Supplemental Information

Dietary lysophosphatidylcholine-EPA enriches both EPA and DHA in the brain: Potential treatment for depression

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Supplemental Table S1: Plasma fatty acid composition (% of total, mean ± SD, n=6 per group)

Fatty acid	Control	Free- EPA	LPC-EPA
14:0	1.23 ±0.09	1.18 ± 0.03	1.27 ± 0.04
16:0	23.83 ±1.51	23.81 ± 0.79	25.08 ± 0.26
16:1(n-7)	2.17 ±0.65	1.26 ± 0.07**	1.35 ± 0.03**
18:0	7.44 ±0.73	7.47± 0.22	8.03 ± 0.06
18:1 (n-9)	18.91 ±1.11	16.05 ± 0.55****	17.07± 0.16**
18:1 (n-7)	2.23 ±0.34	1.73 ± 0.05**	1.87±0.03*
18:2 (n-6)	26.69 ± 0.82	25.07 ± 0.85**	26.74± 0.17 ##
18:3 (n-6)	0.04 ±0.02	0.03 ± 0.02	0.03±0.01
18:3 (n-3)	0.67 ± 0.22	1.22 ± 0.04***	1.28± 0.07***
20:0	0.24 ± 0.04	0.19 ± 0.09	1.36± 0.09
20:1 (n-9)	0.44 ± 0.10	0.47 ± 0.12	0.37± 0.03
20:2 (n-6)	0.52 ± 0.08	0.42 ± 0.06	0.56± 0.05
20:3 (n-6)	0.27 ± 0.19	0.13 ±0.17	0.33± 0.15
20:4 (n-6)	8.89 ± 0.95	4.32 ± 0.16****	4.60± 0.04****
20:5 (n-3)	0.15 ± 0.11	4.27 ± 0.27****	3.92± 0.37****
22:4 (n-6)	1.67 ± 0.21	1.48 ± 0.09	1.60± 0.04
22:5 (n-3)	0.53 ± 0.08	1.06 ± 0.06****	0.86± 0.11**** ##
22:6 (n-3)	2.65 ± 0.33	8.39 ± 0.31****	4.53± 0.18**** ####
16:0 DMA	0.05 ± 0.04	0.02 ± 0.01	0.07± 0.09
18:0 DMA	0.23 ± 0.02	1.19 ± 2.86	0.03± 0.01
18:1 DMA	0.04 ± 0.01	0.04 ± 0.02	0.08± 0.03
Total omega 3 FA (20:5+22:5+22:6)	3.33±0.18	13.72±0.21****	0.07±0.22**** ####

DMA: dimethylacetal

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

Supplemental Table S2: Brain fatty acid composition (% of total, mean ± SD, n=6 per group)

Fatty acid	Control	Free- EPA	LPC-EPA
14:0	0.28 ± 0.12	0.22 ±0.14	0.40 ± 0.19
16:0	24.32 ± 0.44	24.79 ± 0.22	20.58 ± 0.21**** ####
16:1(n-7)	0.54 ± 0.01	0.58 ± 0.02	0.49 ± 0.03
18:0	19.16 ± 0.15	19.38± 0.10	16.54 ± 0.21**** ####
18:1 (n-9)	16.97 ± 0.17	17.10 ± 0.22	15.31 ± 0.44**** ####
18:1 (n-7)	3.91 ± 0.13	4.09 ± 0.07*	3.40 ± 0.14**** ####
18:2 (n-6)	0.81 ± 0.03	0.82 ± 0.04	0.67 ± 0.05**** ####
18:3 (n-6)	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.03
18:3 (n-3)	0.12 ± 0.06	0.17 ± 0.04	0.13 ± 0.07
20:0	0.25 ± 0.10	0.27 ± 0.11	0.26 ± 0.10
20:1 (n-9)	2.05 ± 0.04	2.16 ± 0.04	2.01 ± 0.19
20:2 (n-6)	0.44 ± 0.02	0.34 ± 0.12	0.38 ± 0.18
20:3 (n-6)	0.40 ± 0.05	0.36 ± 0.06	0.31 ± 0.04
20:4 (n-6)	11.31 ± 0.22	10.06 ± 0.12****	6.70 ± 0.16**** ####
20:5 (n-3)	0.04 ± 0.02	0.56 ± 0.19**	4.14 ± 0.30**** ####
22:4 (n-6)	2.36 ± 0.06	2.39 ± 0.04	2.14 ± 0.12
22:5 (n-6)	0.81± 0.12	0.51 ± 0.05****	0.11 ± 0.05**** ####
22:5 (n-3)	0.09 ± 0.07	0.16 ± 0.11	1.17 ± 0.19**** ####
22:6 (n-3)	11.57 ± 0.18	11.93 ± 0.13	21.10 ± 1.24**** ####
16:0 DMA	1.53 ± 0.11	1.39 ± 0.10	1.53 ± 0.16
18:0 DMA	2.61 ± 0.21	2.26 ± 0.21	2.28 ± 0.24
18:1 DMA	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.04
Total omega 3 FA (20:5+22:5+22:6)	11.70 ± 0.21	12.65 ± 0.34****	26.42 ± 1.18**** ####

