

Manuscript Number: DLD-11-585R3

Title: Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumor necrosis factor- $\alpha$ -lowering property

Article Type: Original Article

Section/Category: Regular Articles

Keywords: atorvastatin; NASH; TNF- $\alpha$ ; inflammation.

Corresponding Author: Dr. Sho-ichi Yamagishi, MD, PhD

Corresponding Author's Institution: Kurume University

First Author: Hideyuki Hyogo

Order of Authors: Hideyuki Hyogo; Sho-ichi Yamagishi, MD, PhD; Sayaka Maeda; Yuki Kimura; Tomokazu Ishitobi; Kazuaki Chayama

**Abstract:** Background: We have previously found that atorvastatin decreases liver injury markers in patients with nonalcoholic steatohepatitis. However, how atorvastatin treatment ameliorates the disease activity in nonalcoholic steatohepatitis patients remains unknown. Aims: We examined here which anthropometric, metabolic and inflammatory variables were improved and related with amelioration of disease activity in atorvastatin-treated nonalcoholic steatohepatitis patients. Methods: Forty-two biopsy-proven nonalcoholic steatohepatitis patients with dyslipidemia were enrolled. Patients were treated with atorvastatin (10 mg/day) for 12 months. Results: Atorvastatin significantly decreased liver transaminase,  $\gamma$ -glutamyl transpeptidase, low-density lipoprotein-cholesterol, triglycerides, type IV collagen, and tumor necrosis factor- $\alpha$  levels, while it increased adiponectin and high-density lipoprotein-cholesterol. Atorvastatin improved nonalcoholic fatty liver disease activity score and increased liver to spleen density ratio. Multiple stepwise regression analysis revealed that  $\gamma$ -glutamyl transpeptidase, tumor necrosis factor- $\alpha$  and liver to spleen density ratio (inversely) were independently associated with nonalcoholic fatty liver disease activity score. Aspartate aminotransferase, low-density lipoprotein-cholesterol and nonalcoholic fatty liver disease activity score were independent determinants of decreased liver to spleen density ratio. Conclusion: The present study suggests that atorvastatin improves the disease activity of nonalcoholic steatohepatitis partly via its tumor necrosis factor- $\alpha$  -lowering property.

December 10, 2011

Professor Mario Angelico  
Editor-in-Chief  
Digestive and Liver Disease

Dear Professor Angelico,  
RE: DLD-11-585R2

Many thanks for your letter of December 10, 2011 with your valuable comments concerning our manuscripts entitled **“Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumor necrosis factor- $\alpha$ -lowering property”**. We are very grateful to you for your prompt arrangement and thorough review.

According to your comments, the manuscript has been completely re-revised and we addressed all of them.

We hope the re-revision will satisfy you and be followed by publication in Digestive and Liver Disease.

**Response to the your comments:**

Thank you very much for your valuable comments.

1. According to your comments. we followed the format guidelines regarding abbreviations and removed abbreviations in the title and abstract of the re-revised manuscript. We uploaded Tables as separate files, removed abbreviations from Table headers, and explained all abbreviations present in the Tables in a footnote below. We also removed bold text from the Tables.

Best regards,

Yours sincerely,

Sho-ichi Yamagishi, M.D., Ph.D.

Department of Pathophysiology and  
Therapeutics of Diabetic Vascular  
Complications

Kurume Univ. School of Medicine

67 Asahi-machi

Kurume 830-0011, Japan

Email: shoichi@med.kurume-u.ac.jp

October 26, 2011

Editor-in-Chief  
Digestive and Liver Disease

Dear the Editor,

I respectfully submit our manuscript entitled **“Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its anti-inflammatory property”** by myself *et al.* for your consideration for publication in *Digestive and Liver Disease*. We confirm that there are no conflicts of interest to disclose in this paper.

Thank you for your consideration.

Best regards,

Yours sincerely,

Hideyuki Hyogo<sup>1</sup>, Sho-ichi Yamagishi<sup>2</sup>, Sayaka Maeda<sup>2</sup>, Yuki Kimura<sup>1</sup>, Tomokazu Ishitobi<sup>1</sup>,  
Kazuaki Chayama<sup>1</sup>

<sup>1</sup>Department of Medicine and Molecular Sciences,  
Graduate School of Biomedical Sciences,  
Hiroshima University, Hiroshima, Japan,

<sup>2</sup>Department of Pathophysiology and Therapeutics  
of Diabetic Vascular Complications, Kurume  
University School of Medicine, Kurume, Japan

**Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumor necrosis factor- $\alpha$ -lowering property**

Hideyuki Hyogo<sup>1</sup>, Sho-ichi Yamagishi<sup>2</sup>, Sayaka Maeda<sup>2</sup>, Yuki Kimura<sup>1</sup>, Tomokazu Ishitobi<sup>1</sup>, Kazuaki Chayama<sup>1</sup>

<sup>1</sup>Department of Medicine and Molecular Sciences, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan, <sup>2</sup>Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Japan

Short running Title: Atorvastatin and NASH

Word counts: 1,930

This work was supported in part by Grants of Collaboration with Venture Companies Project from the Ministry of Education, Culture, Sports, Science and Technology, Japan (S.Y). There are no conflicts of interest to disclose in this paper.

