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***Randomized Controlled Trial***

**Efficacy of poly-unsaturated fatty acid therapy on patients with nonalcoholic steatohepatiti**s

Li YH *et al*. Efficacy of PUFA on NASH patients

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**Abstract**

**AIM:** To examine whether poly-unsaturated fatty acid (PUFA) therapy is beneficial for improving nonalcoholic steatohepatitis (NASH).

**METHODS:** Totally 78 patients pathologically diagnosed as NASH were enrolled and were randomLy assigned into the controlled group and PUFA therapy group (added 50 mL PUFA with 1:1 ratio of EHA and DHA into daily diet). At the initial and six months of PUFA therapy, parameters of interest including liver enzymes, lipid profiles, markers of inflammation and oxidation, and histological changes were evaluated and compared between these two groups.

**RESULTS:** At the initial, in patients with NASH, serum levels of alanine aminotransferase (ALT) and aspartase aminotransferase (AST) were slight elevation. Triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol levels, markers of systemic inflammation [C-reactive protein (CRP)] and oxidation [malondialdehyde (MDA)], as well as fibrosis parameters of type IV collagen and pro-collagen type III pro-peptide were also increased beyond normal range. Six months later, as compared to the controlled group, ALT and AST levels were significantly reduced in the PUFA group. In addition, serum levels of TG and TC, CRP and MDA, and type IV collagen and pro-collagen type III pro-peptide were also simultaneously and significantly reduced. Of note, histological evaluation showed that steatosis grade, necro-inflammatory grade, fibrosis stage, and ballooning score were all profoundly improved in comparison to the controlled group, strongly suggesting that increased PUFA consumption was potential to offset NASH progression.

**CONCLUSION:** Increased PUFA consumption is a potential promising approach for NASH prevention and reversion.

**Key words:** Poly-unsaturated fatty acid; Nonalcoholic steatohepatitis; Management

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**Core tip:** Epidemiologically, it has been reported that the prevalence of non-alcoholic fatty liver disease is increasing significantly and its associated morbidities including non-alcoholic steatohepatitis (NASH) and hepatic failure also impose great healthy and economic burdens on individuals and the whole society. Preliminary data from our study showed that 6 mo’s poly-unsaturated fatty acid (PUFA) therapy was possible to improve NASH as reflected by laboratory examination and histological evaluation. Future study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic failure.

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**INTRODUCTION**

Dyslipidemia, featured by increased plasma levels of triglyceride (TG) and total cholesterol (TC), is associated with a broad range of diseases such as atherosclerosis, metabolic syndrome, non-alcoholic fatty liver diseases (NAFLD), *etc*[1-3]. Epidemiologically, it has been reported that the prevalence of NAFLD is increasing in the past decades, and non-alcoholic steatohepatitis (NASH) and hepatic failure induced by NAFLD impose great burdens on the patients and the whole society[1,4,5]. Previously, some studies using lipid-modified medications to evaluate whether NAFLD or NASH could be ameliorated, and the outcomes were controversial[6,7]. For example, Laurin *et al*[6] showed that clofibrate treatment was not beneficial for the improvement of liver function and histological changes in patients with hypertriglycemia and NASH. Nevertheless, data from Basaranoglu *et al*[7] suggested that gemfibrozil therapy could profoundly ameliorate liver dysfunction in patients with NASH. Similarly, the outcomes with 3-hydroxy-3-metylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin) therapy in patients with NASH also revealed conflicting[8-10].

Poly-unsaturated fatty acid (PUFA), predominantly comprising eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA), has been broadly used in daily life. Several sources of evidence indicate that PUFA is capable of improving lipid disorder as well as of ameliorating systemic inflammation and oxidation[11-13]. As it is well known that the pathophysiological characteristics of NAFLD and NASH are featured by lipid-overloaded and lobular inflammation within liver tissues[14,15]. Therefore, we hypothesized that PUFA therapy might be useful and beneficial for improving NASH. In order to investigate our hypothesis, we conducted a randomized, prospective, controlled but not blinded trial. We believed that data from our trial could shed promising lights for the future studies in investigating the optimal therapy for NAFLD and NASH.

