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Low-linoleic acid diet and oestrogen enhance the conversion of α-linolenic acid into DHA through modification of conversion enzymes and transcription factors

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Abstract

Conversion of α-linolenic acid (ALA) into the longer chain *n*-3 PUFA has been suggested to be affected by the dietary intake of linoleic acid (LA), but the mechanism is not well known. Therefore, the purpose of this study was to evaluate the effect of a low-LA diet with and without oestrogen on the fatty acid conversion enzymes and transcription factors. Rats were fed a modified American Institute of Nutrition-93G diet with 0 % *n*-3 PUFA or ALA, containing low or high amounts of LA for 12 weeks. At 8 weeks, the rats were injected with maize oil with or without 17β-oestradiol-3-benzoate (E) at constant intervals for the remaining 3 weeks. Both the low-LA diet and E significantly increased the hepatic expressions of PPAR-α, fatty acid desaturase (FADS) 2, elongase of very long chain fatty acids 2 (ELOVL2) and ELOVL5 but decreased sterol regulatory element binding protein 1. The low-LA diet, but not E, increased the hepatic expression of FADS1, and E increased the hepatic expression of oestrogen receptor-α and β. The low-LA diet and E had synergic effects on serum and liver levels of DHA and on the hepatic expression of PPAR-α. In conclusion, the low-LA diet and oestrogen increased the conversion of ALA into DHA by upregulating the elongases and desaturases of fatty acids through regulating the expression of transcription factors. The low-LA diet and E had a synergic effect on serum and liver levels of DHA through increasing the expression of PPAR-α.

Key words: **α-Linolenic acid: Low-linoleic acid diet: Oestrogen: Conversion enzymes**

Long-chain *n*-3 PUFA, such as EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), have a cardioprotective effect[1]. *n*-3 PUFA can be obtained from fish and from the conversion of α-linolenic acid (ALA, 18 : 3*n*-3), which is found in flaxseed and perilla oil[2]. Isotope tracer studies have suggested that the conversion of ALA to DHA is higher in rats (approximately 70 %)[3] than in humans (0·01–9 %)[4] due to the constraint at fatty acid desaturase (FADS) 1, elongase of very long fatty acid (ELOVL) 2 or translocation of twenty-four carbon intermediates[5]. In addition, the conversion rate of ALA to DHA is lower in men (0·01–4 %) than in women (approximately 9 %)[4] due to the oestrogen-mediated upregulation of the conversion[6].

Oestrogen[7–9], dietary content of ALA[10,11], fatty acid composition of the diet[12] and dietary content of linoleic acid (LA, 18 : 2*n*-6)[13,14] have been shown to affect the conversion of ALA into DHA. Previous studies have reported that oestrogen increases the conversion of ALA into DHA in rat[8,9], given that oestrogen increases the hepatic expression of FADS2 through modulation of PPAR-α expression[7]. Increased intake of ALA also enhances the conversion of ALA into EPA and DHA in rats and humans[10,11]. It has been shown that ALA increases hepatic expressions of FADS2, FADS1, ELOVL5, ELOVL2 and peroxisomal acyl-coenzyme A oxidase 1 (ACOX1) through regulating PPAR-α[15,16]. In addition, an increased LA:ALA ratio decreases the conversion of ALA into EPA and DHA in rat[12], because LA competes with ALA for conversion processes including desaturation, elongation and peroxisomal β-oxidation[17]. The conversion enzymes are known to be regulated by transcription factors such as PPAR-α and sterol regulatory element binding protein 1 (SREBP1)[16].

Previous studies reported that high-LA diets[18–20] and a high-SFA diet[21] decreased plasma phospholipid levels of EPA and DHA as compared with a low-LA diet and a low-SFA diet, respectively. A high-LA diet and a high-SFA diet decreased the expressions of the FADS[22,23] and ELOVL[14,24,25] in female or male rodents. A high-SFA diet consistently decreased the expression of PPAR-α[21] but increased SREBP1[26] in male rodents. However, the effect of a high-LA diet on the transcription factors was controversial. In male rats, diets containing 9·7–16·6 % LA from total energy intake (with 0·2–6·0 % ALA) decreased hepatic expressions of PPAR-α and increased SREBP1 when compared with diets containing 4·6–9·7 % LA

Abbreviations: ACOX1, acyl-coenzyme A oxidase 1; ALA, α-linolenic acid; E, 17β-oestradiol-3-benzoate; ELOVL, elongase of very long fatty acid; ER, oestrogen receptor; FADS, fatty acid desaturase; LA, linoleic acid; SREBP1, sterol regulatory element binding protein 1.

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(with 6·0–12·6 % ALA)[21,27]. On the other hand, Tu *et al.*[28] reported no significant differences between the diets containing 6 % LA from total energy intake (with 0·6 % ALA) and 0·4 % LA (with 0·03 % ALA). In the previous studies, dietary intake of LA and ALA were changed, suggesting that the effect on the expression of conversion enzymes and transcription factors was due to either LA or ALA. Thus, the purpose of this study was to investigate the hypothesis that a high-LA diet decreases the conversion of ALA to EPA and DHA by reducing the expression of the conversion enzymes by modulating the expression of transcription factors, while the ALA content is the same in low- and high-LA diets. In addition, we compared the synergic effect of oestrogen and a low-LA diet on the increased conversion of ALA into EPA and DHA in ovariectomised rats.