Correspondence Author; Sho-ichi Yamagishi, MD, PhD, Department of Pathophysiology, and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume 830-0011, Kurume, Japan

Tel: +81-942-31-7873

Fax: +81-942-31-7895

E-mail: [shoichi@med.kurume-u.ac.jp](mailto:shoichi@med.kurume-u.ac.jp)

## Abstract

**Background:** We have previously found that atorvastatin decreases liver injury markers in patients with nonalcoholic steatohepatitis. However, how atorvastatin treatment ameliorates the disease activity in nonalcoholic steatohepatitis patients remains unknown. **Aims:** We examined here which anthropometric, metabolic and inflammatory variables were improved and related with amelioration of disease activity in atorvastatin-treated nonalcoholic steatohepatitis patients. **Methods:** Forty-two biopsy-proven nonalcoholic steatohepatitis patients with dyslipidemia were enrolled. Patients were treated with atorvastatin (10 mg/day) for 12 months. **Results:** Atorvastatin significantly decreased liver transaminase,  $\gamma$ -glutamyl transpeptidase, low-density lipoprotein-cholesterol, triglycerides, type IV collagen, and tumor necrosis factor- $\alpha$  levels, while it increased adiponectin and high-density lipoprotein-cholesterol. Atorvastatin improved nonalcoholic fatty liver disease activity score and increased liver to spleen density ratio. Multiple stepwise regression analysis revealed that  $\gamma$ -glutamyl transpeptidase, tumor necrosis factor- $\alpha$  and liver to spleen density ratio (inversely) were independently associated with nonalcoholic fatty liver disease activity score. Aspartate aminotransferase, low-density lipoprotein-cholesterol and nonalcoholic fatty liver disease activity score were independent determinants of decreased liver to spleen density ratio. **Conclusion:** The present study suggests that atorvastatin improves the disease activity of nonalcoholic steatohepatitis partly via its tumor necrosis factor- $\alpha$ -lowering property.

**Key words;** atorvastatin, NASH, TNF- $\alpha$ , inflammation

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease in the western world and is histologically categorized into steatosis and steatohepatitis [1]. The latter histological entity, nonalcoholic steatohepatitis (NASH) may be a potentially progressive hepatic disorder that can lead to liver cirrhosis and hepatocellular carcinoma [2]. Insulin resistance increases visceral adipocyte lipolysis and subsequently releases free fatty acids into the portal circulation, where they are rapidly translocated to the liver, thereby eliciting hepatic steatosis [2]. Fatty acids stimulate reactive oxygen species generation in the liver, promoting the disease progression to NASH by enhancing both lipid peroxidation and inflammatory cytokine production [2]. Necro-inflammatory component of NASH could be modulated by various factors that stimulate the biological activity of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [3]. Further, pentoxifylline, an inhibitor of TNF- $\alpha$ , is reported to ameliorate insulin resistance and decrease liver transaminase in patients with NASH [4-6]. These observations suggest that inflammatory cytokine-evoked insulin resistance may be the most consistent pathogenic factor of NASH.

We, along with others, have recently found that statins such as atorvastatin, one of the most widely used drugs for the treatment of hypercholesterolemia, are effective in



patients with NASH with dyslipidemia; atorvastatin not only decreases liver transaminase, but also suppresses hepatic steatosis in these patients [7-12]. However, how atorvastatin could ameliorate NASH remains unknown. We first examined here whether atorvastatin treatment significantly improved anthropometric, metabolic and inflammatory variables, which might be related with disease activity of NASH, and then investigated the independent predictors of NAFLD activity score (NAS) in atorvastatin-treated NASH patients.