**MATERIALS AND METHODS**

***Participants and strategies of research***

All participants pathologically diagnosed as NASH according to the criteria were enrolled and written inform consents were obtained prior to randomization[16]. Current study was conducted in conformity to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics and research committees of our hospital. All participants were definitely ruled out of secondary causes of NASH such as alcohol-induced (alcohol consumption ≥ 20 g/wk), medications-caused (such as tamoxifen and amiodarone), viral hepatitis and autoimmune diseases (primary biliary cirrhosis). Totally 78 participants were enrolled and randomLy assigned into controlled group (prescribed normal saline) and PUFA therapy group (added 50 mL PUFA with 1:1 ratio of EHA and DHA into daily diet) for 6 mo. Additionally, both groups were recommended taking modest physical exercise of 30 min at least 5 d per week. Low-fat and low-cholesterol, and low carbohydrate diet were also recommended.

***Comparison of parameters of interest***

All working staffs involving in parameters evaluations were blinded to the information of both groups. Fasting venous blood sample was drawn and parameters of interest, including lipid profiles [TG; TC; low-density lipoprotein-cholesterol (LDL-C); and high-density lipoprotein-cholesterol (HDL-C)], serum levels of liver enzymes [alanine aminotransferase (ALT) and aspartase aminotransferase (AST)], total and direct billirubin, fasting blood glucose (FBG) and C-reactive protein (CRP) were assessed by using the standard techniques of clinical chemistry laboratories. Serum levels of malondialdehyde (MDA), type IV collagen and pro-collagen type III pro-peptide (P-III-P) were detected in accordance to previous reports[17,18]. Body mass index (BMI), smoking status, family history of NASH and other demographic data were simultaneously collected by questionnaire. All above parameters were evaluated at the initial and at 6 months of PUFA therapy.

***Pathological evaluation***

At the initial and at 6 months of PUFA therapy, liver biopsy was performed to evaluate the changes of hepatic tissues. Notably, steatosis grade, necro-inflammatory grade, fibrosis stage and ballooning score of these two groups were evaluated by two expertise of pathology.

***Statistical analysis***

Continuous variable was presented as mean ± standard deviation and compared by the Student’s t-test when data was normally distributed, otherwise compared by Wilcoxon rank-sum test. Categorical data was presented as percentage and compared by χ2 test. Statistical analyses were performed by using SPSS software version 18.0 (SPSS, Inc., Chicago, Illinois). A value of *P* < 0.05 was considered significant.

**RESULTS**

***Baseline characteristics evaluation of all participants***

As shown in Table 1, all parameters were comparable between the controlled and the PUFA therapy groups at the initial. Of note, male was predilection with NASH in present research, and most of participants were overweight or obese. Serum levels of ALT and AST were slight elevation in both groups. Lipid profiles revealed significant increased serum levels of TG, TC and LDL-C. Markers of inflammation (CRP) and oxidation (MDA) were also elevated in patients with NASH. Fibrotic parameters such as type IV collagen and pro-collagen type III pro-peptide were also higher than the normal range in patients with NASH.

***Comparison of parameters at 6 mo of PUFA therapy***

As presented in Table 2, after 6 mo’s therapy, as compared to the controlled group, liver function was significantly improved in the PUFA group, as suggesting by the significant reduction of ALT and AST levels. In addition, serum levels of TG and TC, CRP, MDA, type IV collagen and pro-collagen type III pro-peptide were also significantly reduced in the PUFA group as compared to the controlled group. Of note, both the BMI and the percentage of smoking were reduced, while the exercise time per week was increased in both groups when compared to the initial, and there was no significant difference of these improvements between the controlled and the PUFA therapy groups.

***Histological evaluation at the initial and at 6 mo of PUFA therapy***

Briefly, as shown in Figure 1, at the initial, the parameters indicating the histological characteristics of NASH were comparable between the controlled and the PUFA therapy groups as shown in Table 3. Nevertheless, with 6 mo’s PUFA therapy, all parameters revealing the severity of NASH were significantly improved when compared to the controlled group.