DMA: dimethylacetal

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

Supplemental Table S3: Liver fatty acid composition (% of total; mean ± SD; n=6 per group)

Fatty acid	Control	Free- EPA	LPC-EPA
14:0	0.50 ±0.05	0.19 ±0.20	0.08 ± 0.05
16:0	21.67±1.26	23.45 ± 0.36 **	22.23± 0.30 #
16:1(n-7)	2.43 ±0.64	3.11± 0.19 *	2.56 ± 0.11
18:0	3.64 ±0.78	4.65± 0.15 **	4.36 ± 0.14 *
18:1 (n-9)	20.64 ±2.45	24.09± 0.22 **	21.03± 0.22 ##
18:1 (n-7)	2.03 ±1.23	3.42± 0.13 *	2.87±0.15
18:2 (n-6)	27.29 ± 4.15	23.39± 0.58 *	22.48± 0.97 *
18:3 (n-6)	0.29 ±0.30	0.13 ± 0.15	0.20±0.19
18:3 (n-3)	2.61± 2.29	0.28 ± 0.33 *	0.29± 0.12 *
20:0	0.20 ± 0.07	0.39 ± 0.16	0.31± 0.15
20:1 (n-9)	0.56 ± 0.36	1.05 ± 0.25 *	0.86± 0.19
20:2 (n-6)	0.55 ± 0.15	0.49 ± 0.35	0.47± 0.09
20:3 (n-6)	1.06 ± 0.11	1.04 ±0.48	1.21± 0.22
20:4 (n-6)	12.67 ± 1.28	4.04 ± 0.17 ****	4.96± 0.26 ****
20:5 (n-3)	0.17 ± 0.17	2.85 ± 0.49 ****	5.65± 0.43 **** ####
22:4 (n-6)	0.35 ± 0.09	4.64 ± 0.20	0.44± 0.08
22:5 (n-3)	2.69± 0.09	2.13± 0.54 ****	3.64± 0.15 **** ####
22:6 (n-3)	2.74± 0.45	3.70 ± 0.24 ***	5.50± 0.19 **** ####
16:0 DMA	0.02 ± 0.01	0.11 ± 0.09	0.05± 0.04
18:0 DMA	0.08 ± 0.08	0.07 ± 0.06	0.08± 0.06
18:1 DMA	0.05 ± 0.03	0.10 ± 0.07	0.11± 0.08
Total omega 3 FA (20:5+22:5+22:6)	3.60±0.53	8.68±0.91***	14.78±0.66 **** ####

DMA: dimethylacetal

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

Supplemental Table S4: Fatty acid composition in heart lipids (% of total; mean ±SD; n=6 per group)