### **Research design and methods**

Forty-two biopsy-proven NASH patients with dyslipidemia (28 males and 14 females,  $50.5 \pm 12.7$  years old) were enrolled in the present study. All patients were negative for serology and viral hepatitis, and had no history of liver diseases. Current and past daily alcohol intake of the subjects was less than 20 g per week. Further, we excluded any patients with drug-induced hepatitis, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and biliary obstruction. Fourteen subjects were type 2 diabetes mellitus (DM), 13 impaired glucose tolerance (IGT) and 19 essential hypertension (HT). Four patients have already received oral hypoglycemic agents for the treatment of DM (DM medication) ( $\alpha$ -glucosidase inhibitor N=3, Sulfonylurea N=1),

and 14 subjects have received anti-hypertensive agents (HT medication) (angiotensin II type 1 receptor blockers N=6 (telmisartan N=1), calcium channel blockers N=12). Within a couple of weeks after the first liver biopsies, all patients started taking atorvastatin (10 mg once daily). After 12 month-atorvastatin therapy, post-treatment second liver biopsies were performed. Then computed tomography (CT) scanning was performed for quantitatively evaluating fat content in the liver. Liver fat content was shown as CT density ratio of liver to spleen (L/S density ratio) as described previously [13]. During the study period, subjects were instructed to improve their life styles and to continue taking the same dose of any concomitant drugs. Nobody received other anti-dyslipidemic agents than atorvastatin. Informed consent was obtained from all patients, and the study was conducted in conformity to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics and research committees of our hospital.

Blood was drawn after 12-hour fasting from the antecubital vein in the morning for determinations of lipid profiles; total cholesterol (T-Chol), LDL-cholesterol (LDL-C), triglycerides (TG), and HDL-cholesterol (HDL-C), fasting plasma glucose (FPG), fasting plasma insulin (IRI), glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP), TNF- $\alpha$ ,

adiponectin, type IV collagen and ferritin. These blood chemistries were measured with standard enzymatic methods or enzyme-linked immunosorbent assay kits as described previously [10,11]. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR index was calculated from the values of FPG (mg/dL) and IRI ( $\mu$ U/ml) using the following formula  $[(\text{FPG} \times \text{IRI})/405]$ .

Patients enrolled in this study underwent a percutaneous liver biopsy under ultrasonic guidance before and after atorvastatin treatment when the informed consent was obtained. All the specimens were examined by an experienced pathologist who was unaware of the clinical and biochemical data of the patients. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes and were classified as either steatosis or steatohepatitis. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus as described previously [14]. NAS was calculated as the unweighted sum of the scores for steatosis, lobular inflammation, and ballooning as reported by Kleiner et al [15].

A correlation between NAS, L/S density ration, TNF- $\alpha$ , and other clinical variables was determined by a linear regression analysis. To determine independent determinants of NAS, L/S density ration and TNF- $\alpha$ , multiple stepwise regression analysis was

performed. Statistical significance was defined as  $p < 0.05$ . All statistical analyses were performed with the use of the SPSS system (SPSS Japan Inc., IBM company, Tokyo, Japan). Statistical analysis was done on all the data including both pre- and post-treatments of atorvastatin.

## Results

Clinical and laboratory variables of all subjects at baseline and after atorvastatin treatment are shown in Table 1. Atorvastatin significantly decreased BMI, AST, ALT,  $\gamma$ -GTP, T-Chol, TG, LDL-C, FPG, type IV collagen and TNF- $\alpha$ , while it increased albumin, HDL-C and adiponectin. Although atorvastatin did not affect HOMA-IR, it significantly improved NAS and increased L/S density ratio. When the data of male and female were analyzed separately, there were some differences in atorvastatin effects between them. As shown in Table 2, atorvastatin significantly decreased FPG and increased HDL-C and adiponectin levels in male, but not female, while the effects of atorvastatin on HbA1c, type IV collagen and ferritin were significant in female, but not male.

As shown in Table 3, in univariate analyses, NAS was correlated with AST, ALT,  $\gamma$ -GTP, adiponectin (inversely), TNF- $\alpha$  and L/S density ratio (inversely), but not LDL-C

level. Because these parameters could be closely correlated with each other, to determine independent determinants of NAS, multiple stepwise regression analysis was performed. This analysis showed that  $\gamma$ -GTP, TNF- $\alpha$ , and L/S density ratio (inversely) were independently correlated with NAS ( $R^2=0.403$ ).

Further, as shown in Table 4, L/S density ratio inversely associated with AST, ALT, T-Chol, LDL-C, NAS, and TNF- $\alpha$ , while it was positively correlated with the presence of HT. Multiple stepwise regression analysis revealed that AST, LDL-C and NAS were independent correlates of L/S density ratio ( $R^2=0.515$ ).

We next investigated the determinants of TNF- $\alpha$  in our subjects. T-Chol, LDL-Chol, NAS, adiponectin (inversely) and L/S density ratio (inversely) were correlated with TNF- $\alpha$  in univariate analysis (Table 5). BMI, DM medication or HT medication was not associated with TNF- $\alpha$  in univariate analysis (Table 5). NAS and adiponectin were independently associated with TNF- $\alpha$  ( $R^2=0.254$ ).