**DISCUSSION**

The prevalence of NAFLD and NASH is gradually increasing and its associated morbidity and mortality impose great burden on the whole society[5,19]. Currently, there are no specific and highly-effective therapy in treating NAFLD and NASH. Additionally, previous epidemiological studies revealed the controversial outcomes with lipid-lowering therapy on patients with NASH[6-10]. Importantly, data from our current study shows that as compared to the controlled group, 6 months of PUFA therapy significantly decrease serum levels of liver enzymes. In addition, other crucial parameters including CRP, MDA, type IV collagen and pro-collagen type III pro-peptide are also profoundly reduced. Histological assessment at 6 mo further corroborates the potential benefits of PUFA therapy on NASH.

In the past decades, many risk factors associated with NAFLD and NASH development has been identified[15,20,21]. Notably, dyslipidemia, resulting from over-consumption of cholesterol and triglyceride, is one the most critical elements for NASH development[20-22]. Knowingly[23,24], lipid accumulating in liver tissues is the first hit of NAFLD and HASH development. Subsequently, second hit in terms of hepatocyte injury, inflammation, oxidation and fibrosis ensues and accelerates NASH progression. Therefore, in light of the underlying mechanisms, many medications especially lipid-lowering agents have been applied for the treatment of NAFLD and NASH. In addition, other medications such as insulin-sensitizing drugs (metformin and thiazolidinedione)[25,26] and antioxidants (Vitamin C and E) have also been tested in clinical studies[27], and disappointedly the outcomes were quite inconsistent.

Basically, PUFA is an essential nutrition for keeping organs function properly, and previous studies also reveal that increased PUFA consumption is not only beneficial for lipid-modification, but is also effective in glycemic-control, hypertension-management, endothelium-protection and inflammation-amelioration[28]. Taken together, we considered that it was reasonable to postulate that increased PUFA consumption might also be effective for NAFLD and NASH management. In our current research, we showed that with 6 mo of PUFA therapy, as compared to the controlled group, both the laboratory parameters of liver function and histological changes of hepatic tissues were profoundly improved, strongly suggesting that PUFA might be a potential promising candidate for NAFLD and NASH management. In light of previous reports regarding the underlying mechanisms associated with PUFA benefits, we speculated that the following mechanisms might be responsible for our favorable findings. In the first place, as aforementioned that dyslipidemia plays a continuous role on NAFLD and NASH development. While PUFA is capable to ameliorate lipid disorder[28], and data from present study also revealed that with 6 months’ PUFA therapy, serum levels of TG and TC were significantly reduced as compared to the controlled group. Importantly, after 6 mo of PUFA therapy, the steatosis grade was reduced, which also directly suggested that lipid accumulation in hepatic tissues could be improved with PUFA therapy. Secondly, the second hit in the process of NASH development is characterized by hepatic inflammation and oxidation. Therefore, amelioration of inflammation and oxidation might be possible to prevent or retard NASH progression. In our present study, we showed that after 6 mo’s PUFA therapy, serum levels of CRP and MDA were profoundly declined as compared to the controlled group. Histological parameter such as necro-inflammatory grade was profoundly improved which also supported the notion that PUFA therapy was beneficial for ameliorating hepatic inflammation in patients with NASH[29]. Last but not the least, through inhibiting lipid accumulation and ameliorating inflammation, PUFA offset hepatic fibrosis as suggested by the decline of serum levels of type IV collagen and pro-collagen type III pro-peptide. Histological evaluation further substantiated that PUFA therapy was beneficial for improving hepatic fibrosis. Nevertheless, the mechanism associated with PUFA therapy on preventing and retarding hepatic fibrosis needs future investigation.

In conclusion, preliminary data from our study shows that 6 mo of PUFA therapy is beneficial for improving NASH as reflected by the laboratory examination and histological evaluation. Further study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic failure.