Methods

Animals and diets

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Hanyang University (HY-IACUC-16-0027). In all, 3-week-old female Wistar rats (Jung-Ang Lab. Animal Inc., Seoul, Korea) were housed in individually ventilated cages in an air-conditioned room maintained at 22 ± 1°C and 40–50 % humidity, with a 12 h light–12 h dark cycle. During a week of acclimation, rats (n 42) were provided a standard chow diet and fresh tap water *ad libitum*. After acclimation, rats were provided experimental diets and weighed weekly, and food intake was measured daily. The experimental diets were modified American Institute of Nutrition-93G diets containing 15·8 % fat with 10 % LA and 0 % ALA (control diet) or with 9·1 % LA and 1 % ALA (low-LA diet) or a high-fat diet containing 40 % fat with 24·5 % LA and 1 % ALA (high-LA diet expressed as percentage total energy) (Table 1). Grape seed oil (Sajo Haepyo) was used instead of soyabean oil to limit the level of ALA and provide LA, which is the main competitor of ALA, in the process of conversion to long chain PUFA, and flaxseed oil (Amaflax) was used as the source of ALA.

Experimental design

During the 12 weeks of the experiment, rats were assigned to one of the three diets: low-LA diet containing 0 % n-3 PUFA from energy (n 18), low-LA diet containing 1 % ALA from energy (n 12) and high-LA diet containing 1 % ALA from energy (n 12). At week 8, rats were ovariectomised or underwent a sham surgery under anaesthesia using a combination of zoletil (25 mg/kg) and rompun (10 mg/kg). From 1 week after surgery, rats were subcutaneously injected with 1 ml of maize oil vehicle (Sigma-Aldrich) with or without 10 μg of 17β-oestradiol-3-benzoate (E) to mimic the rat's oestrus cycle, every 4 d during the last 3 weeks of the experiment. Thus, rats were divided into seven groups (n 6/group): 0 % n-3 PUFA diet, without E after sham surgery; 0 % n-3 PUFA diet, without E and with E after ovariectomy; ALA in low-LA diet (9·1 % LA of total energy), without E (low-LA) and with E after ovariectomy (low-LA-E); and ALA in high-LA diet (24·5 % LA of total energy), without E (high-LA) and with E after ovariectomy (high-LA-E).

Table 1. Composition of experimental diets

	Control diet	Low-LA diet	High-LA diet
Energy (% of total energy)			
Carbohydrate	63·90	63·90	43·00
Protein	20·30	20·30	17·00
Fat	15·80	15·80	40·00
Diet ingredients (g/1000 g diet)			
Casein	200·00	200·00	195·00
Maize starch	397·00	397·00	50·00
Sucrose	100·00	100·00	341·00
Dextrose	132·00	132·00	100·00
Cellulose	50·00	50·00	50·00
Mineral mix	35·00	35·00	35·00
Vitamin mix	10·00	10·00	10·00
Choline	2·50	2·50	2·00
Grape seed oil	70·00	61·73	201·61
Flaxseed oil	0·00	8·27	8·38
Fatty acids (%)			
14 : 0	0·15	0·15	0·15
16 : 0	8·52	8·17	8·40
16 : 1n-7	0·09	0·09	0·09
18 : 0	3·50	3·45	3·49
18 : 1n-9	23·25	23·36	23·29
18 : 2n-6	63·10	57·57	61·25
18 : 3n-3	0·51	6·40	2·47
SFA	12·24	11·83	12·10
MUFA	23·51	23·61	23·54
n-3 PUFA	0·56	6·44	2·52
n-6 PUFA	63·14	57·63	61·31

Control diet, 0 % n-3 PUFA diet; LA, linoleic acid; ALA, α-linolenic acid; low-LA diet, 1 % ALA (18 : 3n-3) in 9·1 % LA (percentage of total energy) diet; high-LA diet, 1 % ALA in 24·5 % LA diet.

Tissue and blood collection

After 12 weeks of the experiment, rats were fasted overnight and euthanised using zoletil (25 mg/kg) and rompun (10 mg/kg). Blood and organs were obtained and were stored at –80°C for further analysis.

GC

Liver tissue (100 mg) was homogenised using 5 ml of chloroform–methanol–distilled water (2:2:1, by vol.)[29]. The homogenates were centrifuged at 4000 rpm (Hanil Science Inc.) for 10 min, and the solvents of bottom layer were redissolved in n-hexane and separated by thin-layer chromatography (SIL G-25; Macherey-Nagel GmbH & Co.) in n-hexane–diethyl ether–acetic acid (40:10:1, by vol.). After developing the TLC plate, phospholipids were scraped off. Liver phospholipids and serum without lipid extraction were directly methylated by adding boron trifluoride methanol (B1252; Sigma-Aldrich) and heated at 100°C for 10 and 45 min, respectively. Fatty acid methyl esters of liver phospholipid and serum were analysed using GC (Shimadzu 2010AF; Shimadzu Scientific Instrument) with a 100 m × 0·25 mm inner diameter with 0·20 μm film capillary column (SP2560; Supelco). H_2 was used as the carrier gas at a flow rate of 40 ml/min. The injection and detection

temperatures were 230 and 240°C, respectively. The run temperature began at 180°C and then was increased at 5°C/min increase up to 200°C and then increased by 10°C/min to 240°C. The split ratio was 10:1. GC was calibrated with an external standard composed of defined fatty acids (GLC-727; Nu-Check Prep). Qualified GC evaluation was carried out by measuring CV of sum of EPA and DHA composition in quality control material[30], and the CV was 2·4 %.

