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Eicosapentaenoic acid improves hepatic steatosis independent of PPARα activation through inhibition of SREBP-1 maturation in mice

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ABSTRACT

Eicosapentaenoic acid (EPA) in fish oil is known to improve hepatic steatosis. However, it remains unclear whether such action of EPA is actually caused by peroxisome proliferator-activated receptor α (PPARα) activation. To explore the contribution of PPARα to the effects of EPA itself, male wild-type and *Ppara*-null mice were fed a saturated fat diet for 16 weeks, and highly (>98%)-purified EPA was administered in the last 12 weeks. Furthermore, the changes caused by EPA treatment were compared to those elicited by fenofibrate (FF), a typical PPARα activator. A saturated fat diet caused macrovesicular steatosis in both genotypes. However, EPA ameliorated steatosis only in wild-type mice without PPARα activation, which was evidently different from numerous previous observations. Instead, EPA inhibited maturation of sterol-responsive element-binding protein (SREBP)-1 in the presence of PPARα through down-regulation of SREBP cleavage-activating protein and site-1 protease. Additionally, EPA suppressed fatty acid uptake and promoted hydrolysis of intrahepatic triglycerides in a PPARα-independent manner. These effects were distinct from those of fenofibrate. Although fenofibrate induced NAPDH oxidase and acyl-coenzyme A oxidase and significantly increased hepatic lipid peroxides, EPA caused PPARα-dependent induction of superoxide dismutases, probably contributing to a decrease in the lipid peroxides. These results firstly demonstrate detailed mechanisms of steatosis-ameliorating effects of EPA without PPARα activation and ensuing augmentation of hepatic oxidative stress.

1. Introduction

Recent lifestyle alterations, such as increased consumption of saturated fats and decreased physical activity, have raised the prevalence of obesity, metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD) [1,2]. Nonalcoholic steatohepatitis (NASH) is the progressive type of NAFLD and may develop into cirrhosis, liver cancer, and ultimately death [1–4]. Since NAFLD is also associated with a high susceptibility to atherosclerosis and ischemic heart disease [3,5], the increased prevalence of NAFLD is becoming a pressing issue worldwide. Thus, establishment of strategies to treat and prevent NAFLD and related metabolic disturbances is required.

Eicosapentaenoic acid (EPA) is one of the major components of n-3 polyunsaturated fatty acids (PUFA) preferentially contained in fish oil. From the first report of high EPA levels in the diet and blood of the Greenland Inuit [6], who rarely exhibit atherosclerotic diseases, numerous epidemiological and clinical studies have been

Abbreviations: ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; apo, apolipoprotein; AOX, acyl-CoA oxidase; AST, aspartate aminotransferase; CoA, coenzyme A; CPT-I, carnitine palmitoyl-CoA transferase-I; EPA, eicosapentaenoic acid; FA, fatty acid; FAS, fatty acid synthase; FAT, fatty acid translocase; FATP, fatty acid transport protein; FF, fenofibrate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; GPx, glutathione peroxidase; 4-HNE, 4-hydroxynonenal; HTGL, hepatic triglyceride lipase; Insig, insulin-induced gene product; LACS, long-chain acyl-CoA synthase; L-FABP, liver fatty acid-binding protein; LXR, liver X receptor; MCAD, medium-chain acyl-CoA dehydrogenase; MDA, malondialdehyde; mRNA, messenger RNA; MTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEFA, non-esterified fatty acid; NL, neutral lipase; Nrf2, nuclear factor-E2-related factor 2; PGC, PPARγ coactivator; PMP, peroxisomal membrane protein; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SCAP, SREBP cleavage-activating protein; S1P, site-1 protease; SD, standard deviation; SOD, superoxide dismutase; SREBP, sterol regulatory element-binding protein; TG, triglyceride; TNF, tumor necrosis factor.

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Table 1
Changes in anthropometric and biochemical parameters from a 16-week saturated fat diet.

Genotype	*Ppara* (+/+)		*Ppara* (−/−)	
Diet	Con ($n=6$)	Sat ($n=6$)	Con ($n=6$)	Sat ($n=6$)
Body weight (g)	23.9 ± 1.9	28.3 ± 1.5*	26.5 ± 2.7	41.0 ± 5.2$^{**,\#\#}$
Liver/body weight (%)	3.8 ± 0.2	4.6 ± 0.4*	4.4 ± 0.2	5.2 ± 0.6*
Epididymal fat/body weight (%)	2.5 ± 0.3	3.7 ± 0.6*	3.0 ± 1.5	6.1 ± 0.5$^{**,\#\#}$
Serum TG (mg/dL)	61 ± 1	123 ± 41*	124 ± 50	233 ± 49$^{**,\#\#}$
Serum NEFA (mEq/L)	0.75 ± 0.30	1.33 ± 0.3*	1.19 ± 0.25	1.54 ± 0.15$^{\#}$
Serum glucose (mg/dL)	92 ± 23	89 ± 24	98 ± 14	103 ± 22
Serum insulin (ng/mL)	0.51 ± 0.09	1.21 ± 0.58	0.48 ± 0.06	2.24 ± 0.46**
Serum AST (U/L)	129 ± 66	243 ± 62	149 ± 92	203 ± 46
Serum ALT (U/L)	13 ± 6	43 ± 16*	18 ± 10	99 ± 21$^{**,\#}$
Liver TG (mg/g)	10 ± 1	30 ± 3**	17 ± 3	52 ± 7$^{**,\#\#}$