## Discussion

In the present study, NAS was correlated with AST, ALT,  $\gamma$ -GTP, adiponectin (inversely), TNF- $\alpha$  and L/S density ratio (inversely), but not LDL-C level in univariate analysis (Table 3). Further, multiple stepwise regression analysis showed that  $\gamma$ -GTP, TNF- $\alpha$ ,

and L/S density ratio (inversely) were independently correlated with NAS. These observations suggest that although cholesterol level was associated with L/S density ratio (Table 4), atorvastatin treatment for 12 months could suppress the disease activity of NASH in a cholesterol lowering-independent manner. In this study, although atorvastatin did not affect insulin sensitivity measured by HOMA-IR index, it improved adipocytokine profiles in NASH patients. Further, TNF- $\alpha$ , but not adiponectin, was independently associated with NAS. These findings suggest that TNF- $\alpha$ -reducing property of atorvastatin rather than its adiponectin-elevating and insulin sensitizing effects may exert beneficial effects on NASH in our patients. Jarrar et al. reported that among various adipocytokines, TNF- $\alpha$  was the only independent predictor of fibrosis in NASH [16], thus further supporting the pathological role of TNF- $\alpha$  in the progression of NASH.

In the present study, BMI was significantly decreased after the atorvastatin treatment (Table 1). However, given that statins use is not associated with weight loss in clinical practice, it is probable that patients changed their dietary and physical habits in association with the atorvastatin treatment, which could lead to the reduction of BMI in our subjects. Further, in this study, BMI was not correlated with NAS, L/S density ratio or TNF- $\alpha$  in univariate analyses (Tables 3-5). It is in sharp contrast with previous

well-done studies on NASH showing that over weight and obesity are the mile stone in the pathogenesis of NASH and life style changes including weight loss and dietary changes are considered first-line therapeutic option [17,18]. The present study included only NASH patients with dyslipidemia and all patients were treated with atorvastatin. Therefore, the low number of patients and the selection bias of this study may have influenced the results. In the present study, DM medication or HT medication was not associated with NAS, L/S density ratio or TNF- $\alpha$  (Tables 3-5). So, it is unlikely that DM medication or HT medication could be associated with TNF- $\alpha$  reduction and improvement of NASH in our subjects.

In this study, the magnitude of LDL-C reduction obtained by atorvastatin treatment was independently correlated with that of increase in L/S density ratio (Table 4). Therefore, it is probable that atorvastatin not only blocks hepatic steatosis by reducing LDL-C levels, but also inhibits the disease progression of NASH partly via the reduction of TNF- $\alpha$ .

We have previously shown that advanced glycation end products (AGEs) evoke inflammatory and proliferative reactions in hepatic stellate cells, thus participating in the progression of NASH [19]. Further, serum levels of AGEs were significantly elevated in NASH patients compared with simple steatosis [14], and atorvastatin

treatment decreased circulating levels of AGEs in NASH subjects with dyslipidemia [11]. Oxidative stress participates in the formation of AGEs, and hydroxyl metabolites of atorvastatin have been shown to have anti-oxidative properties [20]. Given the present findings that HbA1c level was unchanged by the atorvastatin treatment, atorvastatin may reduce serum AGEs levels via its anti-oxidative property, which could partly explain the TNF- $\alpha$ -lowering effects of this agent in our NASH patients. The previous literature data on effect of lipid lower agents showed that such drugs partially improve only transaminase and they have no advantage over lifestyle changes [21,22]. Therefore, due to its unique AGEs- and TNF- $\alpha$ -lowering effects, atorvastatin may be a promising strategy for the treatment of NASH patients with dyslipidemia.

## Limitations

This study is not a randomized study. Thus without a control group (NASH without dyslipidemia or not treated with statin), many factors may have confounded the results. However, in this study, multiple stepwise regression analysis utilizing all the data (both before and after treatments) revealed that  $\gamma$ -GTP, TNF- $\alpha$ , and L/S density ratio (inversely) were independently correlated with NAS, while AST, LDL-C and NAS were independent determinants of L/S density ratio. Therefore, our present study suggests



that atorvastatin may improve the disease activity of NASH and hepatic steatosis partly via its TNF- $\alpha$ -lowering property.

Because of the selection bias, the results of this study could not be extrapolated to the entire population of NASH. Whether atorvastatin in association with weight loss may have a beneficial effect on NASH should be evaluated in future studies. Further, it would be interesting to evaluate separately the effects of atorvastatin on NASH patients with and without improvement of visceral obesity. The eventual differences between the two groups may suggest the protective role of atorvastatin in NASH subjects.

