Shrimp oil extracted from shrimp processing by-product is a rich source of omega-3 fatty acids and astaxanthin-esters, and reveals potential anti-adipogenic effects in 3T3-L1 adipocytes

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Supplementary data

Supplementary S1
Vehicle controls had no significant effect on the mRNA expression of *Pparγ, Srebp1c, Dgat2, Fasn, Scd1* and *Glut-4*, compared to the untreated cells.

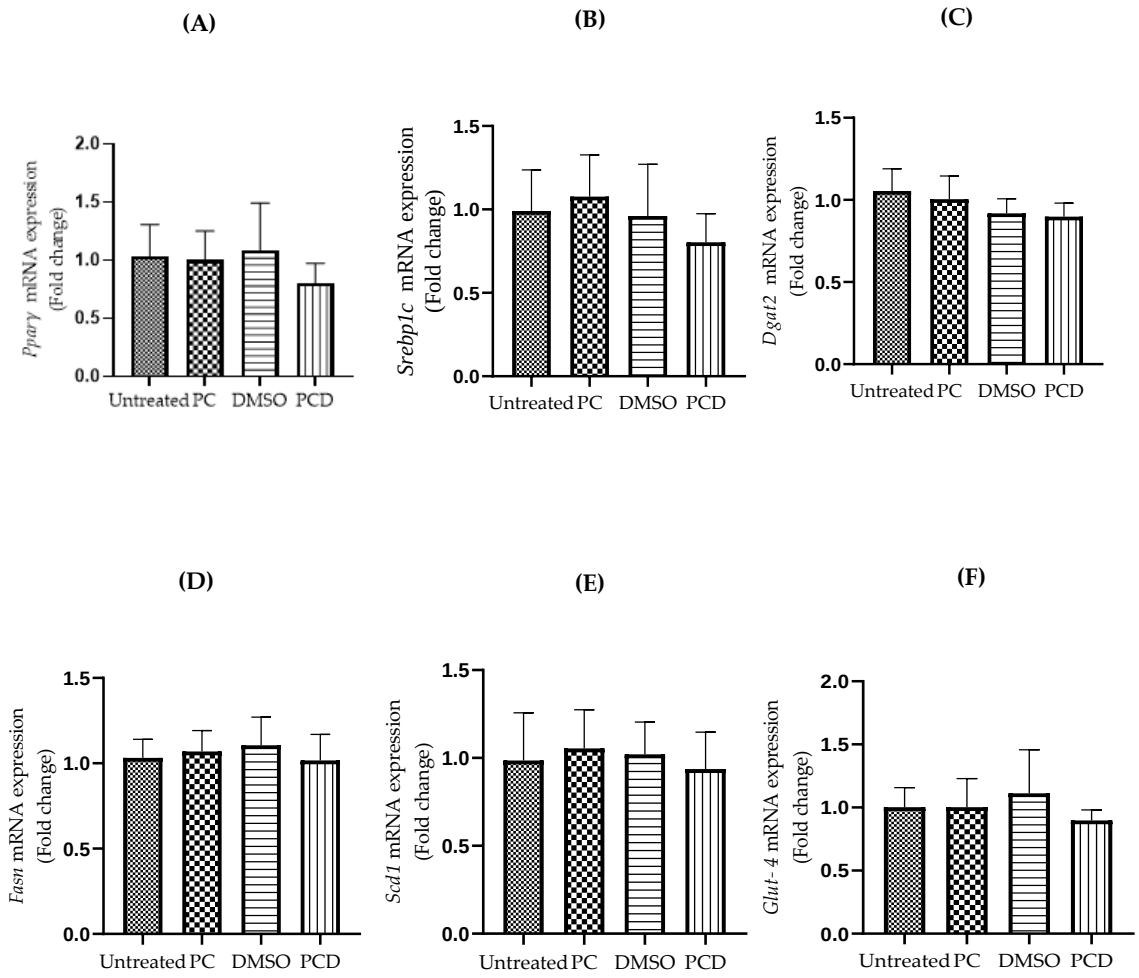

Figure S1. The cells were differentiated to mature adipocytes, for 8 days, as explained in the methods section. Total RNA was extracted, and the mRNA expression analysis of (**A**) Peroxisome proliferator-activated receptor (*Pparγ*), (**B**) sterol regulatory element-binding protein (*Srebp1c*), (**C**) diacylglycerol O-acyltransferase 2 (*Dgat2*), (**D**) Fatty acid synthase (*Fasn*), (**E**) Stearoyl-CoA desaturase-1 (*Scd1*), and (**F**) Glucose transporter type 4 (*Glut-4*) genes was performed. Expression of target genes was normalized to RPLP0 as the reference