Acta Biochim Biophys Sin, 2016, 48(5), 482–484
doi: 10.1093/abbs/gmw016
Advance Access Publication Date: 29 March 2016
New Phenomenon

New Phenomenon

Docosahexaenoic acid supplementation failed to attenuate chronic alcoholic fatty liver in mice

Yi Wang†, Yuying Zhao†, Mingzhuo Li, Yunxuan Wang, Shuqing Yu, and Tao Zeng*

Institute of Toxicology, School of Public Health, Shandong University, Jinan 250012, China

†These authors contributed equally to the work.
*Correspondence address. Tel: +86-531-88382132; Fax: +86-531-88382553; E-mail: zengtao@sdu.edu.cn

The roles of *n*-3 polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid (DHA) in alcoholic fatty liver (AFL) have been investigated in a series of studies. DHA and DHA-rich fish oil were found to provide protection against acute AFL [1,2], while their effects on chronic AFL is still a controversial issue. Although Song *et al*. [3] found that DHA and arachidonic acid prevented chronic AFL, several

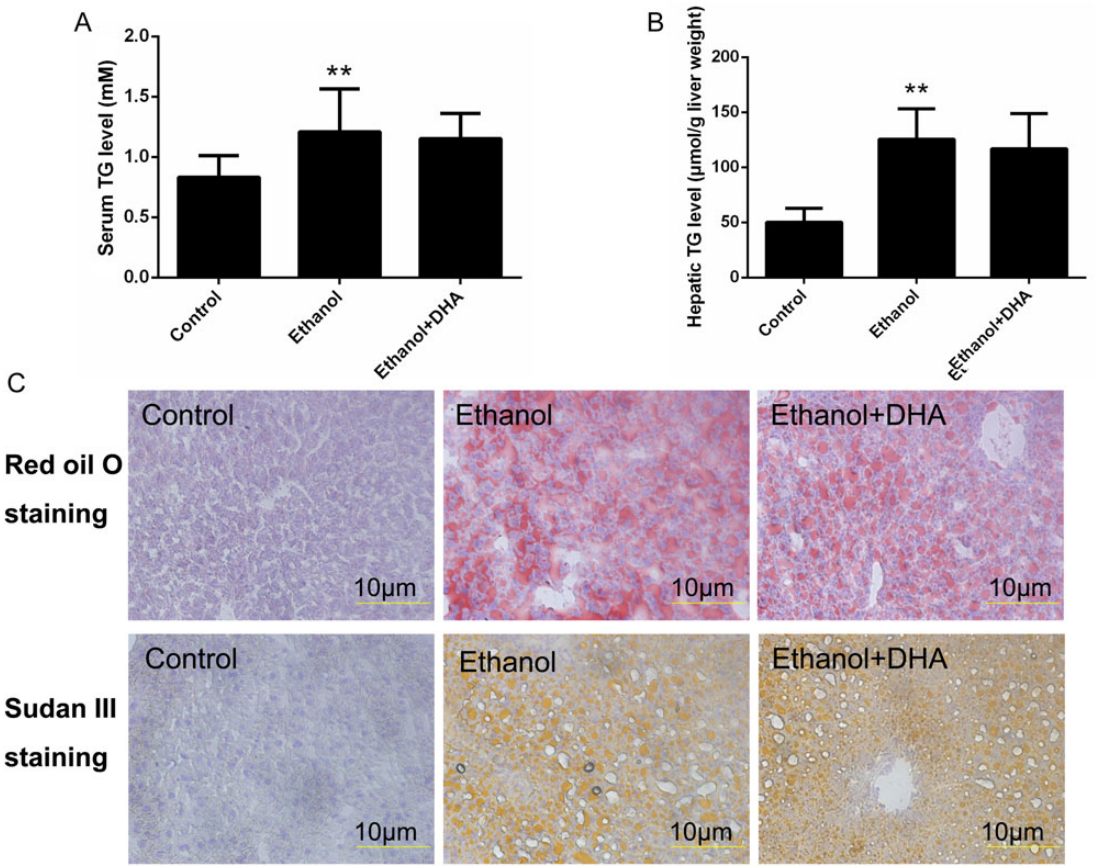

Figure 1. DHA supplementation failed to prevent chronic ethanol-induced increase of serum and hepatic TG levels, and the fat accumulation in the liver (A) Serum TG level. (B) Hepatic TG level. (C) Histopathological examination by oil red O staining and Sudan III staining. Data were presented as mean ± SD. $^{**}P < 0.01$, compared with the control group mice.