Only one subject received telmisartan, an angiotensin II type 1 receptor blocker with peroxisome proliferator-activated receptor-gamma-modulating activity [23]. Therefore, we could not evaluate here the exact role of telmisartan in TNF- $\alpha$  reduction. Further, lipopolysaccharides (LPS) may be associated with NASH and induce increased level of TNF- $\alpha$  [24]. Therefore, it would be interesting to examine the effects of atorvastatin on LPS level in NASH patients.

## **Acknowledgements**

This work was supported in part by Grants of Collaboration with Venture Companies

Project from the Ministry of Education, Culture, Sports, Science and Technology, Japan

(S.Y). There are no conflicts of interest to disclose in this paper.

## References

1. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. *Hepatology* 2009;49:306-17
2. Bugianesi E, Moscatiello S, Ciaravella MF, et al. Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des* 2000;16:1941-51
3. Copaci I, Micu L, Voiculescu M. The role of cytokines in non-alcoholic steatohepatitis. A review. *J Gastrointestin Liver Dis* 2006;15:363-73
4. Zein CO, Yerian LM, Gogate P, et al. Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *Hepatology* 2011 Jul 11. doi: 10.1002/hep.24544
5. Van Wagner LB, Koppe SW, Brunt EM, et al. Pentoxifylline for the treatment of non-alcoholic steatohepatitis: a randomized controlled trial. *Ann Hepatol* 2011;10:277-86
6. Musso G, Gambino R, Cassader M, et al. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010;52:79-104
7. 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

8. Riley P, Al Bakir M, O'Donohue J, et al. Prescribing statins to patients with nonalcoholic fatty liver disease: real cardiovascular benefits outweigh theoretical hepatotoxic risk. *Cardiovasc Ther* 2009;27:216-20

9. Argo CK, Loria P, Caldwell SH, et al. Statins in liver disease: a molehill, an iceberg, or neither? *Hepatology* 2008;48:662-9

10. Hyogo H, Tazuma S, Arihiro K, et al. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism* 2008;57:1711-8

11. 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

12. Gómez-Domínguez E, Gisbert JP, Moreno-Monteagudo JA, et al. A pilot study of atorvastatin treatment in dyslipemid, non-alcoholic fatty liver patients. *Aliment Pharmacol Ther* 2006;23:1643-1647

13. Ricci C, Longo R, Gioulis E, et al. Noninvasive in vivo quantitative assessment of fat content in human liver. *J Hepatol* 1997;27:108-13.

14. Hyogo H, Yamagishi S, Iwamoto K, et al. Elevated levels of serum advanced glycation end products in patients with non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2007;22:1112-9
15. Kleiner DE, Brunt EM, Van Natta M, et al. Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313-21
16. Jarrar MH, Baranova A, Collantes R, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008;27:412-421
17. Yki-Järvinen H. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. *Curr Opin Clin Nutr Metab Care* 2010;13:709-14
18. Tilg H, Moschen A. Weight loss: cornerstone in the treatment of non-alcoholic fatty liver disease. *Minerva Gastroenterol Dietol* 2010;56:159-67
19. Iwamoto K, Kanno K, Hyogo H, et al. Advanced glycation end products enhance the proliferation and activation of hepatic stellate cells. *J Gastroenterol* 2008;43:298-304
20. Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Curr Pharm Des* 2005;11:2279-99

21. 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
22. Duvnjak M, Tomasic V, Gomercic M, et al. Therapy of nonalcoholic fatty liver disease: current status. *J Physiol Pharmacol* 2009;60 Suppl 7:57-66
23. Yamagishi S, Nakamura K, Matsui T. Potential utility of telmisartan, an angiotensin II type 1 receptor blocker with peroxisome proliferator-activated receptor-gamma (PPAR-gamma)-modulating activity for the treatment of cardiometabolic disorders. *Curr Mol Med* 2007;7:463-9
24. Kudo H, Takahara T, Yata Y, et al. Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine non-alcoholic steatohepatitis model. *J Hepatol* 2009;51:168-75

December 10, 2011

Professor Mario Angelico  
Editor-in-Chief  
Digestive and Liver Disease

Dear Professor Angelico,  
RE: DLD-11-585R2

Many thanks for your letter of December 10, 2011 with your valuable comments concerning our manuscripts entitled “**Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumor necrosis factor- $\alpha$ -lowering property**”. We are very grateful to you for your prompt arrangement and thorough review.

According to your comments, the manuscript has been completely re-revised and we addressed all of them.

We hope the re-revision will satisfy you and be followed by publication in Digestive and Liver Disease.

