The exogenous natural phospholipids, EPA-PC and EPA-PE, contributes to ameliorate inflammation and promote macrophages polarization

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Methods

1.1 Preparation and analysis of EPA-PC

Phospholipids enriched with EPA was extracted from sea cucumber *(Cucumaria frondosa*, Nanshan Aquatic Market, Qingdao, China). Briefly, sea cucumber were ground into powder after vacuum freeze-drying. Then, the powder was extracted with a 20-fold volume of chloroform–methanol solution (2:1, v/v) overnight. The extracted solution was mixed with a one-fourth volume of water after filtration. The mixture was placed into a separating funnel and maintained for 24 h; then, the chloroform layer containing the total lipids was collected and evaporated to dryness under vacuum. Then, phospholipids enriched with EPA were separated from the total lipids by silica-gel column chromatography using chloroform, acetone, chloroform/methanol (9:1, v/v), chloroform/methanol (2:1, v/v) and methanol sequentially as eluents. The chloroform/methanol (2:1, v/v) eluent and methanol eluent were collected; then, EPA-PL were obtained after removal of the organic solvents under vacuum. EPA-PC were purified from EPA-PL respectively by silica-gel column chromatography. The fatty acid composition of EPA-PC was determined using an Agilent 6890 Gas Chromatograph with a flame-ionization detector. The column was a HPINNOW-AX capillary column (30 m × 0.32 mm × 0.25 m). The temperature of the detector and injector were kept constant at 250 °C and 240 °C, respectively, and the oven temperature was increased from 170 °C to 240 °C at 3 °C min^{-1} and maintained at 240 °C for 15 min. Nitrogen was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. EPA-PC contained EPA about 48.8%.

The lipid analysis of EPA-PC and EPA-PE was performed by RPLC-Q Exactive-MS/MS system (Thermo Fisher, Waltham, MA, USA). An Acquity UPLC BEH C18 column

(2.1mm×100mm, 1.7 μm) (Waters, MA, USA) was applied. Mobile phase A consisted of acetonitrile/water (60:40, v/v) with 5 mmol ammonium formate and 0.2% ammonia; mobile phase B was isopropanol/acetonitrile (90:10, v/v). The elution gradient was set as follows: 25% B for 2 min; 25% to 50% B for 6 min; 50% to 90% B for 5 min; 90% to 99% B for 6 min and keeping the condition (99% B) for 6 min; 99% to 25% B for 1 min and keep the condition (25% B) for 5 min. The flow rate was set at 0.35 mL/min; the column oven was maintained at 45°C. An aliquot of 5 μL was injected into the RPLC-Q Exactive-MS/MS system. Full Scan-ddMS2 positive-ion mode was applied with HESI source; source temperature of 300°C; Ion transfer tube temperature of 3.5 kV; Sheath gas flow of 38 arbitrary units; Auxiliary gas flow of 10 arbitrary units; a range of m/z 200-1000 for MS scans with a resolution of 35000, and a range of m/z 100-700 for MS/MS scans with a resolution of 17500. The lipid analyses revealed the peaks of PC (16:0/20:5, $[M+HCOO]^+$ = 824.56; 18:0/20:5; $[M+HCOO]^+$ = 852.56), and PE (16:0/20:5, $[M-H]^+$ = 736.50; 18:0/20:5; $[M+HCOO]^+$ = 764.50).

Table S1 The list of qPCR primer sequences used in this publication.

Gene	Forward or Reverse	sequences
IL-6	Forward primer (5’-3’)	AACGATGATGCACTTGCAGA
	Reverse primer (5’-3’)	GAGCATTGGAAATTGGGGTA
TNFα	Forward primer (5’-3’)	TCCCCAAAGGGATGAGAAGTTC
	Reverse primer (5’-3’)	TCATACCAGGGTTTGAGCTCAG
IL-10	Forward primer (5’-3’)	GATTTTAATAAGCTCCAAGACCAAGGT
	Reverse primer (5’-3’)	CTTCTATGCAGTTGATGAAGATGTCAA
IL-1β	Forward primer (5’-3’)	GCAACTGTTCCTGAACTCAACT
	Reverse primer (5’-3’)	ATCTTTTGGGGTCCGTCAACT
36B4	Forward primer (5’-3’)	CGTCCTCGTTGGAGTGACA
	Reverse primer (5’-3’)	CGGTGCGTCAGGGATTG
Tgf-β	Forward primer (5’-3’)	CTTCAATACGTCAGACATTCGGG
	Reverse primer (5’-3’)	GTAACGCCAGGAATTGTTGCTA
Mcp1	Forward primer (5’-3’)	GGCTCAGCCAGATGCAGTTAAC
	Reverse primer (5’-3’)	AGCCTACTCATTGGGATCATCTTG
Arg1	Forward primer (5’-3’)	GCTGGTCTGCTGGAAAAACTT
	Reverse primer (5’-3’)	CCGTGGGTTCTTCACAATTT
iNOS	Forward primer (5’-3’)	TCCTGTTGTTTCTATTCCTTTGTT
	Reverse primer (5’-3’)	CATCAACCAGTATTATDDCTCCT

Table S2 The tissue weight in HFSD-fed mice.

	Chow diet	HFSD	EPA-PC	EPA-PE
Liver weight (g)	0.81 ± 0.08	1.43 ± 0.21$^{\#\#}$	0.98 ± 0.11$^{\#}$	0.86 ± 0.09$^{\#}$
iWAT weight (g)	0.569 ± 0.10	1.36 ± 0.14$^{\#\#}$	1.12 ± 0.24	1.24 ± 0.18

$^{\#\#}$ P <0.01, $^{\#}$ P <0.05, vs chow diet mice.

*P <0.05, vs HFSD-fed mice.

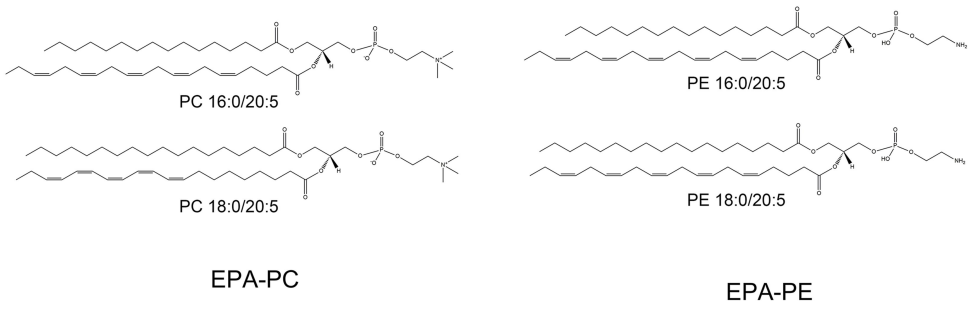

Fig. S1 The structure of EPA-PC and EPA-PE.