

RESPONSE TO REVIEWER 02444949:

We wish to express our strong appreciation to the Reviewer for his or her insightful comments on our manuscript. The Reviewers comments have helped us to improve our manuscript.

Comment 1: Why did you design 150 mg for 2 patients and 300 mg for 4 patients?

Response: In Japan, vitamin E is covered by health insurance for treating atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation, but is not covered for NAFLD/NASH. Therefore we included the patients who had NAFLD/NASH and were treated with vitamin E for the diseases covered by insurance in this study. Dosage of vitamin E followed a health insurance, such as 150-300 mg for atherosclerosis or diabetic retinopathy, 300-600 mg for prevention of lipid peroxidation.

We have changed the following text (p. 8, lines 12-14):

from “Vitamin E was administered for >1 year to 38 patients with NAFLD caused by atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015.”

to “Vitamin E was administered for >1 year to 38 patients with NAFLD as treatments for atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015.”

Furthermore, we have changed the following text (p.9, lines 9-11):

from “However, the maximum dosage of vitamin E accepted by health insurance in Japan is 600 mg per day.”

to “However, the dosage of vitamin E accepted by health insurance in Japan is 150-300 mg for atherosclerosis or diabetic retinopathy, and 300-600 mg for prevention of lipid peroxidation.”

Comment 2: P 13. 4 line from the bottom: markedlyimporve? markedly improve.

Response: In accordance with the Reviewer's comment, we have changed the following text (p.14, line 21):

from “markedlyimprove” to “markedly improve”

Thank you again for your comments on our manuscript. We believe that the revised manuscript is suitable for publication.

RESPONSE TO REVIEWER 00503443:

We wish to express our strong appreciation to the Reviewer for his or her insightful comments on our manuscript. The Reviewers comments have helped us to improve our manuscript.

Comment 1: Abstract section page 3 line 10: there is no need to specify here the machine used and how the result is acquired. This is specified in Materials and Methods. So you may delete from “using a Siemens .. to .. meter/second (m/s)”.

Response: In accordance with the Reviewer's comment, we have deleted the following text (p.5, line 10):

“using a Siemens ACUSON S2000 (Siemens Medical Systems Co., Ltd., Tokyo, Japan). Ten successful acquisitions at different locations were performed on each patient, and the results are expressed as the median value in meters/second (m/s).”

Comment 2: page 3 line 21: “after baseline” can be deleted because is a repetition, page 4 line 4: “also showed significant improved”

Response: In accordance with the Reviewer's comment, we have deleted the following text: “after baseline” (p.5, line 18) and “also showed significant improved” (p.5, line 23). We have also deleted “after baseline” in other section.

Comment 3: please correct Materials and Methods section, page 6, first line: “Vitamin E was ..... with NAFLD because of atherosclerosis diabetic .....”. Nafld is not caused by atherosclerosis or diabetic retinopathy, but it is associated with these conditions. Please correct Vitamin E administration.

Response: In Japan, vitamin E is covered by health insurance for treating atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation, but is not covered for NAFLD/NASH. Therefore we included the patients who had NAFLD/NASH and were treated with vitamin E for the diseases covered by insurance in this study.

We have changed the following text (p. 8, lines 12-14):

from “Vitamin E was administered for >1 year to 38 patients with NAFLD caused by atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January

2011 to July 2015.”

to “Vitamin E was administered for >1 year to 38 patients with NAFLD as treatments for atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015.”

Comment 4: page 7: the dose of vitamin E is not clear. It seems that 150, 300 or 600 mg are given 3 times a day. Please specify that the total dose of 150, 300 or 600 mg is given into 3 administrations per day.

Response: In accordance with the Reviewer's comment, we have changed the following text (p. 9, lines 3-4):

from “The following doses of vitamin E were orally administered three times per day after each meal for >1 year”

to “The total doses of 150, 300 or 600 mg vitamin E were orally given into 3 administrations per day after each meal for >1 year”

Comment 5: Results section: Effect of Vitamin E on serum AST levels, ... on serum ALT levels and ... on serum Gamma-GTP levels can be putted together, because repetitive.