Fatty acid	Control	Free EPA		LPC-EPA	
14:0	1.18 ± 0.19	1.11 ± 0.10		1.13 ± 0.06	
16:0	22.44 ± 1.96	16.56 ± 0.27	****	16.39 ± 0.23	****
16:1(n-7)	2.64 ± 0.90	4.11 ± 0.14	***	4.19 ± 0.09	***
18:0	7.46 ± 1.29	3.94 ± 0.24	****	3.98 ± 0.12	****
18:1 (n-9)	19.33 ± 1.57	20.36 ± 0.18		20.24 ± 0.20	
18:1(n-7)	2.49 ± 0.42	3.55 ± 0.25	****	3.66 ± 0.09	****
18:2 (n-6)	24.68 ± 2.23	27.35 ± 0.34	**	26.94 ± 0.35	*
18:3 (n-6)	0.25 ± 0.05	0.19 ± 0.01		0.19 ± 0.01	
18:3 (n-3)	1.73 ± 0.61	3.73 ± 0.27	****	3.73 ± 0.11	****
20:0	0.40 ± 0.02	0.08 ± 0.01		0.08 ± 0.00	
20:1 (n-9)	0.51 ± 0.09	0.45 ± 0.09		0.37 ± 0.03	
20:2 (n-6)	0.12 ± 0.07	0.29 ± 0.25		0.54 ± 0.05	
20:3 (n-6)	0.40 ± 0.10	0.23 ± 0.04	***	0.23 ± 0.03	**
20:4 (n-6)	8.87 ± 1.24	4.59 ± 0.25	****	4.75 ± 0.32	****
22:0	0.03 ± 0.03	0.01 ± 0.00		0.01 ± 0.01	
20:5 (n-3)	0.085 ± 0.08	4.90 ± 0.70	****	4.95 ± 0.52	****
22:0	0.07 ± 0.05	0.01 ± 0.01		0.01 ± 0.01	
22:4 (n-6)	1.92 ± 0.81	0.35 ± 0.05	***	0.37 ± 0.03	***
22:5(n-3)	0.553 ± 0.23	0.96 ± 0.21		0.93 ± 0.15	
22:6 (n-3)	4.667 ± 0.49	7.14 ± 0.72	****	7.23 ± 0.29	****
24:1 (n-9)	0.05 ± 0.03	0.02 ± 0.01		0.02 ± 0.01	
Total omega 3 FA (20:5+22:5+22:6)	5.30 ± 0.67	13.00 ± 1.53	****	13.11 ± 0.86	****

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)

Note: There were no significant differences between free EPA and LPC-EPA

Supplemental Table S5: Adipose tissue (peri-gonadal) fatty acid composition (% of total; mean ± SD, n=6 per group)

Fatty acid	Control	Free- EPA	LPC-EPA
14:0	1.02± 0.08	1.17 ±0.11	1.11 ± 0.11
16:0	19.69± 0.27	19.04 ± 0.30	19.42± 0.16
16:1(n-7)	4.10 ±0.21	4.05± 0.58	3.70 ± 0.65
18:0	4.01 ±0.10	4.13± 0.29	4.40 ± 0.27 #
18:1 (n-9)	24.87 ±0.25	23.76± 0.34 ****	24.37± 0.06 * ##
18:1 (n-7)	3.54 ±0.10	3.17± 0.15 *	3.72±0.32 ##
18:2 (n-6)	34.18 ± 0.36	33.25± 0.76	34.54± 1.15 #
18:3 (n-6)	0.19 ±0.01	0.17 ± 0.01	0.20±0.13
18:3 (n-3)	3.63± 0.25	3.83 ± 0.28	4.21± 0.36
20:0	0.09 ± 0.01	0.08 ± 0.02	0.14± 0.12
20:1 (n-9)	0.38 ± 0.03	0.36 ± 0.03	0.44± 0.07
20:2 (n-6)	0.62 ± 0.07	0.32 ± 0.01	0.29± 0.10
20:3 (n-6)	0.26 ± 0.06	0.19 ±0.03	0.27± 0.02
20:4 (n-6)	1.71 ± 0.17	1.28 ± 0.13 *	1.48± 0.32
20:5 (n-3)	0.12 ± 0.13	3.70 ± 0.43 ****	0.15± 0.08 ####
22:4 (n-6)	0.38 ± 0.06	0.30 ± 0.14	0.41± 0.05
22:5 (n-3)	0.33± 0.06	0.25± 0.05	0.31± 0.06
22:6 (n-3)	0.62± 0.09	0.71 ± 0.27	0.62± 0.16
16:0 DMA	0.02 ± 0.01	0.12 ± 0.01	0.03± 0.02
18:0 DMA	0.05 ± 0.04	0.03 ± 0.02	0.02± 0.02
18:1 DMA	0.04 ± 0.03	0.03 ± 0.01	0.03± 0.02
Total omega 3 FA (20:5+22:5+22:6)	1.07±0.06	4.66±0.25****	1.08±0.10 ####

DMA: dimethylacetal

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

Supplemental Table S6: Erythrocytes fatty acid composition (% of total; mean ± SD; n=6 per group)