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**COMMENTS**

***Background***

Poly-unsaturated fatty acid (PUFA) has been used for the therapy of dyslipidemia, which is a key risk factor for nonalcoholic steatohepatitis (NASH). Whether PUFA is beneficial for improving NASH is unknown.

***Research frontiers***

Increased PUFA consumption may be beneficial for NASH improvement.

***Innovations and breakthroughs***

Preliminary data from our study showed that 6 mo’s PUFA therapy was possible to improve NASH as reflected by laboratory examination and histological evaluation. Further study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic failure.

***Applications***

Current research may provide preliminary data for future studies in investigating whether long-term PUFA therapy could further improve and reverse NASH.

***Terminology***

Dyslipidemia, featured by increased plasma levels of triglyceride and total cholesterol, is associated with non-alcoholic fatty liver diseases (NAFLD) and NASH. Several sources of evidence have indicated that PUFA is capable of improving lipid disorder as well as ameliorating systemic inflammation and oxidation. As it is well known that the pathophysiological characteristics of NAFLD and NASH are featured by lipid-overloaded and lobular inflammation within liver tissues. Therefore, it is reasonable to postulate that PUFA therapy may be useful and beneficial to improve NASH.

***Peer-review***

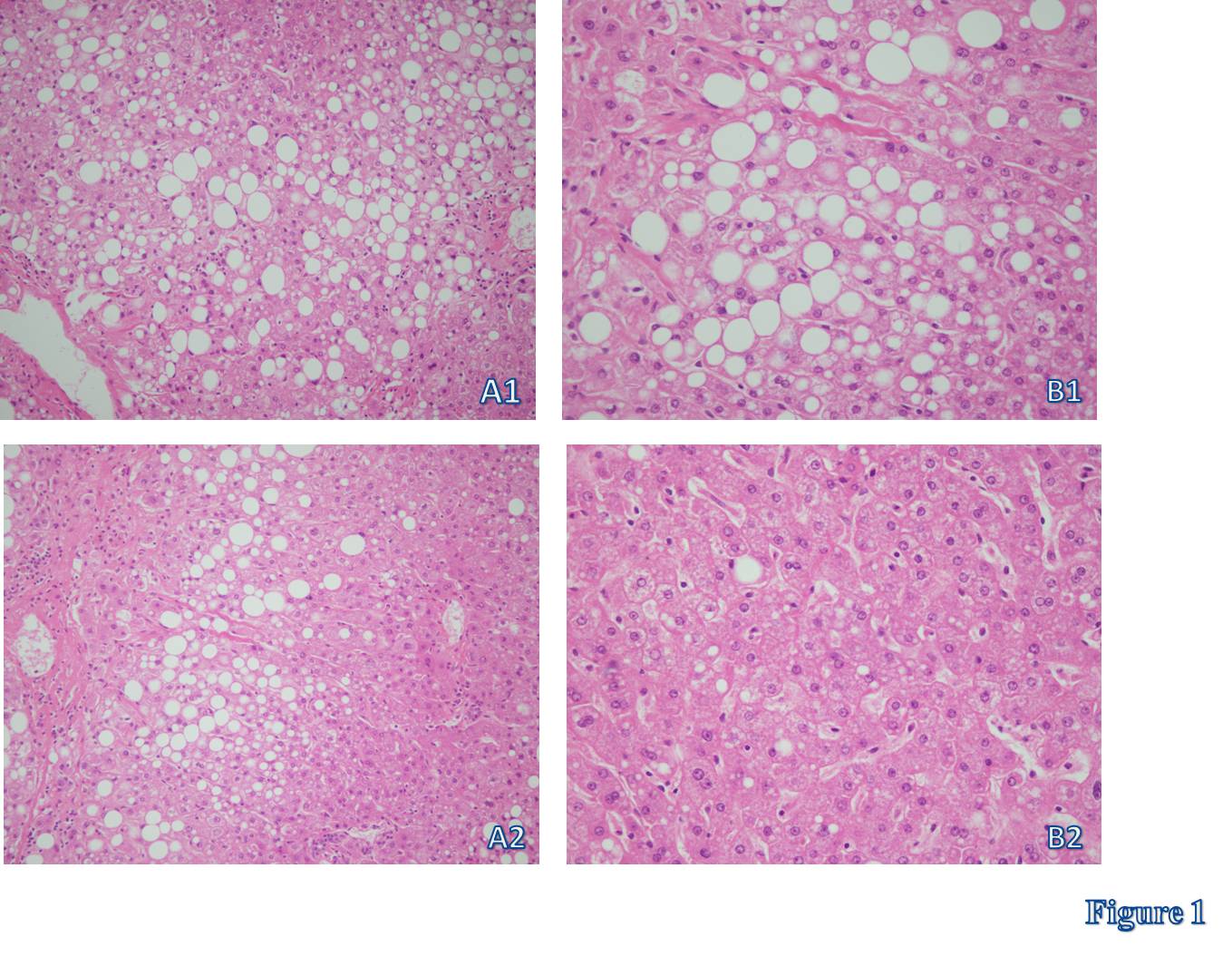
The study objective is strongly justified as it is assumed that dyslipidemia resulting from over-consumption of cholesterol and triglycerides is a major risk factor for this pathology. In the future, this may have major clinical implications as potential validation of antifibrotic therapies in patients with this pathology.

