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17-α-ethynylestradiol alters conversion of α-linolenic acid to longer chain PUFA in rat hepatocarcinoma cells

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Female human subjects and rats have higher DHA (22:6*n* − 3) status and greater capacity to convert α-linolenic acid (18:3*n* − 3) to the longer chain PUFA EPA (20:5*n*–3) and 22:6*n* − 3 than males[(1)]. Previous studies have indicated that 18:3*n* − 3 is regulated by oestrogen. Increased exposure of male transsexuals to synthetic estrogens[(2)] and use of an 17-α ethynylestradiol (EE_2)-based oral contraceptive pill increased 22:6*n* − 3 status and synthesis compared with untreated individuals[(3)]. The aim of this study was to investigate the mechanism by which oestrogen alters 18:3*n* − 3 conversion.

Rat hepatocarcinoma CC1 cells were seeded at approximately 75% confluence and maintained in DMEM with 5% fetal bovine serum at 37°C. Cultures were supplemented with ethyl-linolenate (100 μM) and with EE_2 (0–7 nM) for 72 h. Cells were harvested by scraping into Hanks balanced salt solution. Total lipids were extracted with chloroform and methanol, transmethylated with methanolic sulphuric acid and the proportions of individual fatty acids were measured by GC[(4)]. In order to determine the effect of EE_2 on FADS 1 and 2 mRNA expression, cells treated for 72 h with either zero or EE_2 (700 pM) and RNA was extracted using Trizol. cDNA was prepared and the levels of FADS 1 and FADS 2 mRNA were determined by real time RT-PCR[(5)]. Ct values were normalised to cyclophilin.

	17 α-Ethynylestradiol concentration (pM)								ANOVA (*P*)
	0		70		700		7000		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Proportion of fatty acids (g/100 g total fatty acids)									
18:3*n* − 3	10.8	1.6	12.1	1.6	11.3	1.0	10.0	0.8	0.12
20:5*n*3	1.5*	0.2	0.8†	0.2	1.5*	0.2	1.7*	0.1	<0.0001
22:6*n* − 3	1.9*	0.3	3.1†	0.7	4.0†	0.7	4.1†	0.6	<0.0001
mRNA expression ($\log_{10}$ ΔΔCt)									*t*-test (*P*)
FADS 1	0.22	0.19			0.68	0.17			0.014
FADS 2	0.38	0.13			0.78	0.37			0.09

Fatty acid concentrations were compared by one-way ANOVA with Bonferroni's *post-hoc* test. mRNA levels were compared by Student's unpaired *t*-test. *n* 5 per treatment.

Treatment with EE_2 did not alter 18:3*n* − 3 concentration significantly at any of the concentrations tested. 70 pM EE_2 decreased significantly the proportion of 20:5*n* − 5 compared with untreated cells, but there was no difference at the higher EE_2 concentrations. EE_2 increased the proportion of 22:6*n* − 3 significantly at all concentrations compared with untreated cells. 700 pM EE_2 induced a significant increase in FADS 1, and a trend towards an increase in FADS 2 mRNA expression. Together these findings suggest that oestrogen modifies 18:3*n* − 3 metabolism through a mechanism that involves altered transcription of FADS 1 and 2. One implication is that sex differences in PUFA metabolism involves differential regulation of transcription by sex hormones that may alter over the life course leading to age-related changes in PUFA status.

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