Fatty acid	Control	Free- EPA	LPC-EPA
14:0	2.71± 0.13	1.46 ±0.03****	1.36 ± 0.05****
16:0	33.16± 0.65	28.91 ± 0.43****	28.50± 0.88****
16:1(n-7)	1.53 ±0.03	1.22± 0.03****	1.88 ± 0.11**** ####
18:0	13.95 ±0.69	10.88± 0.32****	9.28 ± 0.45**** ###
18:1 (n-9)	13.53 ±0.47	14.17± 0.22**	17.76± 0.22**** ####
18:1 (n-7)	2.72 ±0.17	2.30± 0.13**	2.42±0.30
18:2 (n-6)	7.11 ± 0.72	12.30± 0.41****	20.34± 0.91**** ####
18:3 (n-6)	0.11 ±0.06	0.11 ± 0.04	0.08±0.04
18:3 (n-3)	0.20± 0.11	0.62 ± 0.05****	1.22± 0.08**** ####
20:0	0.24 ± 0.05	0.22 ± 0.07	0.18± 0.04
20:1 (n-9)	1.04 ± 0.14	0.76 ± 0.07	0.58± 0.23
20:2 (n-6)	0.45 ± 0.06	0.40 ± 0.04	0.37± 0.11
20:3 (n-6)	0.50 ± 0.12	0.43 ±0.04	0.41± 0.05
20:4 (n-6)	12.37 ± 0.55	7.80 ± 0.10****	6.73± 0.20**** ###
20:5 (n-3)	0.16 ± 0.12	8.60 ± 0.90****	0.86± 0.19 ####
22:4 (n-6)	3.06 ± 0.09	2.36 ± 0.07	2.03± 0.12
22:5 (n-3)	0.73± 0.06	0.82± 0.07	0.56± 0.12
22:5 (n-6)	0.82± 0.13	0.11± 0.08	0.10± 0.06** ###
22:6 (n-3)	4.29± 0.63	5.95 ± 0.36**	4.79± 0.90 #
16:0 DMA	0.63 ± 0.05	0.16 ± 0.04	0.08± 0.03
18:0 DMA	0.29 ± 0.02	0.07 ± 0.04	0.05± 0.04
18:1 DMA	0.09 ± 0.13	0.03 ± 0.02	0.08± 0.08
Total omega 3 FA (20:5+22:5+22:6)	5.28±0.29	14.66±0.45****	5.75±0.38####

DMA: dimethylacetal

*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

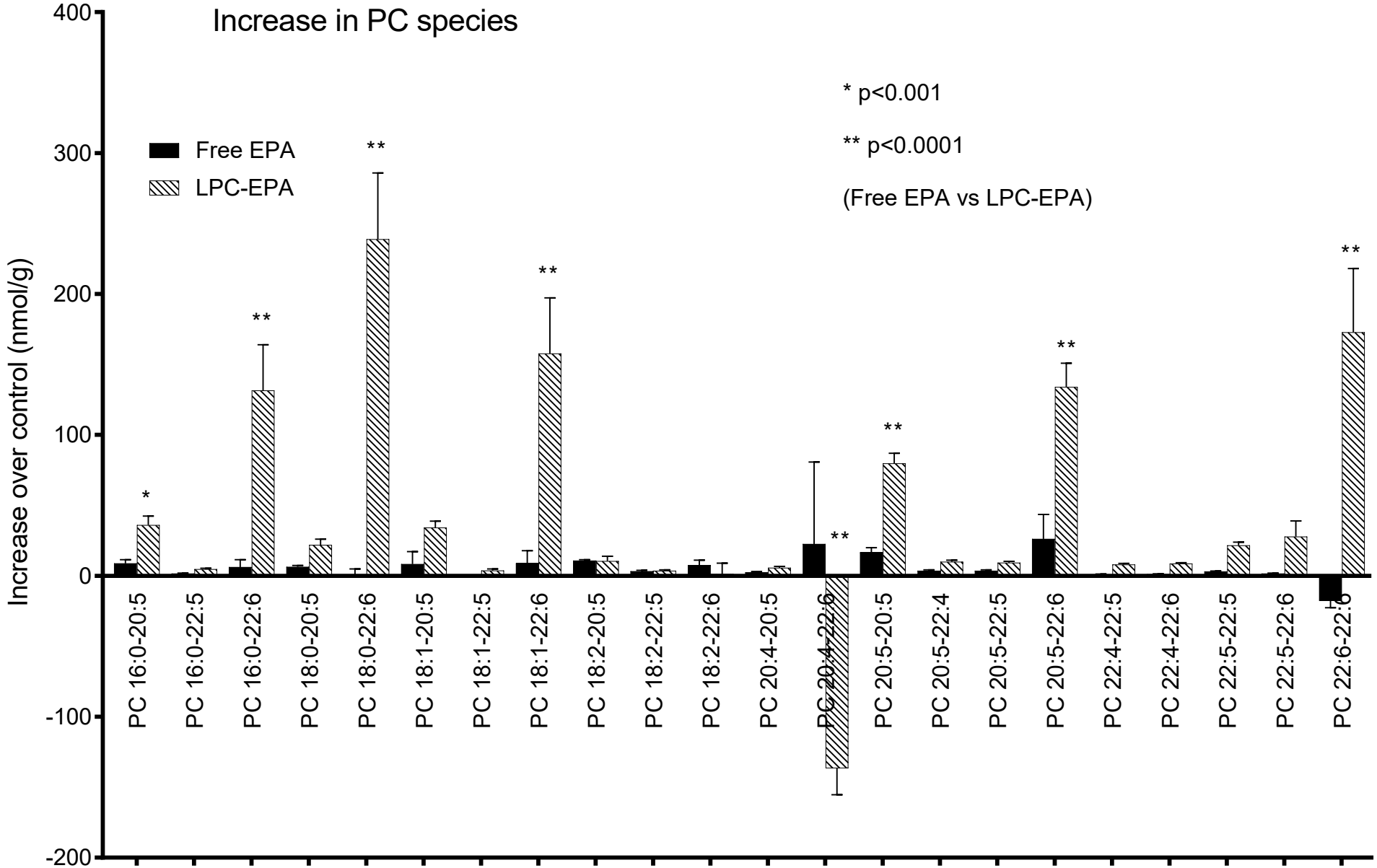
Supplemental Table S7. Retina FA composition

