Supplementary information

Polysaccharides from *Enteromorpha prolifera* alleviate hypercholesterolemia via modulating gut microbiota and bile acid metabolism

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Figure list

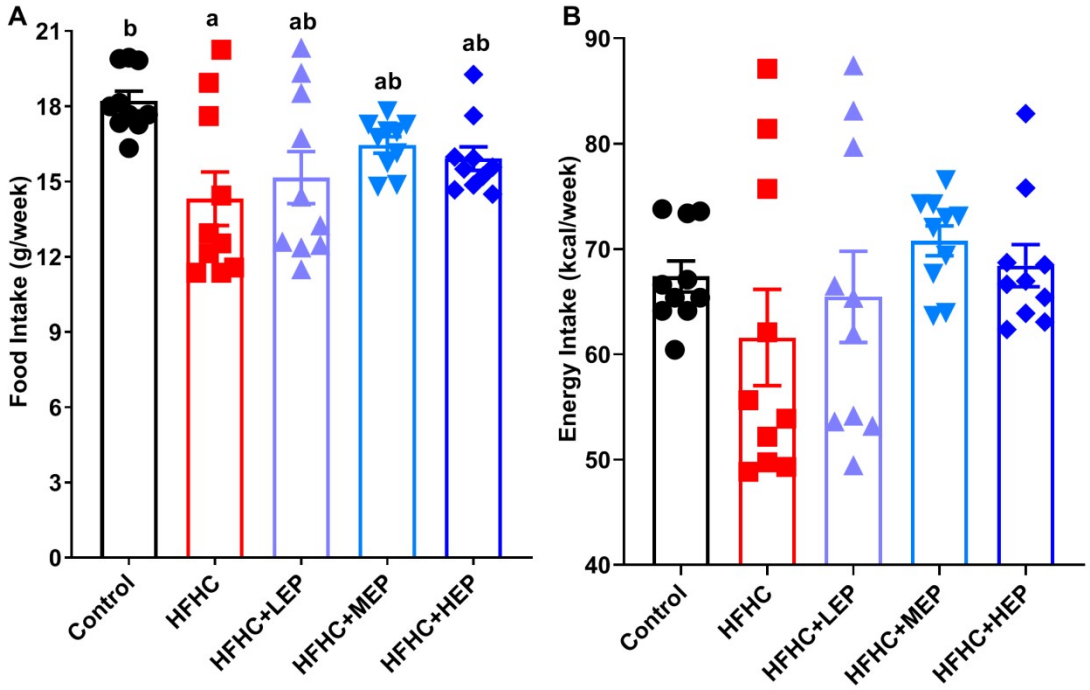

Fig.S1 Effect of EP on energy intake in HFHC-diet fed mice. (A) Food intake. (B) Energy intake. The average weekly energy intake of the Control group was calculated by the weekly food intake (g) × 3.7 (kcal/g, the energy density of the basal diet). Accordingly, the weekly energy intakes of HFHC, HFHC+LEP, HFHC+MEP, and HFHC+MEP groups were calculated by the weekly food intake (g) × 4.3 (kcal/g, the energy density of the HFHC diet). EP, Polysaccharides from *Enteromorpha prolifera*; HFHC, high-fat and high-cholesterol diet; HFHC+LEP, high-fat and high-cholesterol diet with low dose EP; HFHC+MEP, high-fat and high-cholesterol diet with middle dose EP; HFHC+HEP, high-fat and high-cholesterol diet with high dose EP. Data are shown in means ± SEM (n = 10). a, b: Columns not sharing a common letter are significantly different ($P < 0.05$).