**Response to the your comments:**

Thank you very much for your valuable comments.

1. According to your comments. we followed the format guidelines regarding abbreviations and removed abbreviations in the title and abstract of the re-revised manuscript. We uploaded Tables as separate files, removed abbreviations from Table headers, and explained all abbreviations present in the Tables in a footnote below. We also removed bold text from the Tables.

Best regards,

Yours sincerely,

Sho-ichi Yamagishi, M.D., Ph.D.

Department of Pathophysiology and  
Therapeutics of Diabetic Vascular  
Complications

Kurume Univ. School of Medicine

67 Asahi-machi

Kurume 830-0011, Japan

Email: shoichi@med.kurume-u.ac.jp



Table 1. Clinical variables of all subjects before and after atorvastatin treatment

Characteristics	before treatment	after treatment	p-value
Age (years)	50.0±12.7		
Number (male number)	42 (28)		
Body mass index (kg/m <sup>2</sup> )	27.2±2.9	26.3±2.7	p<0.01
AST (IU/L)	48.0±26.7	33.0±18.4	p<0.01
ALT (IU/L)	89.0±61.9	56.6±52.3	p<0.01
γ-GTP (IU/L)	90.4±89.9	65.1±49.9	p<0.05
Total-bilirubin (mg/dL)	0.9±0.3	0.9±0.3	0.765
Direct-bilirubin (mg/dL)	0.4±1.5	0.2±0.3	0.412
Albumin (g/dL)	4.6±0.3	4.8±0.3	p<0.01
T-Chol (mg/dl)	233.9±35.0	177.0±35.5	p<0.01
TG (mg/dl)	206.8±114.8	150.1±73.1	p<0.01
HDL-C (mg/dl)	48.0±12.5	54.1±11.9	p<0.01
LDL-C (mg/dl)	144.5±29.2	92.9±30.8	p<0.01
FPG (mg/dl)	107.9±14.9	104.4±17.3	p<0.05
HbA1c (%)	6.2±0.8	6.1±0.6	0.469
IRI (μU/mL)	12.9±6.7	13.1±7.1	0.560
HOMA-IR	3.2±2.0	3.4±1.9	0.534
NAS	3.9±1.0	3.0±0.9	p<0.01
Adiponectin (μg/mL)	5.5±2.3	6.4±2.8	p<0.01
TNF-α (pg/mL)	17.1±5.8	11.1±7.8	p<0.01
Type IV collagen (ng/mL)	4.2±1.0	3.8±0.8	p<0.05
Ferritin (ng/mL)	221.1±172.0	186.6±174.1	0.085
L/S density ratio	0.5±0.3	0.9±0.2	p<0.01
Number (DM/IGT/HT)	14/13/19	14/13/19	-
DM medication	4	4	-
HT medication	14	14	-

Data are shown as mean ± standard deviation, unless otherwise indicated.

AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density

lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol, FPG; fasting plasma glucose, HbA1c; glycated hemoglobin, IRI; fasting plasma insulin, HOMA-IR; homeostasis model assessment of insulin resistance, NAS; nonalcoholic fatty liver disease activity score, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , L/S; liver to spleen, DM; diabetes mellitus, IGT; impaired glucose tolerance, HT; hypertension.

Table 2. Clinical variables of male and female before and after atorvastatin treatment

