were quantified using noncontrast abdominal CT at the level of the L4 pedicle^{36,37}.

Statistical Analysis

Comparisons between groups (atorvastatin vs placebo) were performed using Student's *t* test for variables that were normally distributed and by Wilcoxon rank-sum test for variables that were not normally distributed. Comparisons between groups among those with NAFLD were assessed by Kolmogorov-Smirnov exact test. For investigating relationships between continuous variables, Pearson correlation coefficient was assessed for normally distributed variables. Two-tailed probability values are reported, and statistical significance was assumed when $P < .05$. Mean and standard deviation or median and interquartile range are used to describe baseline values depending on normality of distribution. Mean and 95% confidence intervals (CIs) are used to report change values. All statistical analyses were performed using SAS JMP and SAS (SAS Institute).

RESULTS

Characteristics of the Participants at Baseline

Baseline characteristics were similar between treatment groups (atorvastatin vs placebo) as previously reported. Baseline liver-to-spleen attenuation ratio was similar between the atorvastatin and placebo groups (1.28 ± 0.30 vs 1.25 ± 0.52 , respectively, $P = .82$). Participants were all on ART, and most had undetectable viremia with similar immunological and virological indices between groups as previously published [8].

Characteristics of the Participants With Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease was identified in 9 participants at baseline using a liver-to-spleen HU attenuation ratio cutoff < 1 . The group with NAFLD ($n = 9$) and the group without NAFLD ($n = 28$) were similar in age, gender, alcohol consumption, body mass index (BMI), VAT, AST, ALT, fasting glucose, total cholesterol, HDL, triglycerides, and ART (Supplementary Table 1). None of the participants reported excessive alcohol consumption. Those with NAFLD had higher levels of MCP-1 compared with those without (406 ± 259 vs 285 ± 79 , $P = .04$), as well as higher levels of direct LDL (153 ± 36 vs 116 ± 30 ,

$P = .004$). Among the 9 participants with NAFLD, all 4 participants in the placebo group and 1 in the atorvastatin group were on nonnucleoside reverse transcriptase inhibitor-based regimens, 2 participants in the placebo group and 3 in the atorvastatin group were on protease inhibitor-based regimens, and 1 participant in the placebo group was on an integrase inhibitor-based regimen.

Relationships to Liver-to-Spleen Attenuation Ratio at Baseline

Among all participants, direct LDL significantly correlated negatively with liver-to-spleen attenuation ratio ($r = -0.35$, $P = .03$), suggesting that higher LDL was related to increased hepatosteatosis at baseline. Among those with NAFLD at baseline, VAT tended to correlate with liver-to-spleen attenuation ($r = -0.61$, $P = .08$), such that those with the most VAT tended to demonstrate more liver fat.

Change in Liver/Spleen CT Attenuation and Lipids by Treatment Group in Participants With Nonalcoholic Fatty Liver Disease

Among participants with NAFLD, change in liver/spleen ratio could be obtained for 7 participants (1 participant dropped out before the final CT scan, and another participant did not have a measurable spleen attenuation value), 3 of whom received atorvastatin. Those treated with atorvastatin demonstrated a mean increase in liver-to-spleen attenuation ratio of 0.46 (95% CI, .16–.77) compared with a mean decrease of -0.04 (95% CI, $-.30$ to $.22$) in those who received placebo ($P = .03$) (Figure 1B). However, among those without NAFLD at baseline, the change in liver/spleen ratio did not differ between placebo (-0.02 ± 0.20) and atorvastatin groups (-0.07 ± 0.17) ($P = .50$). Atorvastatin significantly lowered direct LDL among all participants (-38 mg/dL vs 11 mg/dL, $P < .001$). Atorvastatin also significantly lowered direct LDL in those with NAFLD, with a mean change of -57 mg/dL (95% CI, -89 to -25) vs 3 mg/dL (95% CI, -21 to 28) with placebo ($P = .01$). Changes in BMI, VAT, HDL, triglycerides, fasting glucose, AST, and ALT were similar between treatment groups among all participants and among those with NAFLD.

Relationship of Change in Liver-to-Spleen Attenuation Ratio to Lipids in Participants With Nonalcoholic Fatty Liver Disease

The change in liver-to-spleen attenuation ratio was significantly associated with change in LDL ($r = -0.83$, $P = .02$) (Figure 1C) but not other lipid, metabolic, or inflammatory parameters.

DISCUSSION

In this substudy of a randomized trial in participants with HIV and subclinical coronary atherosclerosis, atorvastatin reduced liver fat among HIV-infected participants with NAFLD, without a concurrent change in BMI or VAT. Of note, the change in hepatosteatosis in response to statin therapy was significantly correlated with decreases in LDL, corroborating a potential statin effect and suggesting a link with reduced lipid levels. To our knowledge, this is the first report of statin therapy reducing liver fat in the HIV population.