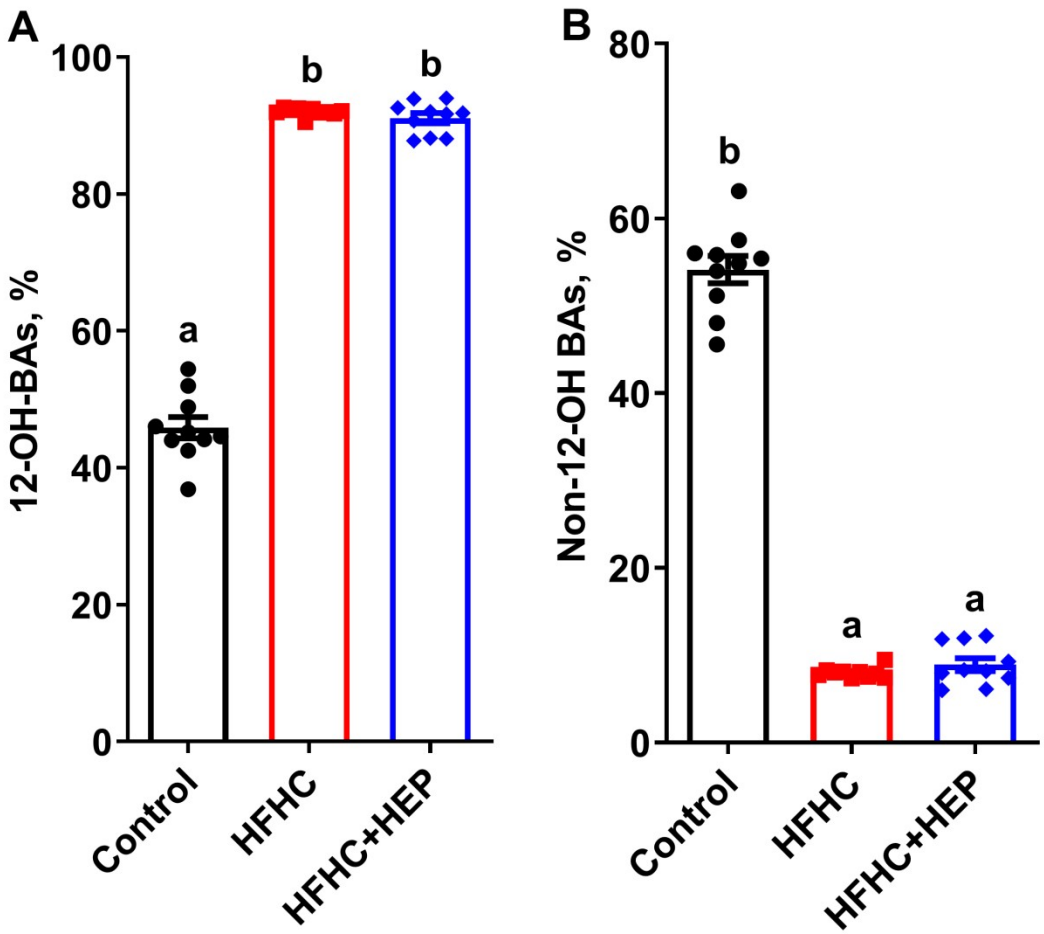

Fig. S2 EP induced changes of fecal bile acids in mice. (A) 12-hydroxylated bile acids (12-OH BAs) proportion. (B) Non-12-hydroxylated bile acids (Non-12-OH BAs) proportion. BAs, bile acids; EP, polysaccharides from *Enteromorpha prolifera*. Data are shown in means ± SEM (n = 10). a, b: Columns not sharing a common letter are significantly different ($P < 0.05$).

Table S1 Primers of genes analyzed by real-time PCR

Gene	Primer Sequence (5’ – 3’)
β-ACTIN	F: CATCCGTAAAGACCTCTATGCCAAC
	R: ATGGAGCCACCGATCCACA
SREBP2	F: CCCACTCAGAACACCAAGCAT
	R: TGGCAGTAGCTCGCTCTCGT
HMGCR	F: TCGTTAGCACTGGTCCAGGAA
	R: GGCTCCATCATTGGCTCTGTA
GPBAR1	F：TGCCCAAAGGTGTCTACGAGTG
	R：GCTGCATTGGCTACTGGTGTG
LDLR	F: TGACCTTCATCCCAGAGCCTTC
	R: GGCATGAGCGGGTATCCATC
APOA1	F: AATGTGTATGTGGATGCGGTCAA
	R: AGCCGTTCCTGCAGCTGACTA
APOB	F: CAGGTGGCCACAGCCAATAA
	R: ACTGCAGGTCTGGCTCAGGA
CYP7A1	F: ACCTCCGGGCCTTCCTAAA
	R: TCAAACATCACTCGGTAGCAGAA
CYP27A1	F: GGACAACCTCCTTTGGGACTTAC
	R: TGCCCTCCTGTCTCATCACTT
NR1H4（FXR）	F: TCTGCTCACAGCGATCGTCA
	R: AAATGCTGCGGGTTCTCAGG

Table S2 List of primary antibodies

Antibody	Host	Dilution	Company	Catalog no.
HMGCR	Rabbit	1:2000	ABclonal Technology, Wuhan, China	A19063
LDLR	Rabbit	1:2000	ABclonal Technology, Wuhan, China	A20808
SREBP-2	Rabbit	1:2000	Proteintech Group, Wuhan, China	28212-1-AP
CYP27A1	Rabbit	1:1000	Proteintech Group, Wuhan, China	14739-1-AP
			Affinity	
CYP7A1	Rabbit	1:2000	Biosciences,Changzhou,China	DF2612
GAPDH	Rabbit	1:10000	ABclonal Technology, Wuhan, China	A19056
HRP Goat	Rabbit	1:10000	ABclonal Technology, Wuhan, China	AS014

Table S3 Bile acid content in the feces of Control, HFHC and HFHC+HEP mice (nmol/L)