Western blot analysis

Hepatic tissues were homogenised in ice-cold lysis buffer (20 mM HEPES, 0·25 M sucrose, 0·5 mM EDTA, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM sodium orthovanadate; pH 7·5) including protease inhibitor cocktail tablets and phosphatase inhibitor cocktails (EDTA-free protease inhibitor and PhosSTOP; Roche Diagnostics GmbH) using a Tissuemiser (Fisher Scientific). The homogenates were centrifuged at 20 000 ***g*** (Eppendorf) for 60 min at 4°C. The protein concentration of supernatants was determined by the Bradford method using bovine serum albumin (Bio-Rad) as the standard. Protein (30 μg) was separated on 8 or 10 % SDS-PAGE, transferred to a polyvinylidene fluoride membrane (0·45 μm; Merck Millipore) and blocked for 1 h at room temperature with 5 % skimmed milk in TRIS-buffered saline containing Tween-20 (TBST). The membranes were incubated overnight with a primary antibody oestrogen receptor (ER)-α (1:1000), ER-β (1:1000), PPAR-α (1:1000), SREBP1 (1:500), FADS2 (1:400), FADS1 (1:500), ELOVL5 (1:200), ELOVL2 (1:10 000) or ACOX1 (1:2000) with 5 % skimmed milk or 5 % bovine serum albumin in TBST at 4°C. The antibodies of ER-α, ER-β, PPAR-α, SREBP1, FADS2, FADS1, ELOVL2 and ACOX1 were purchased from Abcam; ELOVL5, Santa Cruz Biotechnology. After washing in TBST, membranes were incubated with horseradish peroxidise (HRP)-conjugated goat anti-rabbit (Cell Signaling Technology), anti-mouse IgG (Enzo Life Science) or donkey anti-goat IgG-HRP (Santa Cruz Biotechnology) with 5 % skimmed milk in TBST for 1 h at room temperature. Immunoreactive bands were visualised under the UV setting of the ChemiDoc MP Imaging System (Bio-Rad) to estimate the total protein per lanes, and β-actin (1:1000; BD Transduction Laboratories) was used for normalisation. Each Western blot was repeated three times.

Statistical analysis

The sample size was calculated using PASS version 14 (NCSS LLC) based on the previously described effect of high-LA diet consumption on the hepatic level of DHA[31]. Concerning the hepatic level of DHA, a sample size of six animals per group would provide the appropriate power (1–β–0·95) to identify significant differences ($\alpha = 0{\cdot}05$) in the variables analysed with an effect size $d = 2{\cdot}59$, two-sided test and a sample size ratio = 1. Values are expressed as the mean with their standard errors, and differences were considered significant at $P < 0{\cdot}05$. Data were analysed using a two-way ANOVA with factors of ALA and E or LA and E, followed by Duncan's *post hoc* test. Analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc.).

Results

Dietary intake, body weight and organ weights

Regardless of the diet, rats with E by injection or sham surgery consumed significantly less food and had lower final body weights but heavier liver, uterus and kidney weights than rats without E (Table 2). Regardless of E, rats fed the high-LA diet consumed less food than those fed the low-LA diet; however, their body and organ weights were not significantly different.

Fatty acid composition of serum and liver phospholipid

Regardless of the diet, rats with E by injection or sham surgery had lower serum and liver phospholipid levels of 14 : 0, 16 : 0, 16 : 1*n*-7, 18 : 1*n*-9 and MUFA, but higher 18 : 0, 22 : 5*n*-6, 22 : 6*n*-3 and *n*-3 PUFA than rats without E (Tables 3 and 4). However, E had no effect on the serum and liver levels of 20 : 5*n*-3. Regardless of E, rats fed the high-LA diet had lower serum and liver phospholipid levels of 14 : 0, 16 : 0, 16 : 1*n*-7, 18 : 1*n*-9, 18 : 3*n*-3, 20 : 5*n*-3, 22 : 5*n*-3, 22 : 6*n*-3, SFA, MUFA and

Table 2. Dietary intake, organ weights and body weight
(Mean values with their standard errors, *n* 6 per group)

	Sham		OVX		Low-LA		High-LA		OVX-E		Low-LA-E		High-LA-E	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Dietary intake (g/d)	13·65*	0·16	14·82	0·26	14·84	0·21	11·95†	0·11	13·48*	0·23	13·21*	0·18	11·12*†	0·09
Initial body weight (g)	96·42	1·05	96·18	1·82	95·10	1·54	95·60	2·37	94·28	1·08	97·70	1·22	97·45	2·05
Final body weight (g)	264·08*	4·51	302·78	3·24	309·43	4·90	305·82	6·07	259·33*	4·54	261·97*	3·70	266·23*	2·66
Liver weight (g)	6·71	0·11	6·60	0·18	6·89	0·16	6·73	0·16	6·64	0·11	6·76	0·14	6·84	0·05
Uterus weight (g)	0·51*	0·07	0·08	0·00	0·07	0·00	0·08	0·00	0·44*	0·02	0·44*	0·01	0·44*	0·03
Kidney weight (g)	1·59	0·07	1·57	0·01	1·58	0·04	1·58	0·02	1·62	0·02	1·63	0·04	1·62	0·04

E, 17β-oestradiol-3-benzoate; Sham, 0 % *n*-3 PUFA diet without E after sham surgery; OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; LA, linoleic acid; ALA, α-linolenic acid; low-LA and low-LA-E, 1 % ALA (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.