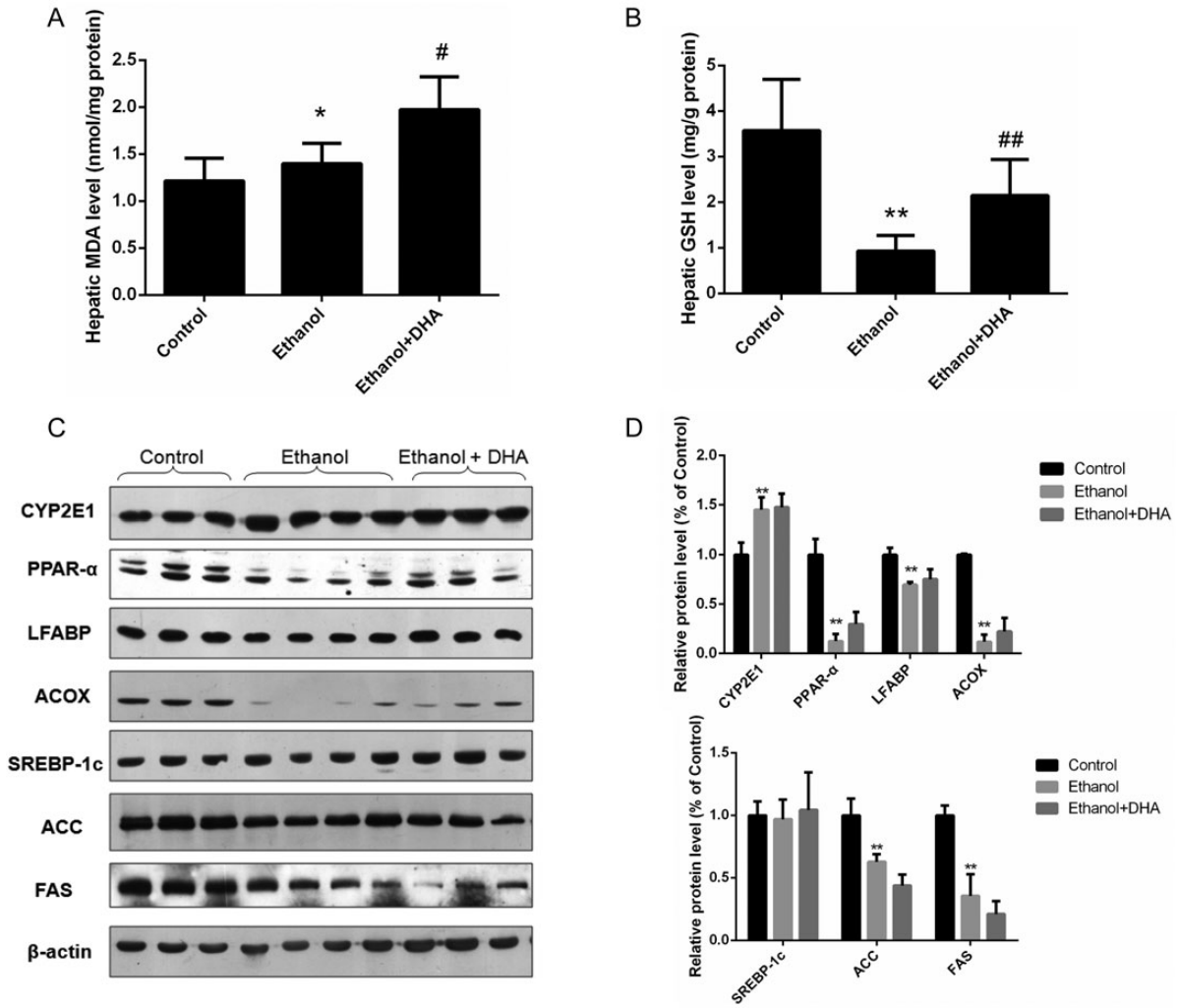

Figure 2. Effects of DHA supplementation on the hepatic MDA level, GSH level, and protein levels of CYP2E1, PPAR-α, LFABP, ACOX, SREBP-1c, ACC, and FAS (A,B) Hepatic MDA and GSH levels were detected using commercial kits. (C) Representative western blot bands for CYP2E1, PPAR-α, LFABP, ACOX, SREBP-1c, ACC, and FAS. (D) Quantitative data analyses of protein levels of CYP2E1, PPAR-α, LFABP, ACOX, SREBP-1c, ACC, and FAS. Data were presented as mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$, compared with the control group mice; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the ethanol group mice.

other studies revealed that fish oil promoted the pathogenesis of chronic AFL, while saturated fatty acids were protective against chronic AFL [4–6]. It was speculated that the doses and quality of *n*-3 PUFAs might be the possible reasons for these conflicting results [1,3]. Anyway, the roles of DHA in chronic AFL remain to be investigated.

Therefore, we investigated the effect of a modest level (250 mg/kg body weight) of purified DHA (purity ≥98%; Sigma, St Louis, USA) on Lieber-DeCarli liquid diet-induced chronic AFL in male C57BL/6 mice. At this dose, DHA exhibited antioxidative and anti-inflammatory effects and ameliorated acute AFL in mice [1,7]. In this study, DHA was stored at −80°C and prepared freshly before use to avoid oxidation.