Class	Full name	Abbreviation	Control	HFHC	HFHC+HEP
Unconjugated	Dehydrolithocholic acid	DHLCA	4.16±0.30	2.54±0.32↓***	2.46±0.54
Unconjugated	Allolithocholic acid	alloLCA	4.26±0.41	2.97±0.31↓***	3.10±0.40
Unconjugated	Isolithocholic acid	isoDCA	4.05±0.29	2.96±0.41↓***	2.93±0.64
Unconjugated	Lithocholic acid	LCA	5.12±0.26	4.69±0.29↓*	4.56±0.58
Unconjugated	23-Nordeoxycholic acid	23-NorDCA	2.41±0.61	2.44±0.21	2.27±0.49
Unconjugated	7-Ketolithocholic acid	7-ketoDCA	3.59±0.12	3.72±0.21	3.85±0.14
Unconjugated	12-Ketolithocholic acid	12-ketoLCA	5.41±0.25	5.53±0.28	5.35±0.64
Unconjugated	Apocholic acid	apoCA	3.99±0.16	4.24±0.21↑*	4.34±0.23
Unconjugated	Ursodeoxycholic acid	UDCA	4.78±0.34	4.25±0.26↓***	4.49±0.11↑#
Unconjugated	Hyodeoxycholic acid	HDCA	5.06±0.25	4.65±0.24↓**	4.63±0.35
Unconjugated	Chenodeoxycholic acid	CDCA	3.90±0.26	4.42±0.27↑***	4.55±0.11
Unconjugated	Deoxycholic acid	DCA	5.70±0.41	6.77±0.20↑***	6.48±0.66
Unconjugated	Dehydrocholic acid	DHCA	2.90±0.69	3.07±0.76	2.92±0.47
Unconjugated	7,12-Diketolithocholic acid	7,12-diketoLCA	3.27±0.36	4.20±0.41↑***	4.32±0.31
Unconjugated	6,7-Diketolithocholic acid	6,7-diketoLCA	3.23±0.96	2.42±0.72↓*	2.43±0.27
Unconjugated	7-Ketodeoxycholic acid	7-ketoDCA	5.28±0.19	6.08±0.38↑***	6.12±0.15
Unconjugated	12-Dehydrocholic acid	12-DHCA	3.96±0.14	5.30±0.35↑***	5.40±0.30
Unconjugated	3-Dehydrocholic acid	3-DHCA	3.58±0.27	5.19±0.33↑***	5.29±0.33
Unconjugated	Ursocholic acid	UCA	3.82±0.37	4.94±0.25↑***	5.83±0.74↓###
Unconjugated	α-Muricholic acid	α-MCA	5.46±0.35	5.73±0.17↑*	5.82±0.13
Unconjugated	β-Muriholic acid	β-MCA	4.98±0.27	5.18±0.29	5.34±0.18
Unconjugated	λ-Muricholic acid	λ-MCA	4.05±0.42	4.04±0.18	4.41±0.24↑#
Unconjugated	Allocholic acid	ACA	4.48±0.82	5.40±0.14↑***	5.32±0.13
Unconjugated	Cholic acid	CA	4.77±0.26	6.82±0.32↑***	6.86±0.19
Conjugated	Glycolithocholic acid	GLCA	2.35±0.55	3.90±0.31↑***	3.25±0.74↓#
Conjugated	Glycocholic acid	GCA	2.33±0.69	4.33±0.53↑***	3.94±0.51
Conjugated	Taurolithocholic acid	TLCA	2.31±0.62	3.02±0.60↑*	2.51±0.65
Conjugated	Tauroursodeoxycholic acid	TUDCA	3.25±0.40	3.79±0.71	3.87±0.62
Conjugated	Taurohyodeoxycholic acid	THDCA	3.20±0.31	3.75±0.65↑*	3.41±0.68
Conjugated	Taurochenodeoxycholic acid	TCDCA	2.95±0.42	3.67±0.77↑*	3.51±0.68
Conjugated	Taurodeoxycholic acid	TDCA	3.58±0.34	5.61±0.58↑***	5.05±0.90
Conjugated	Tauro α-Muricholic acid	T-α-MCA	3.88±0.35	4.84±0.72↑**	4.71±0.58
Conjugated	Tauro β-Muricholic acid	T-β-MCA	4.18±0.36	4.91±0.82↑*	4.88±0.67
Conjugated	Taurocholic acid	TCA	4.42±0.27	6.19±0.75↑***	6.00±0.69

* Means the difference between Control group and HFHC group; # Means the difference between HFHC and HFHC+HEP group. Data are shown in means ± SEM (n = 10). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$.

Method S1. Bile acids quantification

Briefly, fecal samples (25mg) were weighed and reconstituted with 1000 μL of a mixture of MeOH: MeCN: H_2O at a volume ratio of 2:2:1 (v/v/v). For homogenization, samples were vortexed for 30s, grounded at 35Hz for 4 minutes, and ultra-sonicated for 5 minutes in an ice-water bath. Samples were centrifuged after 1 hour of incubation at -40°C. The supernatant was collected and injected for UHPLC-MS/MS analysis. A Q Exactive Focus mass spectrometer coupled with UPLC (Agilent Technologies, Palo Alto, CA, USA) was used and operated in Parallel Reaction Monitoring (PRM) mode.

Peak signal intensity, chromatographic peak shape, and retention time stability were monitored. Linearity (linear correlation coefficient) ranged between 0.9926 – 1 (Table S2). Limits of detection were calculated based on calibration curves for spiked samples at a concentration of maximum signal to noise of 3. The limit of quantification was determined as the lowest standard in the linear calibration curve and the recovery rate was 105.6% - 233.8%.