* Mean values were significantly different between maize oil and E by injection or sham surgery within the same diet ($P < 0{\cdot}05$).

† Mean values were significantly different among low-LA and high-LA with maize oil or E injection, respectively ($P < 0{\cdot}05$).

Table 3. Fatty acid (FA) composition of serum
(Mean values with their standard errors, *n* 6 per group)

	Sham		OVX		Low-LA		High-LA		OVX-E		Low-LA-E		High-LA-E	
FA (%)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	0·26*	0·00	0·41	0·01	0·40	0·00	0·32†	0·00	0·27*	0·00	0·25*	0·00	0·20*†	0·00
16 : 0	12·29*	0·16	13·11	0·12	13·26	0·18	11·29†	0·16	12·03*	0·13	11·17*	0·29	10·20*†	0·20
18 : 0	17·79*	0·25	16·24	0·19	16·14	0·22	16·38	0·27	17·96*	0·44	17·50*	0·33	17·42*	0·55
16 : 1*n*-7	1·03*	0·02	1·61	0·08	1·56	0·04	0·55†	0·07	0·96*	0·02	1·02*	0·07	0·32*†	0·00
18 : 1*n*-9	8·43*	0·26	9·37	0·23	8·61	0·13	7·38†	0·26	7·96*	0·28	8·17*	0·11	6·83*†	0·33
18 : 2*n*-6	14·02	0·41	14·21	0·22	14·45	0·12	17·86†	0·64	13·83	0·26	14·22	0·16	18·21†	1·04
20 : 4*n*-6	35·01	0·52	35·21	0·42	33·59	0·31	36·48†	0·48	34·90	0·38	35·02	0·42	36·07†	0·37
22 : 4*n*-6	0·60	0·00	0·59	0·02	0·30‡	0·01	0·72†	0·01	0·60	0·02	0·29‡	0·01	0·71†	0·00
22 : 5*n*-6	4·31*	0·20	2·62	0·13	0·39‡	0·01	1·17†	0·28	4·68*	0·19	0·67*‡	0·06	2·06*†	0·09
18 : 3*n*-3	0·12	0·00	0·12	0·01	0·59‡	0·01	0·40†	0·01	0·12	0·00	0·59‡	0·00	0·41†	0·01
20 : 5*n*-3	0·13	0·00	0·14	0·01	0·61‡	0·01	0·32†	0·01	0·14	0·00	0·64‡	0·03	0·33†	0·01
22 : 5*n*-3	0·29	0·00	0·31	0·01	0·77‡	0·00	0·58†	0·01	0·28	0·00	0·79‡	0·02	0·60†	0·00
22 : 6*n*-3	1·91*	0·03	1·55	0·01	4·75‡	0·05	4·38†	0·05	1·97*	0·08	6·86*‡	0·19	5·59*†	0·13
SFA	30·72	0·21	30·50	0·21	30·88	0·25	27·86†	0·12	30·61	0·30	29·17	0·32	28·32†	0·46
MUFA	10·34*	0·25	12·71	0·24	12·30	0·11	10·00†	0·27	10·48*	0·21	10·48*	0·14	8·71*†	0·32
n-6 PUFA	54·98	0·34	53·73	0·27	50·22‡	0·35	55·45†	0·88	54·13	0·39	51·42‡	0·51	56·01†	0·96
n-3 PUFA	2·44*	0·03	2·13	0·02	6·72‡	0·05	5·67†	0·05	2·51*	0·08	8·89*‡	0·19	6·92*†	0·14

E, 17*β*-oestradiol-3-benzoate; Sham, 0 % *n*-3 PUFA diet without E after sham surgery; OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; LA, linoleic acid; ALA, *α*-linolenic acid; low-LA and low-LA-E, 1 % ALA (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.
* Mean values were significantly different between maize oil and E by injection or sham surgery within the same diet ($P < 0·05$).
† Mean values were significantly different among low-LA and high-LA with maize oil or E injection, respectively ($P < 0·05$).
‡ Mean values were significantly different among OVX and low-LA with maize oil or E injection, respectively ($P < 0·05$).

Table 4. Fatty acid (FA) composition of liver phospholipids
(Mean values with their standard errors, *n* 6 per group)