Characteristics	Male			Female		
	before treatment	after treatment	p-value	before treatment	after treatment	p-value
Age (years)	47.8±12.4			54.4±12.6		
Number (male number)	28			14		
Body mass index (kg/m <sup>2</sup> )	27.2±3.0	26.3±2.8	p<0.01	27.2±2.9	26.1±2.6	p<0.01
AST (IU/L)	45.9±21.7	34.9±20.0	p<0.01	52.2±35.2	29.1±14.8	p<0.05
ALT (IU/L)	89.3±59.1	63.9±60.0	p<0.01	88.5±69.4	42.1±28.2	p<0.05
γ-GTP (IU/L)	81.3±77.4	66.3±52.8	0.070	108.4±111.9	62.6±45.3	0.068
Total-bilirubin (mg/dL)	0.9±0.4	0.9±0.3	0.778	0.8±0.2	0.9±0.3	0.212
Direct-bilirubin (mg/dL)	0.5±1.8	0.2±0.4	0.403	0.1±0.1	0.2±0.1	0.500
Albumin (g/dL)	4.6±0.3	4.8±0.3	p<0.01	4.5±0.2	4.8±0.4	p<0.01
T-Chol (mg/dl)	228.8±34.0	166.5±31.7	p<0.01	244.0±36.1	198.0±34.3	p<0.01
TG (mg/dl)	214.1±127.9	167.9±81.7	p<0.05	192.3±85.2	114.6±31.0	p<0.01
HDL-C (mg/dl)	44.8±9.7	49.8±9.8	p<0.01	54.5±15.2	62.8±11.3	0.087
LDL-C (mg/dl)	141.2±31.6	83.1±26.8	p<0.01	151.0±23.5	112.4±29.8	p<0.01
FPG (mg/dl)	104.4±11.1	100.6±15.0	p<0.05	114.8±19.2	112.0±19.5	0.483
HbA1c (%)	5.5±0.5	5.6±0.6	0.125	6.4±0.8	6.0±0.6	p<0.05
IRI (μU/mL)	12.7±5.5	14.8±7.4	0.117	13.4±9.0	8.9±4.4	0.325
HOMA-IR	3.0±1.3	3.7±2.0	0.088	3.6±3.0	2.5±1.5	0.572
NAS	3.9±1.0	2.9±0.9	p<0.01	4.0±1.2	3.1±0.9	p<0.05
Adiponectin (μg/mL)	4.8±1.5	6.0±2.5	p<0.01	6.8±3.0	7.6±3.3	0.092
TNF-α (pg/mL)	17.0±5.4	10.1±7.6	p<0.01	18.7±5.2	13.2±7.9	p<0.05
Type IV collagen (ng/mL)	4.0±0.8	3.8±0.7	0.134	4.5±1.3	3.8±1.1	p<0.05
Ferritin (ng/mL)	253.2±187.1	218.0±192.1	0.271	151.9±110.7	106.9±76.1	p<0.05
L/S density ratio	0.6±0.3	1.0±0.2	p<0.01	0.5±0.3	0.9±0.3	p<0.01
Number (DM/IGT/HT)	5/9/10	5/9/10	-	9/4/9	9/4/9	-
DM medication	2	2	-	2	2	-
HT medication	6	6	-	8	8	-

Data are shown as mean ± standard deviation, unless otherwise indicated.

AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyl

transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol, FPG; fasting plasma glucose, HbA1c; glycated hemoglobin, IRI; fasting plasma insulin, HOMA-IR; homeostasis model assessment of insulin resistance, NAS; nonalcoholic fatty liver disease activity score, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , L/S; liver to spleen, DM; diabetes mellitus, IGT; impaired glucose tolerance, HT; hypertension.

**Table 3. Univariate and stepwise multiple regression analyses for determinants of nonalcoholic fatty liver disease activity score**

Factors	Univariate*		Multivariate <sup>+</sup>	
	$\beta$	P	$\beta$	P
Body mass index (kg/m <sup>2</sup> )	0.140	0.268		
AST (IU/L)	0.359	P<0.01		
ALT (IU/L)	0.326	P<0.01		
$\gamma$ -GTP (IU/L)	0.297	P<0.05	0.255	P<0.05
Total-bilirubin (mg/dL)	-0.022	0.861		
Direct-bilirubin (mg/dL)	-0.053	0.676		
Albumin (g/dL)	-0.008	0.949		
T-Chol (mg/dl)	0.174	0.168		
TG (mg/dl)	0.050	0.694		
HDL-C (mg/dl)	0.051	0.688		
LDL-C (mg/dl)	0.153	0.226		
FPG (mg/dl)	0.062	0.625		
HbA1c (%)	0.049	0.703		
IRI ( $\mu$ U/mL)	0.138	0.305		
HOMA-IR	0.188	0.169		
Adiponectin ( $\mu$ g/mL)	-0.272	P<0.05		
TNF- $\alpha$ (pg/mL)	0.389	P<0.01	0.310	P<0.01
Type IV collagen (ng/mL)	0.238	0.059		
Ferritin (ng/mL)	0.132	0.305		
L/S density ratio	-0.549	P<0.01	-0.404	P<0.01
DM	-0.120	0.346		
HT	0.016	0.898		
DM medication	-0.123	0.333		
HT medication	-0.064	0.615		

\*Univariate coefficients.  $\beta$ : Regression coefficients.

<sup>+</sup>A stepwise multivariate regression analysis was performed.

R<sup>2</sup>=0.403

AST; aspartate aminotransferase, ALT; alanine aminotransferase,  $\gamma$ -GTP;  $\gamma$ -glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density

lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol, FPG; fasting plasma glucose, HbA1c; glycated hemoglobin, IRI; fasting plasma insulin, HOMA-IR; homeostasis model assessment of insulin resistance, NAS; nonalcoholic fatty liver disease activity score, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , L/S; liver to spleen, DM; diabetes mellitus, HT; hypertension.