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**Figure 1 Changes of pathological features before and after therapy.** Panel A1 and B1 indicate the pathological features at baseline in the controlled and PUFA groups, and Panel A2 and B2 indicate the pathological features at 6 mo’s therapy in the controlled and PUFA groups. PUFA: Poly-unsaturated fatty acid.

**Table 1 Baseline characteristics evaluation of all participants**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Total | Controlled | PUFA | *P* value |
| *n* | 78 | 39 | 39 |  |
| Age (yr) | 51.9 ± 7.8 | 50.4 ± 7.2 | 52.6 ± 6.6 | 0.306 |
| Male, *n* (%) | 70 (89.7) | 36 (92.3) | 34 (87.2) | 0.178 |
| BMI (kg/m2) | 27.9 ± 1.6 | 27.2 ± 1.3 | 28.0 ± 1.4 | 0.225 |
| Smoking, *n* (%) | 45 (57.7) | 22 (56.4) | 23 (59.0) | 0.154 |
| Family history, *n* (%) | 3(3.8) | 1(2.6) | 2(5.1) | 0.237 |
| ALT (U/L) | 89.9 ± 10.4 | 91.3 ± 10.2 | 89.2 ± 12.4 | 0.313 |
| AST (U/L) | 82.4 ± 11.6 | 83.5 ± 8.9 | 82.0 ± 9.6 | 0.196 |
| Billirubin, total (md/dL) | 1.2 ± 0.3 | 1.2 ± 0.2 | 1.2 ± 0.4 | 0.354 |
| Billirubin, direct (md/dL) | 0.8 ± 0.2 | 0.7 ± 0.3 | 0.8 ± 0.4 | 0.366 |
| FBG (mmol/L) | 6.1 ± 0.3 | 6.1 ± 0.2 | 6.1 ± 0.5 | 0.117 |
| TG (mmol/L) | 2.6 ± 0.4 | 2.6 ± 0.3 | 2.5 ± 0.3 | 0.252 |
| TC (mmol/L) | 5.6 ± 0.7 | 5.5 ± 0.3 | 5.6 ± 0.4 | 0.408 |
| LDL-C (mmol/L) | 3.9 ± 0.7 | 3.9 ± 0.4 | 3.9 ± 0.3 | 0.387 |
| HDL-C (mmol/L) | 1.1 ± 0.2 | 1.1 ± 0.1 | 1.1 ± 0.2 | 0.163 |
| CRP (mg/L) | 10.4 ± 1.3 | 10.6 ± 1.1 | 10.0 ± 1.5 | 0.204 |
| MDA (nmol/mL) | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.6 ± 0.2 | 0.339 |
| Type IV collagen (ng/mL) | 4.9 ± 0.5 | 4.8 ± 0.6 | 4.9 ± 0.2 | 0.286 |
| P-III-P (U/mL) | 0.9 ± 0.3 | 0.9 ± 0.1 | 0.9 ± 0.4 | 0.350 |
| Weekly exercise time (min) | 60.3 ± 7.3 | 61.2 ± 5.6 | 60.1 ± 4.6 | 0.313 |