	Sham		OVX		Low-LA		High-LA		OVX-E		Low-LA-E		High-LA-E	
FA (%)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14 : 0	0·08*	0·00	0·10	0·00	0·10	0·00	0·06†	0·00	0·08*	0·00	0·08*	0·00	0·05*†	0·00
16 : 0	12·42*	0·21	14·01	0·20	13·78	0·25	11·51†	0·09	12·82*	0·31	12·59*	0·12	10·38*†	0·12
18 : 0	35·48*	0·15	33·23	0·20	33·71	0·06	34·46	0·11	35·60*	0·20	35·86*	0·31	35·73*	0·35
16 : 1*n*-7	0·25*	0·00	0·39	0·00	0·42	0·00	0·08†	0·00	0·24*	0·00	0·22*	0·00	0·05*†	0·01
18 : 1*n*-9	3·80*	0·01	4·32	0·03	4·24	0·10	2·80†	0·03	3·77*	0·04	3·43*	0·09	2·59*†	0·06
18 : 2*n*-6	7·45	0·11	7·73	0·09	7·48	0·13	8·71†	0·07	7·30	0·09	7·58	0·09	8·51†	0·13
20 : 4*n*-6	26·34	0·18	26·31	0·33	26·07	0·20	27·82†	0·26	26·2	0·12	25·62	0·14	27·43†	0·24
22 : 4*n*-6	0·75	0·00	0·74	0·00	0·30‡	0·00	0·51†	0·00	0·73	0·01	0·29‡	0·00	0·50	0·01
22 : 5*n*-6	7·38*	0·10	6·34	0·04	0·55‡	0·01	1·27†	0·05	7·47*	0·16	1·33*‡	0·09	3·33*†	0·11
18 : 3*n*-3	0·01	0·00	0·01	0·00	0·08‡	0·00	0·04†	0·00	0·01	0·00	0·08‡	0·00	0·04†	0·00
20 : 5*n*-3	0·02	0·00	0·02	0·00	0·10‡	0·00	0·04†	0·00	0·02	0·00	0·10‡	0·00	0·04†	0·00
22 : 5*n*-3	0·13	0·00	0·13	0·01	0·47‡	0·01	0·42†	0·01	0·13	0·00	0·46‡	0·01	0·43†	0·01
22 : 6*n*-3	4·27*	0·07	3·71	0·03	10·48‡	0·04	9·50†	0·13	4·21*	0·05	13·81*‡	0·21	10·50†	0·04
SFA	48·12	0·14	47·50	0·25	47·74	0·22	46·19†	0·10	48·64	0·41	48·67	0·22	46·29†	0·45
MUFA	4·30*	0·04	5·19	0·06	5·07	0·10	3·25†	0·03	4·18*	0·05	4·04*	0·10	2·98*	0·07
n-6 PUFA	42·58	0·11	42·83	0·20	35·14‡	0·17	39·79†	0·16	43·07	0·27	35·40‡	0·20	40·12†	0·20
n-3 PUFA	4·43*	0·07	3·87	0·04	11·14‡	0·04	9·99†	0·12	4·37*	0·05	14·44*‡	0·20	11·02*†	0·04

E, 17*β*-oestradiol-3-benzoate; Sham, 0 % *n*-3 PUFA diet without E after sham surgery; OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; LA, linoleic acid; ALA, *α*-linolenic acid; low-LA and low-LA-E, 1 % *α*-linolenic acid (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.
* Mean values were significantly different between maize oil and E by injection or sham surgery within the same diet ($P < 0·05$).
† Mean values were significantly different among low-LA and high-LA with maize oil or E injection, respectively ($P < 0·05$.
‡ Mean values were significantly different among OVX and low-LA with maize oil or E injection, respectively ($P < 0·05$).

n-3 PUFA but higher 18 : 2*n*-6, 20 : 4*n*-6, 22 : 4*n*-6, 22 : 5*n*-6 and *n*-6 PUFA than rats fed the low-LA diet. Regardless of E and amount of dietary LA, rats fed ALA had lower serum and liver levels of 22 : 4*n*-6, 22 : 5*n*-6 and *n*-6 PUFA but higher 18 : 3*n*-3, 20 : 5*n*-3, 22 : 5*n*-3, 22 : 6*n*-3 and *n*-3 PUFA than rats fed the 0 % *n*-3 PUFA diet. There was a synergic effect of low-LA and E on the increased serum and liver phospholipid levels of 22 : 6*n*-3 ($P = 0·001$ and $P < 0·001$).

Hepatic expressions of enzymes involved in conversion of α-linolenic acid into EPA and DHA

Regardless of the diet, rats with E by injection or sham surgery had higher hepatic expression of FADS2, ELOVL5, ELOVL2, PPAR-*α*, ER-*α* and ER-*β* but lower SREBP1 than rats without E (Fig. 1–3). Regardless of E, rats fed the high-LA diet had higher hepatic expression of SREBP1 but lower FADS2, FADS1, ELOVL5,