gene, and data were expressed as fold change. Vehicles: PC, DMSO, PCD. Data were analyzed using one-way ANOVA and Tukey's post-hoc test. $p<0.05$ was considered significant. n=3. Untreated=Untreated cells, PC=L-α-phosphatidylcholine (30 µg/mL culture medium), DMSO=dimethyl sulfoxide (0.06 %), PCD=PC+DMSO [(30 µg + 0.06 %)/mL culture medium].

Supplementary S2
Schematic of shrimp processing by-product extraction to prepare shrimp extract and shrimp oil

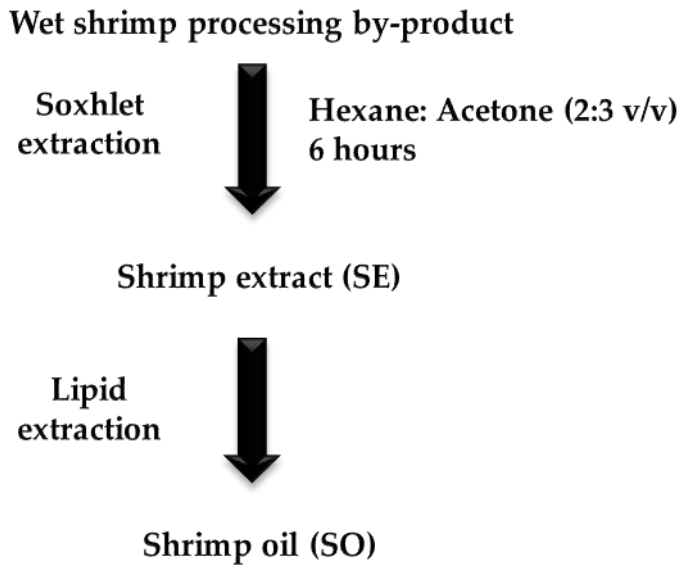

Figure S2. Schematic of shrimp processing by-product extraction to prepare shrimp extract and shrimp oil

Supplementary S3
Particle size analysis of oil emulsions

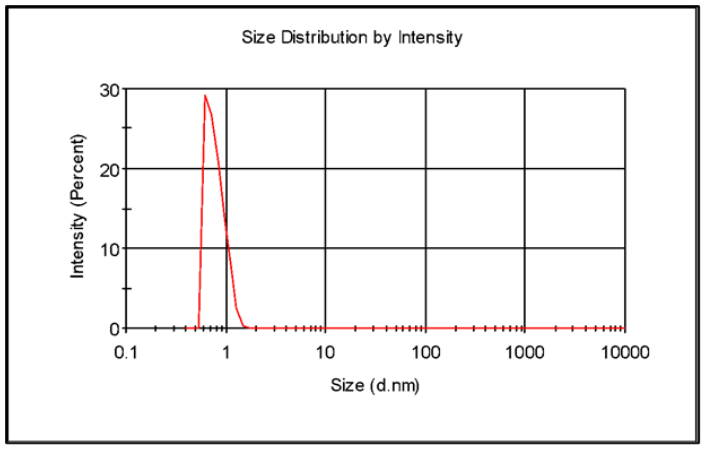

Figure S3. Analysis of particle size distribution of oil emulsions using dynamic light scattering (DLS). DLS measurements were carried out using a Zetasizer 1000Hs/3000Hs (Malvern Instruments, Worcestershire, U.K.) as described in the methods section. The data were expressed as the z-average (d. nm) and polydispersity index (PDI).