ALT: Alanine aminotransferase; AST: Aspartase aminotransferase; BMI: Body mass index; PUFA: Poly-unsaturated fatty acid; MDA: Malondialdehyde; LDL-C: Lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; CRP: C-reactive protein; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol.

**Table 2 Comparison of parameters 6 mo later**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Controlled | PUFA | *P* value |
| *n* | 39 | 39 |  |
| BMI (kg/m2) | 26.4 ± 1.0 | 25.8 ± 1.2 | 0.065 |
| Smoking, *n* (%) | 15(38.5) | 14(35.9) | 0.278 |
| ALT (U/L) | 80.4 ± 7.6 | 67.8 ± 5.3 | < 0.01 |
| AST (U/L) | 75.6 ± 5.8 | 60.3 ± 6.8 | < 0.01 |
| Billirubin, total (md/dL) | 0.8 ± 0.2 | 0.7 ± 0.2 | 0.343 |
| Billirubin, direct (md/dL) | 0.6 ± 0.1 | 0.6 ± 0.2 | 0.338 |
| FBG (mmol/L) | 5.9 ± 0.2 | 5.9 ± 0.3 | 0.285 |
| TG (mmol/L) | 2.4 ± 0.4 | 1.8 ± 0.3 | 0.015 |
| TC (mmol/L) | 5.2 ± 0.6 | 4.7 ± 0.3 | 0.040 |
| LDL-C (mmol/L) | 3.5 ± 0.5 | 3.1 ± 0.6 | 0.062 |
| HDL-C (mmol/L) | 1.1 ± 0.3 | 1.3 ± 0.4 | 0.105 |
| CRP (mg/L) | 9.2 ± 0.8 | 7.6 ± 0.4 | 0.045 |
| MDA (nmol/mL) | 0.6 ± 0.1 | 0.4 ± 0.2 | 0.048 |
| Type IV collagen (ng/mL) | 4.6 ± 0.7 | 3.5 ± 0.5 | 0.023 |
| P-III-P (U/mL) | 0.8 ± 0.2 | 0.5 ± 0.3 | 0.039 |
| Weekly exercise time (min) | 107.6 ± 12.3 | 109.5 ± 10.4 | 0.272 |

ALT: Alanine aminotransferase; AST: Aspartase aminotransferase; BMI: Body mass index; PUFA: Poly-unsaturated fatty acid; MDA: Malondialdehyde; LDL-C: Lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; CRP: C-reactive protein; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol.

**Table 3 Histological evaluation at initial and 6 mo later**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Controlled | PUFA | *P* value |
| At the initial | |  |  |
| Steatosis grade | 1.8 ± 0.2 | 1.9 ± 0.2 | 0.355 |
| Necro-inflammatory grade | 1.4 ± 0.2 | 1.4 ± 0.1 | 0.268 |
| Fibrosis stage | 1.7 ± 0.2 | 1.7 ± 0.1 | 0.309 |
| Ballooning score | 1.6 ± 0.3 | 1.6 ± 0.2 | 0.227 |
| Six months later | |  |  |
| Steatosis grade | 1.8 ± 0.1 | 1.4 ± 0.2 | 0.032 |
| Necro-inflammatory grade | 1.5 ± 0.1 | 1.1 ± 0.1 | 0.017 |
| Fibrosis stage | 1.6 ± 0.3 | 1.1 ± 0.2 | 0.02 |
| Ballooning score | 1.6 ± 0.2 | 1.0 ± 0.2 | 0.015 |

PUFA: Poly-unsaturated fatty acid.