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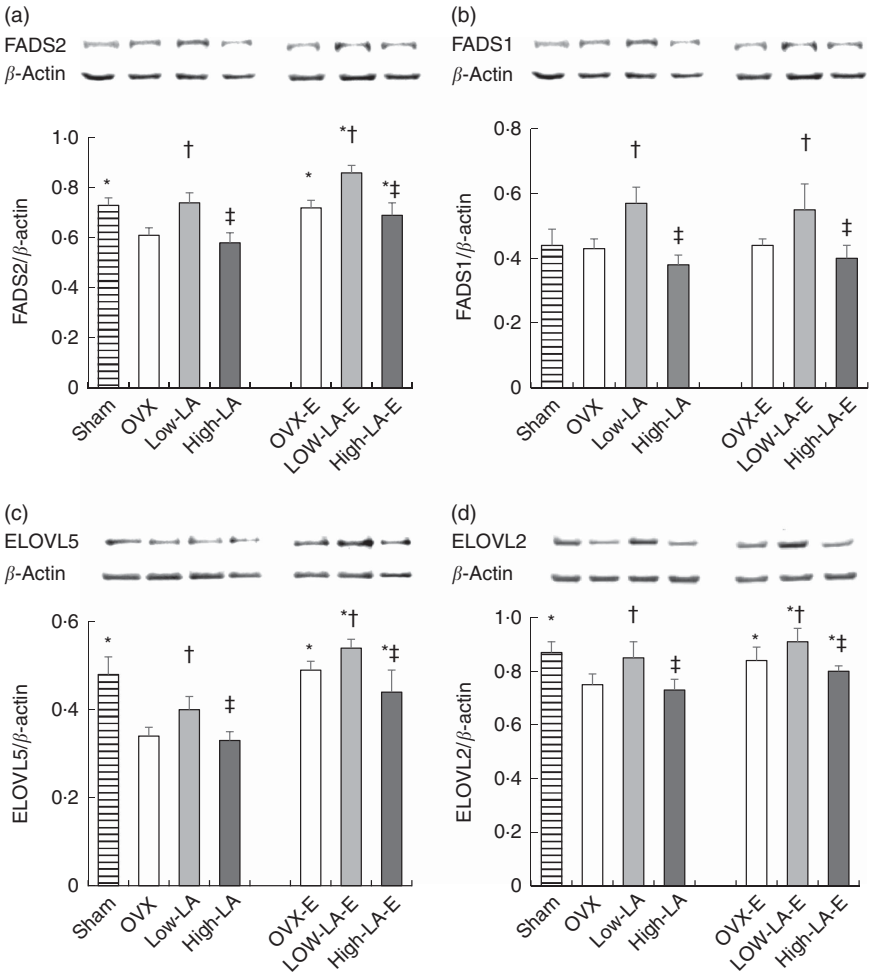

Fig. 1. Effects of 17β-oestradiol-3-benzoate (E), α-linolenic acid (ALA) and linoleic acid (LA) on the hepatic expressions of (a) fatty acid desaturase (FADS)2, (b) FADS1, (c) elongase of very long fatty acid (ELOVL)5 and (d) ELOVL2. Values are means (*n* 6), with their standard errors represented by vertical bars. * Mean values were significantly different between maize oil and E injection or sham surgery within the same diet ($P < 0{\cdot}05$). † Mean values were significantly different among OVX and low-LA with maize oil or E injection, respectively ($P < 0{\cdot}05$). ‡ Mean values were significantly different among low-LA and high-LA with maize oil or E injection, respectively ($P < 0{\cdot}05$). OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; low-LA and low-LA-E, 1 % α-linolenic acid (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.

ELOVL2 and PPAR-α than rats fed the low-LA diet. Regardless of E and amount of dietary LA, rats fed ALA had higher hepatic expression of FADS2, FADS1, ELOVL5, ELOVL2 and PPAR-α but lower SREBP1 than rats fed the 0 % *n*-3 PUFA diet. Hepatic expression of ER-α and ER-β was not affected by the amount of dietary LA or ALA intake. Hepatic expression of ACOX1 was not affected by E, LA or ALA. There was a synergic effect of low-LA and E on the increased hepatic expression of PPAR-α ($P < 0{\cdot}05$).

Discussion

This study demonstrated that a high-LA diet reduced protein expression of FADS2, FADS1, ELOVL5 and ELOVL2 through increased expression of PPAR-α and decreased expression of SREBP1 in ovariectomised rats, suggesting that a high-LA diet decreased the conversion of ALA into EPA and DHA.

Previous studies consistently reported that high-LA diets decrease the expressions of FADS2 and FADS1[22] in female rats and ELOVL5[14,25] in male rats. No previous study has reported the effect of a high-LA diet on ELOVL2. Valenzuela *et al.*[13] reported that a high-LA diet decreased the activity of FADS2 and FADS1 calculated by the ratios of 18 : 3*n*-6:LA and 20 : 4*n*-6:20 : 3*n*-6, respectively. Although the activity or expression of desaturase was not measured directly, the previous study reported that a high-LA diet decreased the conversion of ALA into EPA and DHA by reduced activity of FADS[13]. In addition, a high-SFA diet decreased the hepatic expressions of ELOVL5[14], FADS2, FADS1[22] and ELOVL2, suggesting that high fat with either LA or SFA could decrease all elongases and desaturases.

Two previous studies suggested that diets containing 9·7–16·6 % LA from total energy intake (with 0·2–6·0 % ALA) decreased hepatic expression of PPAR-α but increased SREBP1 as compared with diets containing 4·6–9·7 % LA (with 6·0–12·6 % ALA)[15,27], consistent with the present study. In addition, Tu *et al.*[28] reported no significant differences between the diets containing 6 % LA and 0·4 % LA on the expressions of PPAR-α and SREBP1. This inconsistency could be due to dietary contents of total PUFA, LA and ALA. The high-LA diet