Our results support similar findings of atorvastatin and other statins in reducing hepatosteatosis in the general population. In a substudy of the St. Francis Heart Study, among participants with NAFLD defined as liver/spleen ratio <1, atorvastatin 20 mg daily in combination with vitamins C and E significantly improved liver/spleen ratio by 0.40 after 4 years of treatment [19]. In another study in patients with NAFLD who were randomized to atorvastatin, fenofibrate, or both drugs, the proportion of patients who no longer had evidence of NAFLD at the end of treatment was significantly higher in the atorvastatin groups than in the fenofibrate group [20]. Studies using other lipophilic statins have shown mixed results. A few studies suggest beneficial effects of pitavastatin. In one study, patients with biopsy-proven nonalcoholic steatohepatitis (NASH) treated with pitavastatin for 12 months showed significant improvement in ALT, γ -glutamyl transpeptidase, and lipid profiles [21]. In another study, both pitavastatin and atorvastatin reduced the severity of hepatosteatosis by CT [22]. On the other hand, a randomized placebo-controlled study of simvastatin in 16 patients with NASH demonstrated no statistically significant improvement in serum aminotransferases, hepatic steatosis, necroinflammatory activity, or stage of fibrosis with treatment, despite a 26% reduction in LDL in the simvastatin group [23]. Among the hydrophilic statins, rosuvastatin may have beneficial effects on NAFLD [24–26]. In a pilot study of 23 patients with NAFLD, rosuvastatin treatment for 8 months significantly improved the lipid profile and liver biochemical markers [24]. In another study, repeat liver biopsies and ultrasonography showed improvement of NASH in 19 of 20 patients after 12-month treatment with rosuvastatin [26]. These findings in the general population suggest that the beneficial effect on hepatosteatosis may not be limited to atorvastatin or lipophilic statins; however, there may be differential effects of different statins on NAFLD. Moreover, data on the effects of statins in the HIV population are lacking.

Statins may reduce hepatosteatosis by inhibiting expression of the membrane-bound transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c). Sterol regulatory element-binding protein-1c may play an important role in the pathogenesis of NAFLD as an explanatory link between metabolic dysregulation and triglyceride synthesis. When activated, SREBP-1c enters the nucleus where it upregulates enzymes involved in hepatic lipogenesis [27]. In humans, atorvastatin 80 mg given for 4 weeks reduced messenger RNA expression of SREBP-1c [28]. Thus, one potential mechanism through which statins reduce hepatosteatosis may involve downregulation of SREBP-1c and inhibition of triglyceride synthesis.

We found that MCP-1 concentrations are higher among HIV-infected participants with hepatosteatosis than those without steatosis, which is consistent with existing literature in the general population. In the general population, hepatic expression of MCP-1 has been shown to be upregulated in

nonalcoholic fatty liver disease [29]. Thus, activation of the innate immune system in chronic HIV infection may play an important role in the development of NAFLD, and statins can help to reduce proinflammatory monocytes and macrophages.

This study has limitations. The CT liver density measurement technique assessed for diffuse hepatosteatosis, the most common manifestation of fatty liver. Areas of focal fatty deposition may have been missed, although this potential for sampling error is also shared by liver biopsy, the reference standard for hepatosteatosis [30]. This study was also limited by the small number of participants with NAFLD who could be included in the analysis. Larger trials are needed to corroborate this observed effect of statin therapy on hepatosteatosis in patients with HIV and NAFLD and to elucidate potential mechanisms, including LDL-lowering, metabolic, or inflammatory effects.

CONCLUSIONS

Leveraging CT data obtained from a randomized trial of atorvastatin, in this new analysis, we report an improvement in hepatosteatosis with statin therapy in HIV-infected participants with NAFLD, possibly related to a reduction in LDL or via effects on immune activation. This preliminary observation suggests that statins may confer potential beneficial effects on NAFLD through their effects on lipid metabolism and inflammation in the HIV patient population, warranting future investigation.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

Acknowledgments

We thank the patients who generously donated their time to participate in this study, the nurses and bionutritionists at the Massachusetts General Hospital Clinical Research Center of the Harvard Clinical and Translational Science Award, and Dr. Hang Lee for statistical support.

Financial support. This work was funded by National Institutes of Health (NIH) grants K23HL092792 (to J. L.), 5T32HL076136 (to M. T. L.), 5T32DK007028-40 (to E. N.), 5K24HL113128 (to U. H.), R01HL095123 (to S. K. G.), and P30 DK040561 (to S. K. G.). The project described was also supported by NIH 1 UL1 RR025758-04, Harvard Clinical and Translational Science Center, National Center for Research Resources, Massachusetts General Hospital Executive Committee on Research, and the American Roentgen Ray Society.

Potential conflicts of interests. S. K. G. has consulted with Navidea, BMS, Merck, Gilead, and Theratechnologies and received grant support from Gilead, Amgen, KOWA Pharmaceuticals, and Theratechnologies, unrelated to this manuscript. J. L. has consulted with Gilead, unrelated to this manuscript. M. T. L. received grant support from the American College of Radiology, unrelated to this manuscript. U. H. received grant support from Siemens Healthcare, American College of Radiology Imaging Network, and HeartFlow Inc., unrelated to this manuscript. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. *Clin Infect Dis* 2010; 50:1387–96.

2. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* **2007**; 25:883–9.
3. Moyle G, Carr A. HIV-associated lipodystrophy, metabolic complications, and antiretroviral toxicities. *HIV clinical trials* **2001**; 3:89–98.
4. Joshi D, O'Grady J, Dieterich D, et al. Increasing burden of liver disease in patients with HIV infection. *Lancet* **2011**; 377:1198–209.
5. Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med* **2005**; 352:48–62.
6. Vodkin I, Valasek M, Bettencourt R, et al. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: a case-control study. *Aliment Pharmacol Ther* **2015**; 41:368–78.
7. Matthews L, Kleiner DE, Chairez C, et al. Pioglitazone for hepatic steatosis in HIV/hepatitis C virus coinfection. *AIDS Res Hum Retroviruses* **2015**; 31:961–6.
8. Lo J, Lu MT, Ihenachor EJ, et al. Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet HIV* **2015**; 2:e52–63.
9. Ekstedt M, Franzén LE, Mathiesen UL, et al. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol* **2007**; 47:135–41.
10. Pastori D, Polimeni L, Baratta F, et al. The efficacy and safety of statins for the treatment of non-alcoholic fatty liver disease. *Dig Liver Dis* **2015**; 47:4–11.
11. de Keyser CE, Koehler EM, Schouten JN, et al. Statin therapy is associated with a reduced risk of non-alcoholic fatty liver in overweight individuals. *Dig Liver Dis* **2014**; 46:720–5.
12. Raff GL, Abidov A, Achenbach S, et al. SCCT guidelines for the interpretation and reporting of coronary computed tomographic angiography. *J Cardiovasc Comput Tomogr* **2009**; 3:122–36.
13. Boyce CJ, Pickhardt PJ, Kim DH, et al. Hepatic steatosis (fatty liver disease) in asymptomatic adults identified by unenhanced low-dose CT. *AJR Am J Roentgenol* **2010**; 194:623–8.
14. Park YS, Park SH, Lee SS, et al. Biopsy-proven nonsteatotic liver in adults: estimation of reference range for difference in attenuation between the liver and the spleen at nonenhanced CT. *Radiology* **2011**; 258:760–6.
15. Speliotes EK, Massaro JM, Hoffmann U, et al. Liver fat is reproducibly measured using computed tomography in the Framingham Heart Study. *J Gastroenterol Hepatol* **2008**; 23:894–9.
16. Zeb I, Li D, Nasir K, et al. Computed tomography scans in the evaluation of fatty liver disease in a population based study: the multi-ethnic study of atherosclerosis. *Acad Radiol* **2012**; 19:811–8.
17. Assy N, Djibre A, Farah R, et al. Presence of coronary plaques in patients with nonalcoholic fatty liver disease. *Radiology* **2010**; 254:393–400.
18. Puchner SB, Lu MT, Mayrhofer T, et al. High-risk coronary plaque at coronary CT angiography is associated with nonalcoholic fatty liver disease, independent of coronary plaque and stenosis burden: results from the ROMICAT II trial. *Radiology* **2015**; 274:693–701.
19. 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.
20. Athyros VG, Mikhailidis DP, Didangelos TP, et al. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study. *Curr Med Res Opin* **2006**; 22:873–83.
21. Hyogo H, Ikegami T, Tokushige K, et al. Efficacy of pitavastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: an open-label, pilot study. *Hepatol Res* **2011**; 41:1057–65.
22. Han KH, Rha SW, Kang HJ, et al. Evaluation of short-term safety and efficacy of HMG-CoA reductase inhibitors in hypercholesterolemic patients with elevated serum alanine transaminase concentrations: PITCH study (PITavastatin versus atorvastatin to evaluate the effect on patients with hypercholesterolemia and mild to moderate hepatic damage). *J Clin Lipidol* **2012**; 6:340–51.
23. 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.
24. Antonopoulos S, Mikros S, Mylonopoulou M, et al. Rosuvastatin as a novel treatment of non-alcoholic fatty liver disease in hyperlipidemic patients. *Atherosclerosis* **2006**; 184:233–4.
25. Nakahara T, Hyogo H, Kimura Y, et al. Efficacy of rosuvastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: An open-label, pilot study. *Hepatol Res* **2012**; 42:1065–72.
26. Kargiotis K, Athyros VG, Gioulema O, et al. Resolution of non-alcoholic steatohepatitis by rosuvastatin monotherapy in patients with metabolic syndrome. *World J Gastroenterol* **2015**; 21:7860–8.
27. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* **2002**; 109:1125–31.
28. 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.
29. Westerbacka J, Kolak M, Kiviluoto T, et al. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* **2007**; 56:2759–65.
30. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* **2005**; 128:1898–906.