Supplementary S4
Effect of fish oil on adipogenesis in 3T3-L1 adipocytes

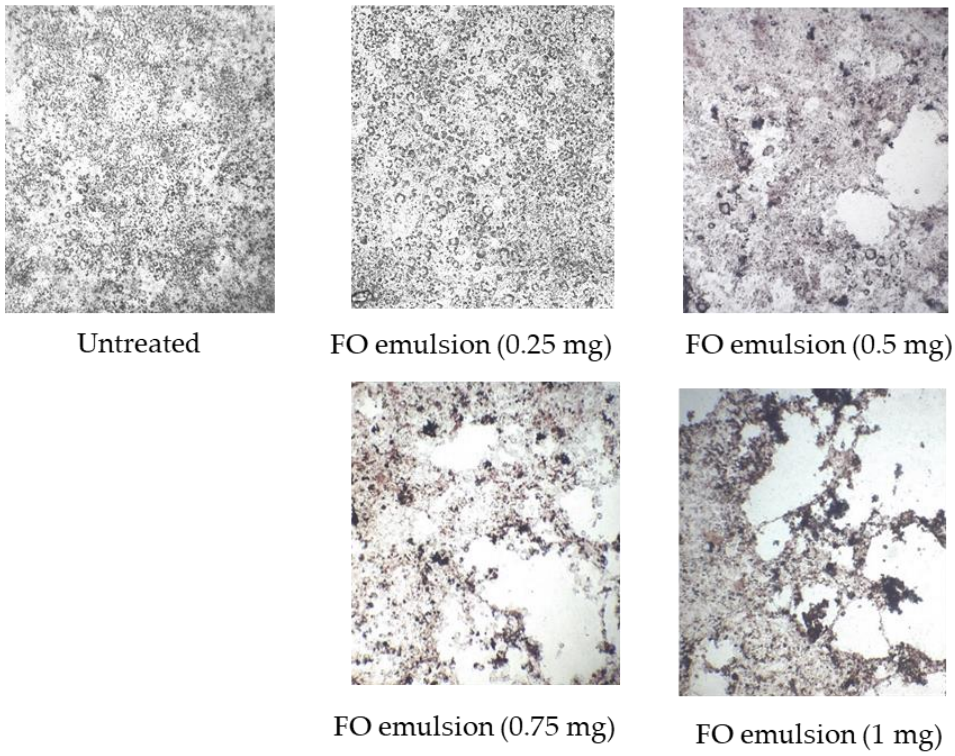

Figure S4. Preadipocytes were differentiated to mature adipocytes in the presence or absence of different concentrations of fish oil emulsions (0.25, 0.5, 0.75 and 1 mg/mL culture medium), for 8 days, as explained in the methods section. On day 8, the cells were viewed using a Leica DMIL LED Microscope at 40x magnification, and Infinity Camera Analyze Software (version 6.5.5) was used for capturing the images. Untreated=Untreated cells, FO=Fish oil.

Supplementary S5
Effect of lipid emulsions on the cell metabolic activity of 3T3-L1 preadipocytes

