

Table S1 Diet Ingredients

Ingredient	Diet Group (g/100g Dry Weight)			
	ALA-1 diet	ALA-2.5 diet	ALA-5 diet	ALA-7.5 diet
<b>Variable diet fat</b>				
Palm Oil	8.90	6.95	3.71	0.47
Safflower Oil	0	0	0	0
Olive Oil	2.45	1.92	1.02	0.13
Flaxseed Oil <sup>1</sup>	1.65	4.13	8.26	12.40
EPA Oil <sup>2</sup>	0	0	0	0
<b>Fixed diet ingredients</b>				
Wheat Fl			30	
Dextrin			10	
Sucrose			10	
Casein			10	
Lactalbumin			10	
Alphacel			11	
$\beta$ -Sitosterol			0.015	
Mineral Mix <sup>3</sup>			4.7	
Vitamin Mix <sup>4</sup>			1	
Choline Bitartrate			0.25	
Tenox 20A			0.006	
Carbohydrates/% Calories			46.7/47.1	

Protein/% Calories 23.2/23.4

Fat/% Calories 13/29.5

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1. Omega-3 H<sup>TM</sup> EPA: 70% EPA & 10% DHA from Huatai Biopharm Inc. (<http://www.huatai-biopharm.com/>).

2 ALA oil: 65% ALA from News agency merchant natural flavor oil refinery (<https://yikangxl.1688.com/>)

3. AIN-93M; TD94049.

4. AIN-93; TD 94047.

Table S2 Diet Ingredients

Ingredient	Diet Group (g/100g Dry Weight)				
	0.25E+5A	0.5E+5A	1E+5A	EPA	Ctl
	diet	diet	diet	diet	diet
<b>Variable diet fat</b>					
Palm Oil	3.45	3.20	2.68	7.06	6.50
Safflower Oil	0	0	0	0.47	0
Olive Oil	0.95	0.88	0.74	2.83	6.50
Flaxseed Oil <sup>1</sup>	8.26	8.26	8.26	0	0
EPA Oil <sup>2</sup>	0.33	0.66	1.32	2.64	0
<b>Fixed diet ingredients</b>					
Wheat Fl			30		
Dextrin			10		
Sucrose			10		
Casein			10		
Lactalbumin			10		
Alphacel			11		
$\beta$ -Sitosterol			0.015		
Mineral Mix <sup>3</sup>			4.7		
Vitamin Mix <sup>4</sup>			1		
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Carbohydrates/% Calories 46.7/47.1

Protein/% Calories 23.2/23.4

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Table S3 Fatty acid composition (%) of ALA diets

Fatty acids	Ctl diet	A-1 diet	A-2.5 diet	A-5 diet	A-7.5 diet
FA/TFA (%)					
C14:0	1.47±0.01	1.55±0.38	1.78±0.12	1.50±0.27	0.96±0.06
C16:0	29.06±1.25	30.23±0.32	27.47±0.35	19.61±0.57	16.07±0.12
C18:0	5.54±0.19	4.95±0.39	5.90±0.37	4.75±0.02	4.16±0.22
C18:1	56.33±0.51	44.88±0.53	39.15±1.61	30.18±0.47	20.71±0.31
C18:2	7.31±0.11	12.24±0.04	11.49±0.30	12.86±0.42	12.95±0.44
C18:3 ALA	0.00±0.00	6.13±0.04	14.23±1.31	31.11±0.33	45.17±1.03