Response: In accordance with the Reviewer's comment, we have put those results together. We have changed the following text (p. 11, lines 20- p. 12, lines 6):

from “**Effect of vitamin E on serum AST levels**

Serum AST levels in all patients significantly decreased from baseline to 6 and 12 months after baseline ( $P < 0.001$  and  $P < 0.001$ , respectively). Serum AST levels in the CC/CG group significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.004$  and  $P < 0.001$ , respectively). Serum AST levels in the GG group also significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.045$  and  $P = 0.011$ , respectively; Fig. 2a).

**Effect of vitamin E on serum ALT levels**

Serum ALT levels in all patients significantly decreased from baseline to 6 and 12 months after baseline ( $P < 0.001$  and  $P < 0.001$ , respectively). Serum ALT levels in the CC/CG group significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.022$  and  $P < 0.001$ , respectively). Serum ALT levels in the GG group also significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.004$  and  $P < 0.001$ , respectively; Fig. 2b).

**Effect of vitamin E on serum  $\gamma$ -GTP levels**

Serum  $\gamma$ -GTP levels in all patients significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.019$  and  $P < 0.001$ , respectively). Serum  $\gamma$ -GTP levels in the CC/CG group significantly decreased from baseline to 6 and 12 months after baseline ( $P = 0.047$  and  $P = 0.003$ , respectively). Serum  $\gamma$ -GTP in the GG group significantly decreased from baseline to 12 months after baseline ( $P = 0.005$ ; Fig. 2c)."

to **"Effect of vitamin E on serum AST, ALT, and  $\gamma$ -GTP levels**

Serum AST, ALT and  $\gamma$ -GTP levels in all patients significantly decreased from baseline to 6 months ( $P < 0.001$ ,  $P < 0.001$ , and  $P = 0.019$ , respectively) and 12 months ( $P < 0.001$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively). Those in the CC/CG group also significantly decreased from baseline to 6 months ( $P = 0.004$ ,  $P = 0.022$ , and  $P = 0.047$ , respectively) and 12 months ( $P < 0.001$ ,  $P < 0.001$ , and  $P = 0.003$ , respectively). Serum AST and ALT levels in the GG group significantly decreased from baseline to 6 months ( $P = 0.045$ , and  $P = 0.004$ , respectively) and 12 months ( $P = 0.011$ , and  $P < 0.001$ , respectively), and serum  $\gamma$ -GTP levels in the GG group significantly decreased from baseline to 12 months ( $P = 0.005$ ) (Fig. 2a, 2b, and 2c)."

Comment 6: Effect of vitamin E on Vs, page 11: the group CG/GG does not exist; it is, perhaps, CC/CG? The same error is at page 21 in "Figure 4". Please verify.

Response: In accordance with the Reviewer's comment, we have changed from CG/GG to CC/CG (p. 13, lines 1 and p. 26, lines 17).

Comment 7: Discussion section: The discussion section is a little prolix; there is often a repetition of results and it is not sufficiently evidenced that the GG group include a number of patients with liver cirrhosis, which may justify why Fib 4 in this group does not reduce.

Response: We are uncertain why FIB-4 in GG group does not reduce. The small number of the patients may be the reason. A larger scale study is necessary to confirm the results of the present study.

Comment 8: Furthermore the statement that "ARFI is more sensitive the liver biopsies for detecting ..." is too hard, so it needs to be mitigate or avoided.

Response: In accordance with the Reviewer's comment, we have added the following text (p. 14, lines 24-p.15, lines 1): "There may be possibility that the reduction of Vs is

attributed to factors other than reduction of fibrosis.”

Thank you again for your comments on our manuscript. We believe that the revised manuscript is suitable for publication.

## RESPONSE TO REVIEWER 02541391:

We wish to express our strong appreciation to the Reviewer for his or her insightful comments on our manuscript. The Reviewers comments have helped us to improve our manuscript.