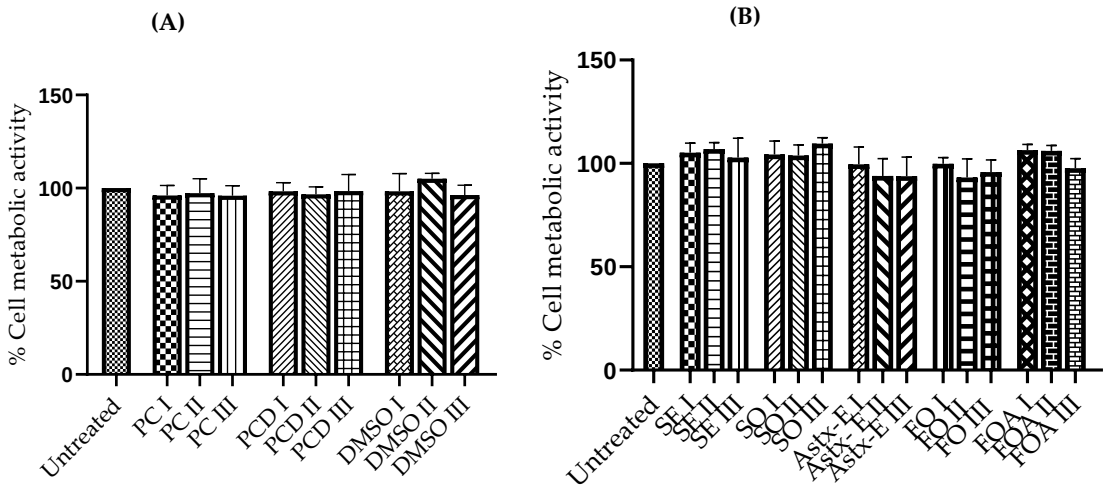

Figure S5. Cell metabolic activity of 3T3-L1 preadipocytes treated with various concentrations of treatments and vehicles. Cells were treated with various concentrations of treatments, and the vehicles, as explained in the methods section. (**A**) Vehicles: PC, DMSO, PCD; (**B**) Treatments: SE, SO, Astx-E, FO, and FO+Astx-E. Data were analyzed using one-way ANOVA to determine significance ($P<0.05$). Values are expressed as means ± SD, n=3. Untreated=Untreated cells, PC=L-α-phosphatidylcholine (I, II, III represents 15, 30, and 60 μg/mL culture medium, respectively), PCD=PC+DMSO [(I, II, III represents 15 μg + 0.03%, 30 μg + 0.06%, 60 μg + 0.1%)/mL culture medium, respectively], DMSO=dimethyl sulfoxide (I, II, III represents 0.03, 0.06, and 0.1%, respectively), SE=shrimp extract (I, II, III represents 0.125, 0.25, and 0.5 mg/mL culture medium, respectively), SO=shrimp oil (I, II, III represents 0.125, 0.25, 0.5 mg/mL culture medium, respectively), Astx-E=esterified astaxanthin (I, II, III represents 7.95, 15.9, and 31.8 ng/mL culture medium, respectively), FO=fish oil (I, II, III represents 0.125, 0.25, and 0.5 mg/mL culture medium, respectively), FOA= FO+Astx-E [(I, II, III represents 0.125 mg + 7.95 ng, 0.25 mg + 15.9 ng, 0.5 mg + 31.8 ng)/mL culture medium, respectively].

Supplementary S6
Primer sequences for Real-time quantitative polymerase chain reaction

Table S1. Primer sequences for Real-time quantitative polymerase chain reaction

Gene	Sequence		Ascension No
Pparγ	5′-GAGCTGACCCAATGGTTGCTG-3′	Forward	XM_017321456.1
	5′-GCTTCAATCGGATGGTTCTTC-3′	Reverse	
Srebp1c	5′-CGGCTCTGGAACAGACACTG-3′	Forward	NM_001313979.1
	5′-TGAGCTGGAGCATGTCTTCG-3′	Reverse	
Scd1	5′-CACCTGCCTCTTCGGGATTT-3′	Forward	NM_009127.4
	5′-CTTGACAGCCGGGTGTTTG-3′	Reverse	
Dgat2	5′-CTGCTGTTGGCTGGTTTCAC-3′	Forward	NM_026384.3
	5′-CAGGAGGATATGCGCCAGAG-3′	Reverse	
Fasn	5′-CTGCGGAAACTTCAGGAAATG-3′	Forward	NM_007988.3
	5′-GGTTCGGAATGCTATCCAGG-3′	Reverse	
Glut-4	5′-GATTCTGCTGCCCTTCTGTC-3′	Forward	AB_008453.1
	5′-ATTGGACGCTCTCTCTCCAA-3′	Reverse	
Rplp0	5′-AATTTCAATGGTGCCTCTGG-3′	Forward	NM_007475.5
	5′-TCACTGTGCCAGCTCAGAAC-3′	Reverse	

Primers used in qPCR were designed using NCBI primer blast and purchased from IDT Technologies. Pparγ: peroxisome proliferator-activated receptor-gamma; Srebp1c: sterol regulatory element-binding protein 1; Scd1: stearoyl-CoA desaturase 1; Dgat2: diacylglycerol O-acyltransferase 2; Fasn: fatty acid synthase; Glut-4: Glucose transporter type 4; Rplp0: ribosomal protein large.