	Control	Free EPA	LPC-EPA
14:0	0.15 ± 0.05	0.17 ± 0.03	0.18 ± 0.08
16:0	18.83 ± 2.49	19.72 ± 1.25	14.89 ± 0.71* ###
16:1(n-7)	0.35 ± 0.13	0.46 ± 0.05	0.45 ± 0.03
18:0	18.45 ± 2.39	18.88 ± 1.45	14.58 ± 1.18* #
18:1 (n-9)	15.93 ± 3.15	16.77 ± 2.26	14.13 ± 2.12
18:1(n-7)	3.38 ± 0.51	3.90 ± 0.64	3.19 ± 0.44
18:2 (n-6)	0.69 ± 0.06	0.83 ± 0.10	0.62 ± 0.05
18:3 (n-6)	0.07 ± 0.05	0.05 ± 0.01	0.11 ± 0.04
18:3 (n-3)	0.06 ± 0.05	0.05 ± 0.01	0.06 ± 0.03
20:0	0.38 ± 0.15	0.39 ± 0.05	0.38 ± 0.11
20:1 (n-9)	1.60 ± 0.71	1.99 ± 0.80	2.33 ± 0.72
20:2 (n-6)	0.19 ± 0.09	0.15 ± 0.10	0.21 ± 0.08
20:3 (n-6)	0.41 ± 0.06	0.33 ± 0.04	0.32 ± 0.06
20:4 (n-6)	11.80 ± 1.09	10.20 ± 1.55	5.65 ± 0.45*** ##
20:5 (n-3)	0.14 ± 0.14	0.46 ± 0.28	5.07 ± 0.98*** ###
22:4 (n-6)	3.11 ± 0.53	2.61 ± 0.39	1.97 ± 0.19** #
22:5(n-6)	1.37 ± 0.22	0.73 ± 0.28	0.10 ± 0.05** ##
22:5 (n-3)	0.32 ± 0.19	0.40 ± 0.20	1.78 ± 0.55** ##
22:6 (n-3)	19.27 ± 1.45	18.79 ± 0.52	32.95 ± 3.63** ###
16:0 DMA	0.22 ± 0.16	0.13 ± 0.07	0.40 ± 0.14
18:0 DMA	0.04 ± 0.04	0.08 ± 0.05	0.26 ± 0.12
18:1 DMA	0.07 ± 0.02	0.06 ± 0.03	0.13 ± 0.15
20:5+22:5+22:6	19.73 ± 1.34	19.65 ± 0.80	39.81 ± 4.67*** ###