Comment 1: Minor language polishing is required.

Response: We have deleted repetition phrases, such as “after baseline” and “also showed significant improved”. In the results sections, we have briefly put same results together (p. 11 lines 20-p. 12, lines 6, “Effect of vitamin E on serum AST, ALT, and  $\gamma$ -GTP levels”).

Thank you again for your comments on our manuscript. We believe that the revised manuscript is suitable for publication.

RESPONSE TO REVIEWER 02534481:

We wish to express our strong appreciation to the Reviewer for his or her insightful comments on our manuscript. The Reviewers comments have helped us to improve our manuscript.

Comment 1: The used criteria to establish the diagnosis of NAFLD in these patients is not clear.

Response: We have changed the following text (p. 8, lines 17-20):

from “The diagnosis of NAFLD was confirmed by liver biopsy or ultrasonic examination and clinical history.”

to “The diagnosis of NAFLD was confirmed by liver biopsy in 10 patients, by ultrasonic examination in 23 patients, and by presence of cirrhosis with no obvious etiology and with metabolic risk factors such as obesity and metabolic syndrome in 5 patients<sup>[11]</sup>.”

Comment 2: It's not clear what this means "caused by atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation"

Response: In Japan, vitamin E is covered by health insurance for treating atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation, but is not covered for NAFLD/NASH. Therefore we included the patients who had NAFLD/NASH and were treated with vitamin E for the diseases covered by insurance in this study.

We have changed the following text (p. 8, lines 12-14):

from “Vitamin E was administered for >1 year to 38 patients with NAFLD caused by atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015.”

to “Vitamin E was administered for >1 year to 38 patients with NAFLD as treatments for atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation from January 2011 to July 2015.”

Comment 3: Should add the IRB number for approval

Response: In accordance with the Reviewer's comment, we have added the following text (p. 2, lines 16-19): “Institutional review board statement: This study was reviewed



and approved by the Ethics Committee of the Fujita Health University Hospital (IRB number: 14-020).”, and we attach a copy of ethics approval document in PDF format.

Comment 4: Not clear on what basis the Vit E dose was selected.

Response: Dosage of vitamin E followed a health insurance, such as 150-300 mg for atherosclerosis or diabetic retinopathy, 300-600 mg for prevention of lipid peroxidation.

We have changed the following text (p.9, lines 9-11):

from “However, the maximum dosage of vitamin E accepted by health insurance in Japan is 600 mg per day. ”

to “However, the dosage of vitamin E accepted by health insurance in Japan is 150-300 mg for atherosclerosis or diabetic retinopathy, and 300-600 mg for prevention of lipid peroxidation. ”

Comment 5: Why this sample size was chosen and on what based outcome.

Response: This is retrospective study, so there are limitations to collect an applicable patients. There were only thirty-eight NAFLD patients who had NAFLD/NASH and treated with vitamin E for atherosclerosis, diabetic retinopathy, or prevention of lipid peroxidation.

Comment 6: Would the authors think the negative results are due to small size of biased effect?

Response: As the reviewer indicated, the negative results are due to small size of biased effect. A larger scale study is necessary to confirm the results of the present study.

Comment 7: How can this add to the published trial?

Response: The PIVENS trial reported that serum AST, ALT, and  $\gamma$ -GTP levels in the vitamin E group decreased compared with the placebo group, and that vitamin E group had a reduction in steatosis, lobular inflammation, and activity score, whereas fibrosis scores did not markedly improve. The present study showed that a 1-year treatment of vitamin E improved not only laboratory values but also the noninvasive scores related to hepatic fibrosis and liver stiffness in NAFLD patients, and that the treatment

responses were similar between different *PNPLA3* genotypes. The results of our study contribute to non-invasive evaluation of the efficacy of vitamin E treatment for NAFLD/NASH.

We have described them in “Research frontiers” and “Innovations and breakthroughs” (p. 17, lines 17-p. 18, lines 8).

Thank you again for your comments on our manuscript. We believe that the revised manuscript is suitable for publication.