Table S4 Fatty acid composition (%) of diets

Fatty acids	2 E	0.25E+5A	0.5E+5A	1E+5A	A-5
C14:0	1.35±0.08	1.10±0.01	1.06±0.01	0.97±0.05	1.50±0.27
C15:0	0.19±0.05	0.18±0.03	0.16±0.01	0.12±0.01	ND
C16:0	26.42±0.22	16.95±0.15	16.82±0.28	15.03±0.31	19.61±0.57
C16:1	0.36±0.01	0.22±0.01	0.26±0.01	0.21±0.03	ND
C17:0	0.26±0.01	0.21±0.10	0.13±0.01	0.14±0.00	ND
C18:0	4.37±0.10	4.26±0.06	4.19±0.05	4.08±0.01	4.75±0.02
C18:1	43.20±0.11	27.64±0.12	27.65±0.15	26.04±0.24	30.18±0.96
C18:2	10.79±0.01	12.44±0.04	12.23±0.19	12.24±0.04	12.86±0.42
C18:3 ALA	0.51±0.21	33.89±0.02	33.66±0.21	33.75±0.18	31.11±0.33
C20:0	0.30±0.04	0.51±0.02	0.47±0.04	0.48±0.12	ND
C20:1	0.27±0.02	ND	0.19±0.04	0.16±0.01	ND
C20:4 n-6	0.55±0.01	ND	0.24±0.06	0.30±0.01	ND
C20:4 n-3	0.42±0.08	0.06±0.01	0.12±0.01	0.18±0.03	ND
C20:5 EPA	10.07±0.15	1.25±0.35	2.57±0.06	5.37±0.10	ND
C22:0	ND	0.10±0.05	0.12±0.01	0.15±0.04	ND
C22:5 n-6	ND	ND	ND	ND	ND
C22:5 n-3	0.25±0.01	ND	ND	0.20±0.03	ND
C22:6 DHA	0.99±0.03	0.15±0.10	0.29±0.00	0.62±0.02	ND

ND: no detected.

Table S5 Primer sequences used for RT-PCR

mRNA	Sense primer sequence	Antisense primer sequence
<i>GAPDH</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Fads2</i>	GACATAAAGAGCCTGCATGTGTT	GGGCAGGTATTCAGCTTCTTC
	T	
<i>Elovl5</i>	GGTGTGTGGGAAGGCAAATAC	TGCGAAGGATGAAGAAAAAGG
<i>Hsd17b12</i>	ATGCTCCCAGTTCCATTGTT	CTTATCCAAAGTCGGCTTCTGTA
<i>Hacd4</i>	CTATTGGGTTAGTTATGCGGGTT	CATTTCTCTTGGACCTCCTCTTGA
<i>Hacd3</i>	CCCAGAGTGGATTGGCATT	ATCAGTGAGGGAACACCAGAAG
<i>Hacd2</i>	GCTGCTTGTC AATAGCCCTG	CACAATCTAACTTGGTCCTGG
<i>Tecr</i>	ATCAACCACCCTCTCTACACACC	ACAGGACACCAACAGGAACAGC
<i>Pecr</i>	ACACTGGAGCGGCAAGAGAA	TGTCCCATTTCGCCATAGTTG
<i>Fads1</i>	TGGTGCCCTTCATCCTCTGT	TGATGTGCTGCTGCGAGA





Table S6 Body weight of five groups during test.

	0 w	1 w	2 w	3 w	4 w	5 w	6 w	7 w
Ctl group	20.9±0.39	22.19±0.58	24.4±0.7	25.96±0.56	26.68±0.64	27.91±0.65	28.8±0.55	29.56±0.56
A-1 group	20.91±0.43	21.96±0.47	24.02±0.51	25.38±0.77	26.63±0.77	28.08±0.8	29.5±1.06	31.33±1.32
A-2.5 group	20.88±0.23	22.03±0.35	23.74±0.55	25.85±0.38	26.52±0.47	27.97±0.61	29.24±0.73	29.67±0.72
A-5 group	20.26±0.22	21.63±0.23	23.91±0.3	25.41±0.46	26.63±0.44	27.57±0.37	28.51±0.47	29.6±0.38
A-7.5 group	20.41±0.16	21.81±0.26	24.63±0.36	25.11±0.57	26.64±0.58	27.43±0.58	28.31±0.63	29.46±0.62

As shown in Table S6, the body weight of all groups were increased during test, but there were no significant differences among the groups at the same time ( $P > 0.05$ ). Therefore, supplementation of different amount of ALA in diets did not affect body weight.