Results are expressed as mean ± SD. Con, control standard diet; Sat, saturated fat diet; TG, triglyceride; NEFA, non-esterified fatty acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
* $P<0.05$ compared with mice of the same genotype fed a control diet.
** $P<0.01$ compared with mice of the same genotype fed a control diet.
$^{\#}$ $P<0.05$ compared with *Ppara* (+/+) mice fed the same diet.
$^{\#\#}$ $P<0.01$ compared with *Ppara* (+/+) mice fed the same diet.

undertaken to show the efficacy of n-3 PUFA and EPA on reducing serum triglyceride (TG) concentrations and preventing cardiovascular events [7–9]. Some data on the steatosis-ameliorating effect of n-3 PUFA have also been obtained [10,11], creating the intriguing possibility that EPA might be beneficial for the treatment of NAFLD.

It has been considered that n-3 PUFA exhibited TG-reducing effects through regulation of peroxisome proliferator-activated receptor α (PPARα) and sterol regulatory element-binding protein (SREBP)-1, which control hepatic fatty acid (FA) catabolism and synthesis, respectively [12]. PPARα is a nuclear receptor expressed primarily in the liver and is involved in not only FA/TG metabolism,

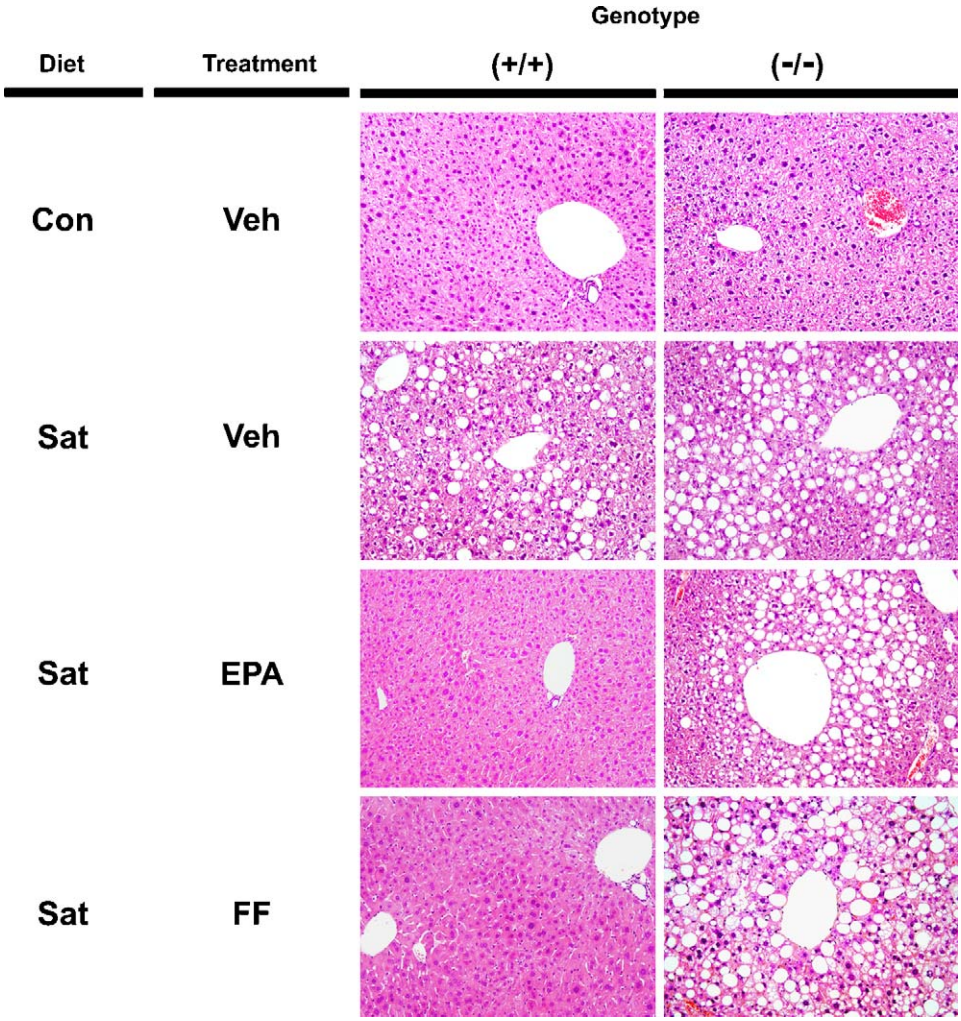

Fig. 1. Histological findings in the livers of wild-type and *Ppara*$^{-/-}$ mice. Male 8-week-old wild-type (+/+) and *Ppara* (−/−) mice were fed a control standard (Con) or saturated fat diet (Sat) for 16 weeks. After 4 weeks on the saturated fat diet, treatment with highly-purified EPA or FF was initiated and continued for 12 weeks. Liver sections were stained by hematoxylin and eosin method. Original magnification, 200×. Veh, vehicle.