It was found that DHA supplementation did not suppress the increase of serum and hepatic triglyceride (TG) levels (**Fig. 1A, B**). Histopathological examination showed that the numbers of lipid droplets in ethanol/DHA group mice were not reduced when compared with the ethanol group mice (**Fig. 1C**). These results suggested that purified DHA (250 mg/kg body weight) could not prevent chronic ethanol-induced fatty liver.

The development of AFL has been demonstrated to be associated with cytochrome P4502E1 (CYP2E1)-induced oxidative stress, the suppression of peroxisome proliferator-activated receptor α (PPAR-α)-regulated fatty acid oxidation, and the enhanced lipogenesis controlled by sterol regulatory element-binding protein-1c (SREBP-1c) [8]. Thus, we further detected the level of malondialdehyde (MDA), and the protein levels of CYP2E1, PPAR-α, and SREBP-1c. It was found that DHA did not inhibit chronic ethanol-induced increase of hepatic CYP2E1 protein level and MDA level, although it significantly suppressed ethanol-induced decrease of hepatic glutathione (GSH) level. Additionally, DHA supplementation had no significant effect on the protein levels of PPAR-α, liver fatty acid-binding protein (LFABP), acetyl acyl-CoA oxidase (ACOX), mature form of SREBP-1c, fatty acid synthase (FAS), and acyl-CoA carboxylase (ACC) (**Fig. 2**). These results suggested that DHA supplementation could not block chronic ethanol-induced suppression of PPAR-α-mediated fatty acid oxidation, and had no effect on chronic ethanol-induced suppression of SREBP-1c pathway.

Our results were inconsistent with previous reports in acute AFL models [1,2]. This means that the results from acute AFL models may not be directly extended to the chronic AFL model, as acute and chronic AFL may have different mechanisms. In a chronic AFL

model, Song *et al*. [3] found that DHA/α-linolenic acid could prevent chronic AFL; however, it is still difficult to determine whether the protective effects can be attributed to DHA because two *n*-3 PUFAs were used in that study. It has been demonstrated that the oxidized *n*-3 PUFAs and higher levels of DHA could trigger oxidative stress and inflammation [9,10]. Thus, some researchers speculated that the conflicting results of DHA on chronic AFL might be related with the quality and doses of DHA. In our study, we considered the quality and the dose of DHA. Although DHA at this dose was found to ameliorate acute AFL [1], results of the current study showed that it failed to prevent chronic AFL in mice. To understand the exact roles of PUFAs in AFL, more PUFAs need to be investigated in both acute and chronic AFL models in the future.

Funding

This work was supported by the grants from the National Undergraduate Training Programs for Innovation and Entrepreneurship (No. 201410422025) and the Young Scholars Program of Shandong University (No. 2015WLJH52).

References

1. Huang LL, Wan JB, Wang B, He CW, Ma H, Li TW, Kang JX. Suppression of acute ethanol-induced hepatic steatosis by docosahexaenoic acid is associated with downregulation of stearoyl-CoA desaturase 1 and inflammatory cytokines. *Prostaglandins Leukot Essent Fatty Acids* 2013, 88: 347–353.
2. Wada S, Yamazaki T, Kawano Y, Miura S, Ezaki O. Fish oil fed prior to ethanol administration prevents acute ethanol-induced fatty liver in mice. *J Hepatol* 2008, 49: 441–450.
3. Song BJ, Moon KH, Olsson NU, Salem N Jr. Prevention of alcoholic fatty liver and mitochondrial dysfunction in the rat by long-chain polyunsaturated fatty acids. *J Hepatol* 2008, 49: 262–273.
4. You M, Considine RV, Leone TC, Kelly DP, Crabb DW. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. *Hepatology* 2005, 42: 568–577.
5. Ronis MJ, Korourian S, Zipperman M, Hakkak R, Badger TM. Dietary saturated fat reduces alcoholic hepatotoxicity in rats by altering fatty acid metabolism and membrane composition. *J Nutr* 2004, 134: 904–912.
6. Nanji AA, Zhao S, Sadrzadeh SM, Dannenberg AJ, Tahan SR, Waxman DJ. Markedly enhanced cytochrome P450 2E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-ethanol-fed rats. *Alcohol Clin Exp Res* 1994, 18: 1280–1285.
7. El-Mesery M, Al-Gayyar M, Salem H, Darweish M, El-Mowafy A. Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides. *Cell Div* 2009, 4: 6.
8. Zeng T, Xie KQ. Ethanol and liver: recent advances in the mechanisms of ethanol-induced hepatosteatosis. *Arch Toxicol* 2009, 83: 1075–1081.
9. Awada M, Soulage CO, Meynier A, Debard C, Plaisancie P, Benoit B, Picard G, *et al*. Dietary oxidized n-3 PUFA induce oxidative stress and inflammation: role of intestinal absorption of 4-HHE and reactivity in intestinal cells. *J Lipid Res* 2012, 53: 2069–2080.
10. Shoji H, Franke C, Demmelmair H, Koletzko B. Effect of docosahexaenoic acid on oxidative stress in placental trophoblast cells. *Early Hum Dev* 2009, 85: 433–437.