DMA: dimethylacetal

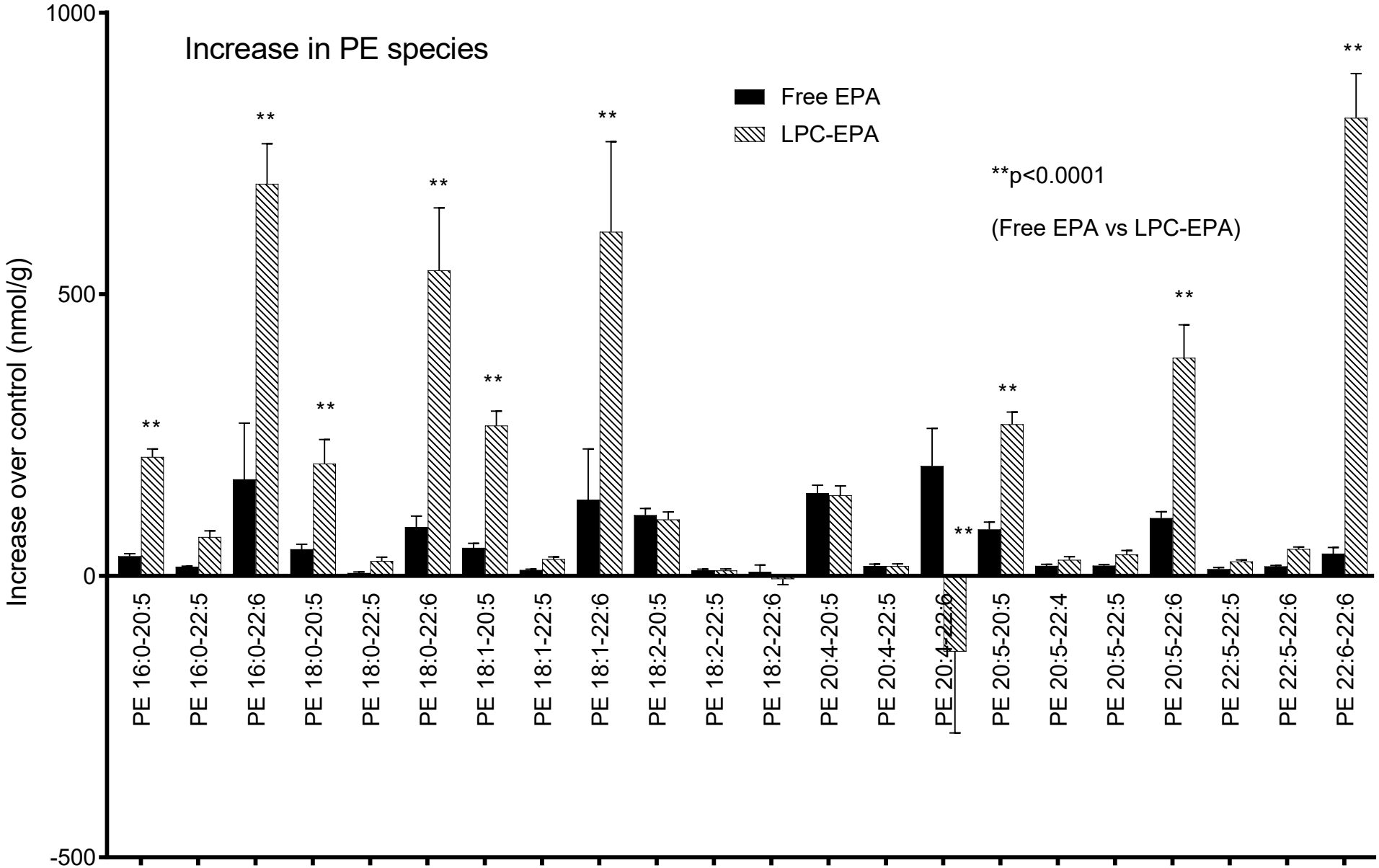
*P< 0.05, **p<0.01; ***p<0.001; ****p<0.0001 compared to control (ANOVA)
# p<0.05; ## p<0.01; ### p<0.001; #### p<0.0001, compared to free EPA (ANOVA)

Supplemental Figure S1.