## Figures

Fig. S1 Hacd 3, Hacd 4, Pecn, Tecd, and Hsd17b12 mRNA in liver of different ALA levels groups

Animals were fed with different ALA content diet and mRNA was isolated in liver and measured by Fluorescence quantitative PCR (qPCR) method. The expression of Hacd 3, Hacd 4, Pecn, Tecd, and Hsd17b12 were kept in a stable level from the ALA percent of 1% to 5%, but relatively high levels at the ALA percent of 7.5%. Bars labeled with different letters are significantly different from each other ( $P < 0.05$ ).

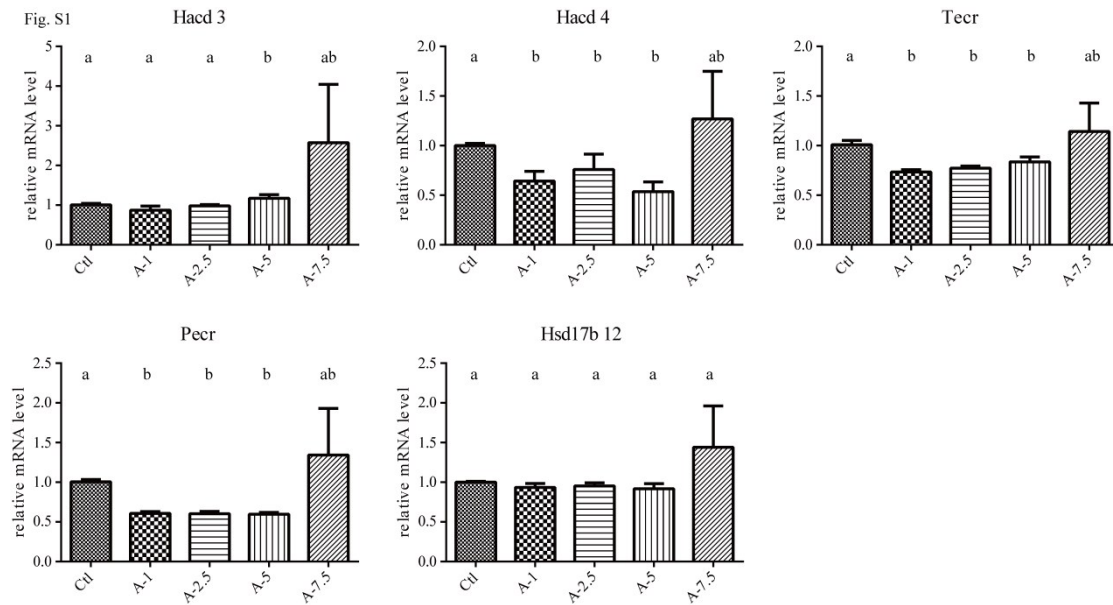


Fig. S2 Hacd 3, Hacd 4, Pecn, Tecd and Hsd17b12 mRNA in liver of different EPA levels groups

Animals were fed with different amount of EPA plus 5% ALA diet and mRNA was isolated from liver and measured by Fluorescence quantitative PCR (qPCR) method. The expression of Hacd 3, Hacd 4, Pecn, Tecd and Hsd17b12 were shown for different groups. Bars labeled with different letters are significantly different from each other ( $P < 0.05$ ).