**Table 4. Univariate and stepwise multiple regression analyses for determinants of liver to spleen density ratio**

Factors	Univariate <sup>*</sup>		Multivariate <sup>+</sup>	
	$\beta$	P	$\beta$	P
Body mass index (kg/m <sup>2</sup> )	-0.181	0.113		
AST (IU/L)	-0.438	P<0.01	-0.192	P<0.05
ALT (IU/L)	-0.371	P<0.01		
$\gamma$ -GTP (IU/L)	-0.185	0.105		
Total-bilirubin (mg/dL)	0.034	0.769		
Direct-bilirubin (mg/dL)	-0.139	0.225		
Albumin (g/dL)	0.171	0.135		
T-Chol (mg/dl)	-0.485	P<0.01		
TG (mg/dl)	-0.072	0.534		
HDL-C (mg/dl)	0.112	0.330		
LDL-C (mg/dl)	-0.549	P<0.01	-0.439	P<0.01
FPG (mg/dl)	0.064	0.578		
HbA1c (%)	0.138	0.229		
IRI ( $\mu$ U/mL)	0.029	0.820		
HOMA-IR	0.069	0.599		
NAS	-0.549	P<0.01	-0.422	P<0.01
Adiponectin ( $\mu$ g/mL)	0.137	0.245		
TNF- $\alpha$ (pg/mL)	-0.340	P<0.01		
Type IV collagen (ng/mL)	-0.101	0.381		
Ferritin (ng/mL)	0.013	0.909		
DM	0.025	0.825		
HT	0.244	P<0.05		
DM medication	0.108	0.346		
HT medication	0.119	0.298		

<sup>\*</sup>Univariate coefficients.  $\beta$ : Regression coefficients.

<sup>+</sup>A stepwise multivariate regression analysis was performed.

R<sup>2</sup>=0.515

AST; aspartate aminotransferase, ALT; alanine aminotransferase,  $\gamma$ -GTP;  $\gamma$ -glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density

lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol, FPG; fasting plasma glucose, HbA1c; glycated hemoglobin, IRI; fasting plasma insulin, HOMA-IR; homeostasis model assessment of insulin resistance, NAS; nonalcoholic fatty liver disease activity score, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , L/S; liver to spleen, DM; diabetes mellitus, HT; hypertension.



**Table 5. Univariate and stepwise multiple regression analyses for determinants of tumor necrosis factor- $\alpha$**

Factors	Univariate <sup>*</sup>		Multivariate <sup>+</sup>	
	$\beta$	P	$\beta$	P
Body mass index (kg/m <sup>2</sup> )	0.006	0.959		
AST (IU/L)	0.146	0.191		
ALT (IU/L)	0.068	0.543		
$\gamma$ -GTP (IU/L)	0.115	0.305		
Total-bilirubin (mg/dL)	0.148	0.185		
Direct-bilirubin (mg/dL)	-0.049	0.660		
Albumin (g/dL)	-0.186	0.095		
T-Chol (mg/dl)	0.252	p<0.05		
TG (mg/dl)	0.136	0.223		
HDL-C (mg/dl)	-0.165	0.139		
LDL-C (mg/dl)	0.269	p<0.05		
FPG (mg/dl)	0.171	0.124		
HbA1c (%)	0.214	0.054		
IRI ( $\mu$ U/mL)	0.034	0.785		
HOMA-IR	0.017	0.896		
NAS	0.389	p<0.01	0.351	p<0.01
Adiponectin ( $\mu$ g/mL)	-0.283	p<0.05	-0.306	p<0.05
Type IV collagen (ng/mL)	0.189	0.088		
Ferritin (ng/mL)	-0.050	0.662		
L/S density ratio	-0.340	p<0.01		
DM	0.170	0.126		
HT	-0.099	0.374		
DM medication	0.142	0.203		
HT medication	-0.155	0.166		

<sup>\*</sup>Univariate coefficients.  $\beta$ : Regression coefficients.

<sup>+</sup>A stepwise multivariate regression analysis was performed.

R<sup>2</sup>=0.254

AST; aspartate aminotransferase, ALT; alanine aminotransferase,  $\gamma$ -GTP;  $\gamma$ -glutamyl transpeptidase, T-Chol; total cholesterol, TG; triglycerides, HDL-C; high-density

lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol, FPG; fasting plasma glucose, HbA1c; glycated hemoglobin, IRI; fasting plasma insulin, HOMA-IR; homeostasis model assessment of insulin resistance, NAS; nonalcoholic fatty liver disease activity score, TNF- $\alpha$ ; tumor necrosis factor- $\alpha$ , L/S; liver to spleen, DM; diabetes mellitus, HT; hypertension.