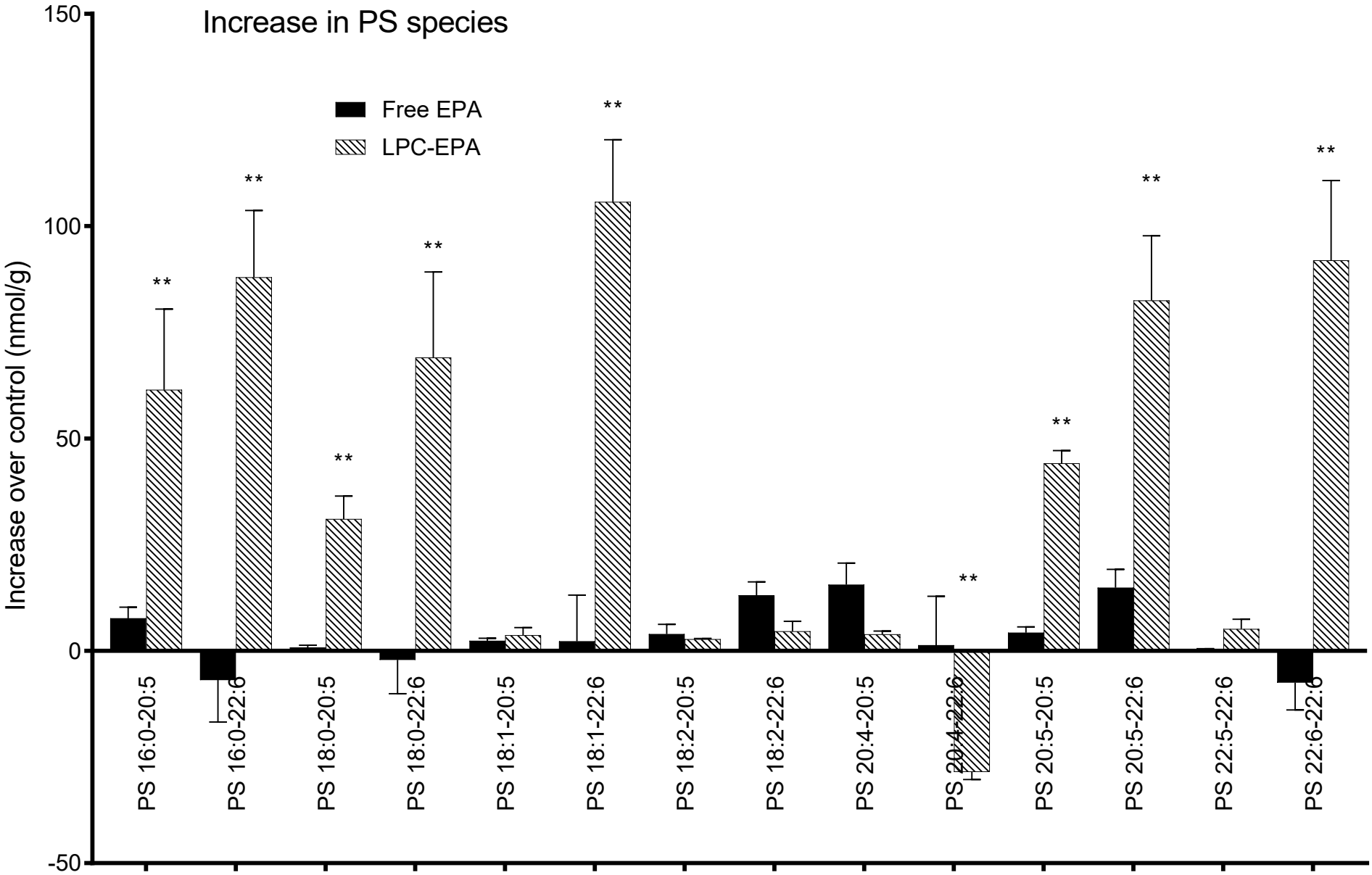
The molecular species composition of omega 3 fatty acid-containing diacyl PCs was determined by LC/MS/MS, as described in the text. The increase of each species above the No-EPA control mean was then calculated. The results shown are mean +/- SD of 6 samples. Statistical significance determined by unpaired t test with Welch's correction (Graphpad Prism)