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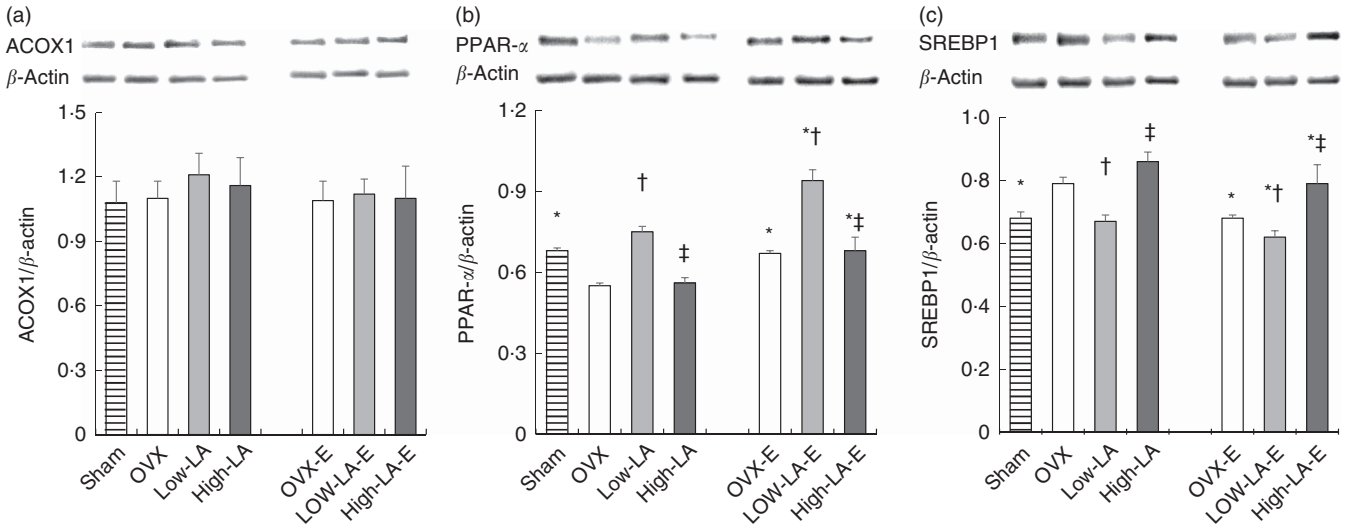

Fig. 2. Effects of 17β-oestradiol-3-benzoate (E), α-linolenic acid (ALA) and linoleic acid (LA) on the hepatic expressions of (a) acyl-coenzyme A oxidase 1 (ACOX1), (b) PPAR-α and (c) sterol regulatory element binding protein 1 (SREBP1). Values are means (*n* 6), with their standard errors represented by vertical bars. * Mean values were significantly different between maize oil and E injection or sham surgery within the same diet ($P < 0.05$). † Mean values were significantly different among OVX and low-LA with maize oil or E injection, respectively ($P < 0.05$). ‡ Mean values were significantly different among low-LA and high-LA with maize oil or E injection, respectively ($P < 0.05$). OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; low-LA and low-LA-E, 1 % α-linolenic acid (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.

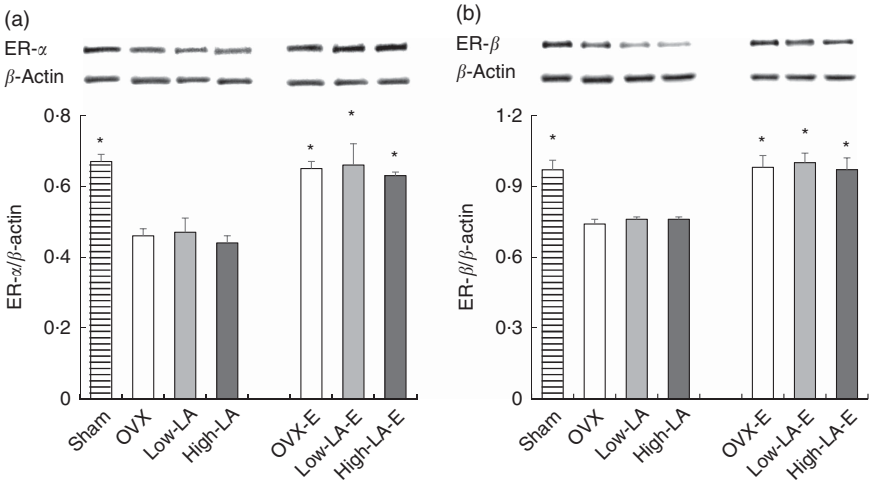

Fig. 3. Effects of 17β-oestradiol-3-benzoate (E), α-linolenic acid (ALA) and linoleic acid (LA) on the hepatic expressions of (a) oestrogen (ER)-α and (b) ER-β. Values are means (*n* 6), with their standard errors represented by vertical bars. * Mean values were significantly different between maize oil and E injection or sham surgery within the same diet ($P < 0.05$). OVX and OVX-E, 0 % *n*-3 PUFA diet without E and with E after ovariectomy; low-LA and low-LA-E, 1 % α-linolenic acid (18 : 3*n*-3) in 9·1 % LA diet without E and with E after ovariectomy; high-LA and high-LA-E, 1 % ALA in 24·5 % LA diet without E and with E after ovariectomy.

containing 6·6 % PUFA had no effect on transcription factors, but the high-LA diet containing approximately 17 % PUFA, which contained a similar amount of PUFA as used presently (25 %), modified the transcription factors. Clarke *et al.*[32] also reported that a diet with 20 % PUFA (from total energy intake) regulated the expression of SREBP1, but a diet with 5 % PUFA did not, suggesting that the amount of PUFA could be important for modification of transcription factors. As mentioned above, PUFA increase the expression of PPAR-α and decreased SREBP1[33], but *n*-3 PUFA were reportedly more potent modulator of PPAR-α and SREBP1 than *n*-6 PUFA[16,17]. In addition, a diet with LA:ALA = 0·5:1 increased the expression of PPAR-α and decreased SREBP1 as compared with a diet with LA:ALA = 10:1 in rats[34]. Previous studies modulated dietary content of LA and ALA, since high-LA diets contained low ALA and low-LA diets contained high ALA. However, in the present study, the high- and low-LA diets contained the same amount of ALA.