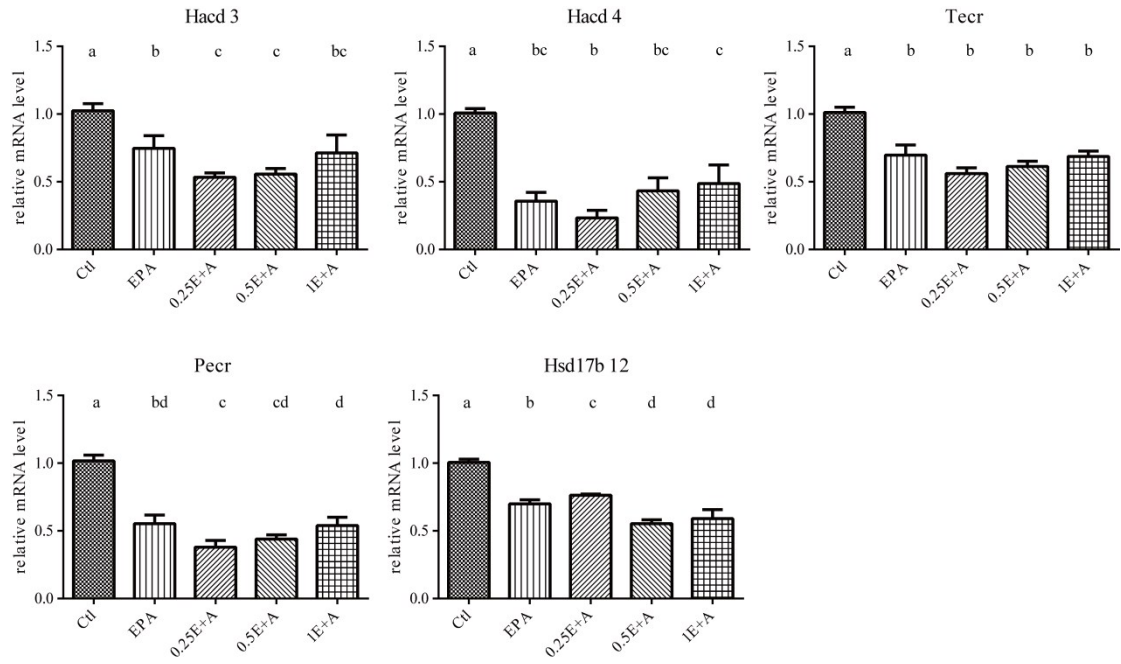
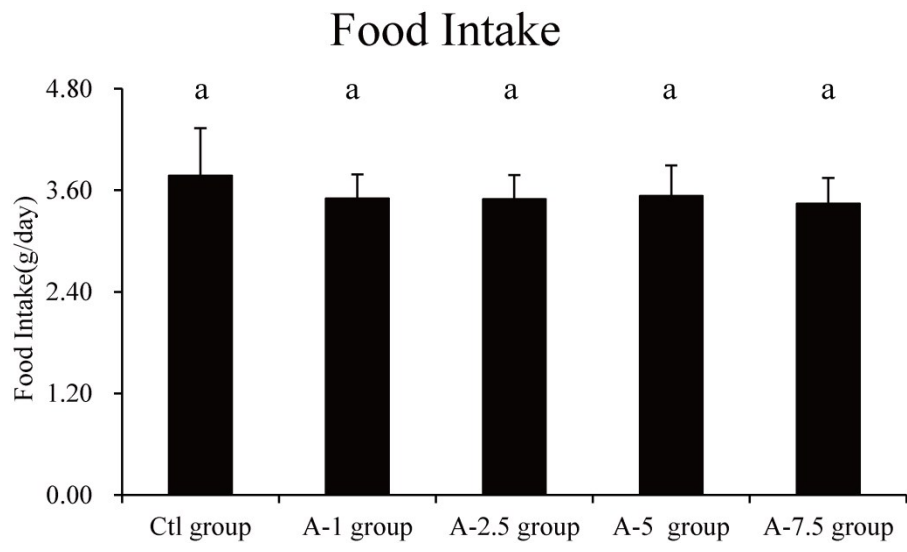


Fig. S3 Food intake of five groups during the experiment.



During the 7 weeks, there were no significant difference among the groups ( $P > 0.05$ ). ALA supplementation did not change the food intake of different groups mice. Bars labeled with different letters are significantly different from each other ( $P < 0.05$ ).