Supplemental Figure S2



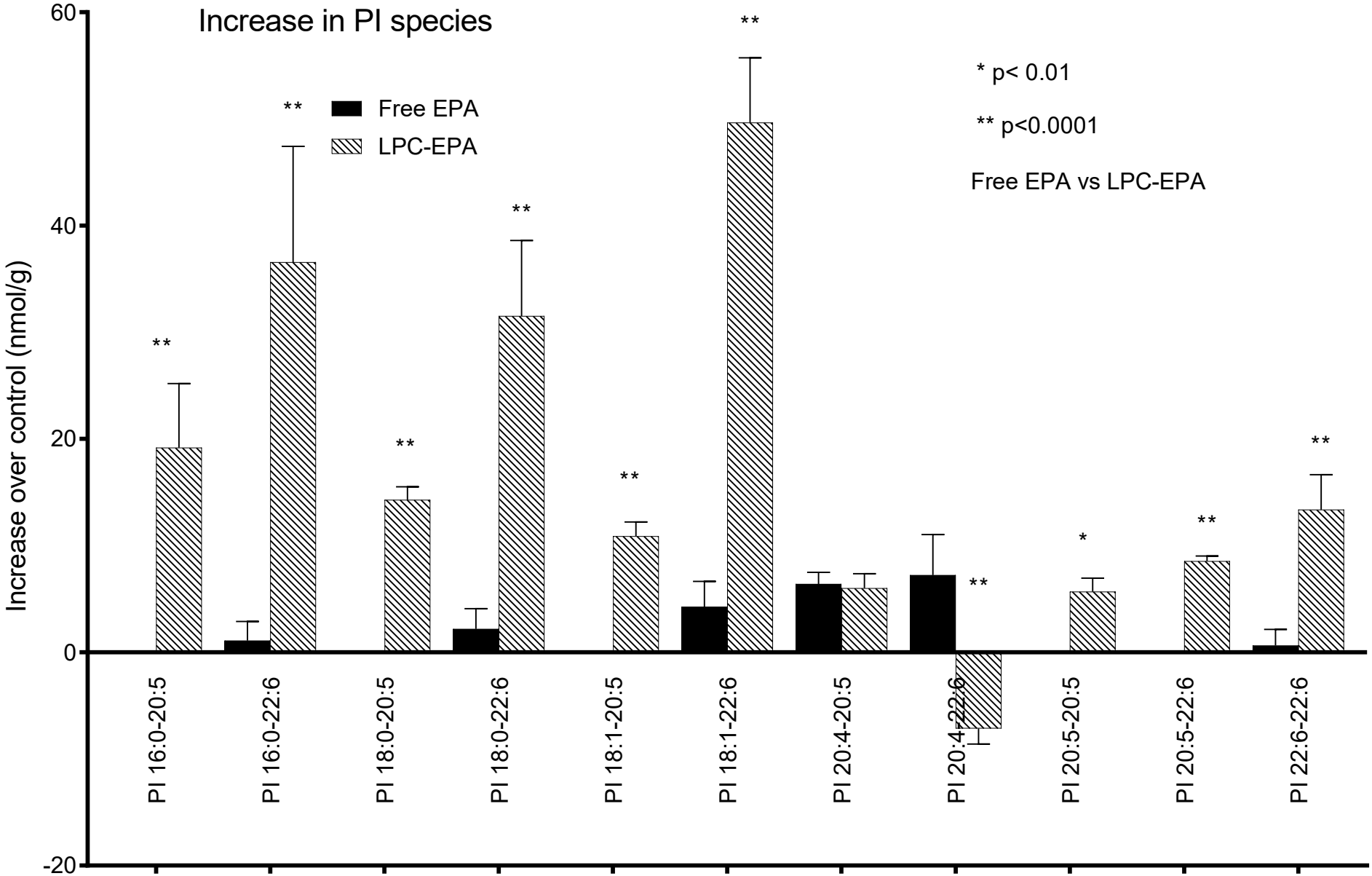
The molecular species composition of omega 3 fatty acid-containing diacyl PEs was determined by LC/MS/MS, as described in the text. The increase of each species above the mean of No-EPA controls was then calculated. The results shown are mean +/- SD of 6 samples. Statistical significance determined by unpaired t test with Welch's correction (Graphpad Prism)

Supplemental Figure S3



The molecular species composition of omega 3 fatty acid-containing diacyl PS was determined by LC/MS/MS, as described in the text. The increase of each species above the mean of No-EPA controls was then calculated. The results shown are mean +/- SD of 6 samples. Statistical significance determined by unpaired t test with Welch's correction (Graphpad Prism)

Supplemental Figure S4



The molecular species composition of omega 3 fatty acid-containing diacyl PIs was determined by LC/MS/MS, as described in the text. The increase of each species above the mean of No-EPA controls was then calculated. The results shown are mean +/- SD of 6 samples. Statistical significance determined by unpaired t test with Welch's correction (Graphpad Prism)