A few studies showed that hepatic and blood levels of EPA and DHA were lower with a high-LA diet (approximately 26 % LA from the total energy intake and 0·4–1 % ALA from the total energy intake) than a low-LA diet (0·4–3·5 % LA from the total energy intake and 0·03–0·4 % ALA from the total energy intake) in male rats[11,28,35], which is consistent with the present study. Wu *et al.*[36] reported that enhanced conversion from ALA to DHA traced by isotope occurred through the increased activity of FADS1 in HepG2 cell. In addition, blood levels of EPA and DHA were increased with the increased expression of conversion enzymes, such as FADS and ELOVL, in rats and

humans[13,37]. An isotope tracer study conducted in humans reported that a high-LA diet reduced the conversion of ALA to EPA as compared with a low-LA diet. A clinical trial also reported that a high-LA diet significantly decreased the plasma phospholipid level of EPA and DHA, although the high-LA diet contained a high level of ALA compared with a low-LA diet[38]. Thus, the results of the present study suggested that a high-LA diet reduced the conversion of ALA into EPA and DHA by decreasing the hepatic expression of both desaturases and elongases.

Contrary to our hypothesis, the high-LA diet had no effect on ACOX1 expression in the present study. Previously, there were inconsistent results regarding the effect of high-LA diets on ACOX1, a peroxisomal β-oxidation enzyme. Consistent with the present study, high-LA diets, containing 3·7 times more LA (12·6 % from energy), without *n*-3 PUFA supplementation, have been shown to decrease the hepatic expression of ACOX1 but high-LA diets with *n*-3 PUFA supplementation did not[39]. Previous studies reported that *n*-3 PUFA increase ACOX1 expression with diets containing LA:ALA ratios of 0·2–0·3, but not LA:ALA ratios of 1·3–387[15,40], suggesting that LA:ALA ratios higher than 1·3 had no effect on ACOX1 expression. In the present study, LA:ALA ratios were 9–124, and ACOX1 expression was not changed. Although ALA had no effect on ACOX1 expression in the present study, previous studies showed that *n*-3 PUFA could allow the recovery of PPAR-α signalling after reduction due to high-LA diets and, consequently, trigger the recovery of ACOX1 expression[39].

Consistent with our results, previous studies reported that oestrogen increases hepatic and blood levels of DHA but not EPA[8,41]. Oestrogen also increases the hepatic expressions of FADS2[8], ELOVL2[42] and ELOVL5[43], which are modulated by increased PPAR-α[7] and decreased SREBP1[44]. However, Kitson *et al.*[8] reported that oestrogen has no effect on the hepatic expression of FADS1, converting 20 : 4*n*-3 to EPA, which is consistent with the present study. Although FADS1 expression was not affected, oestrogen increased the conversion of ALA into DHA, because it increased the expression of FADS2 – the rate-limiting enzyme in the conversion of ALA into DHA[45].

In the present study, oestrogen and the low-LA diet have synergic effects on serum and liver phospholipid levels of DHA and on the hepatic expression of PPAR-α. Consistent with the present study, it has been well known that oestrogen increases the hepatic expressions of ER-α and ER-β[44,46]. The binding of oestrogen to membrane ER directly phosphorylates PPAR-α and increases the intracellular concentration of DHA, which indirectly activates PPAR-α via elevated Ca phospholipase A_2[7]. The present study and previous studies[21,39] consistently showed that low-LA diets increase PPAR-α expression. Therefore, the combination of oestrogen and a low-LA diet have an added effect on PPAR-α expression. Regarding the synergic effect on DHA level, the low-LA diet increased the conversion of ALA into EPA and DHA, but oestrogen only increased the conversion of ALA into DHA. Thus, the synergic effect of oestrogen and low-LA diets was observed on the hepatic level of DHA, but not EPA, in the present study.

The present study had a few limitations. First, the high-LA diet had a higher content of sugar compared with the low-LA diet. Thus, these components could influence the low conversion of ALA into DHA in the high-LA diet. Second, the conversion of ALA to DHA was estimated by fatty acid composition rather than using the stable isotope tracer technique. Third, mRNA levels of transcription factors and conversion enzymes were not measured to confirm our findings at the gene level.

Conclusion

The high-LA diet decreased the conversion of ALA into EPA and DHA by reducing the expression of FADS and elongases through modification of transcription factors such as PPAR-α and SREBP1. In addition, oestrogen enhanced the conversion of ALA into DHA by modifying conversion enzymes and transcription factors, particularly PPAR-α, synergically with the low-LA diet.

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The authors' contributions are as follows: D. K. carried out the experimental work and prepared the manuscript; J.-E. C. conducted the statistical analyses; Y. P. contributed to the interpretation of the results, editing of the manuscript, and to the final text. All authors read and approved the final manuscript.

None of the authors has any conflicts of interest to declare.

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