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Microalga Decreases Plasma Cholesterol by Down-regulation of Intestinal NPC1L1, Hepatic LDL Receptor, and HMG-CoA Reductase

Jingnan Chen,[†] Yue Jiang,[*,†] Ka Ying Ma,[‡] Feng Chen,[#,§] and Zhen-Yu Chen[*,‡]

[†]Kwong Living Trust Food Safety and Analysis Laboratory and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

[‡]Food and Nutritional Sciences Programme, School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China

[#]Institute for Food and Bioresource Engineering, College of Engineering, Peking University, Beijing, China

[§]School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China

ABSTRACT: The present study examined the cholesterol-lowering activity of algal powder (AP), algal lipids (AL), and algal residue (AR) and their interaction with genes of transporters, receptors, and enzymes involved in cholesterol absorption and metabolism. In this experiment, 48 hamsters were fed either control diet or one of the three experimental diets containing 2% AP, 1.0% AL, or 1.0% AR for 6 weeks. Plasma total cholesterol (TC) and non-high-density-lipoprotein-cholesterol (non-HDL-C) were significantly decreased in the AP and AL groups but not in the AR group compared with those in the control hamsters. It was found that the cholesterol-lowering activity of AP and AL was associated with down-regulation of hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, low-density lipoprotein receptor (LDLR), and intestinal Niemann-Pick C1-like 1 (NPC1L1) transporter. It was concluded that the alga possessed the cholesterol-lowering activity and its lipids were the active ingredients. The mechanisms underlying the cholesterol-lowering activity of algae were mediated most likely by increasing the sterol excretion and decreasing the cholesterol absorption and synthesis.

KEYWORDS: microalga, total cholesterol, LDL receptor, HMG-CoA reductase, NPC1L1

INTRODUCTION

Interest in microalgae as functional foods is growing. A large number of microalgal species have been identified, and their compositions present great variation.[1] Microalgae have been used for the production of protein, omega-3 fatty acids, antioxidants, carotenoids, polysaccharides, vitamins, sterols, and squalene.[2−4] In addition, microalgae have been regarded as the possible source of biofuel for potential industrial application in recent years.[5]

High plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) correlate positively, whereas high-density lipoprotein cholesterol (HDL-C) correlates negatively, with the risk of coronary heart disease. Recently, functional foods have attracted much attention as an alternative possible therapy in lowering plasma cholesterol.[6] On the one hand, cholesterol-lowering functional foods may affect the cholesterol absorption process by regulating genes of cholesterol transporters/proteins such as Niemann-Pick C1-like 1 (NPC1L1), intestinal acyl-CoA: cholesterol acyltransferase 2 (ACAT2), microsomal triacylglycerol transport protein (MTP), and ATP-binding cassette transporters subfamily G members 5 and 8 (ABCG5/8). NPC1L1 transports cholesterol from the lumen into enterocytes, whereas ACAT2 converts cholesterol to cholesteryl ester (CE) followed by MTP,[7,8] which packs CE into chylomicrons (CM). Subsequently, CM is transferred into blood through the lymphatic system. ABCG5/8 returns the unabsorbed free cholesterol left in the enterocytes to the lumen for excretion. On the other hand, cholesterol-lowering functional foods may interact with gene expression of proteins, enzymes, and receptors involved in cholesterol homeostasis including sterol regulatory element binding protein-2 (SREBP-2), liver X receptor α (LXRα), 3-hydroxy-3-methylglutaryl reductase (HMG-CoA reductase), low-density lipoprotein receptor (LDLR), and cholesterol-7α-hydroxylase (CYP7A1). SREBP-2 regulates the transcription of HMG-CoA reductase and LDLR, with HMG-CoA reductase acting as the rate-limiting enzyme in cholesterol synthesis and LDLR being responsible for the removal of LDL-C from the plasma. LXRα is responsible for the transcription of CYP7A1, which is a rate-limiting enzyme in the conversion of cholesterol to bile acids in the liver and elimination in the bile fluid.[9,10]

Microalga *Chlorella pyrenoidosa* has been shown to be capable of lowering plasma TC.[11] However, the underlying mechanism is unclear, and the active ingredients responsible for the activity have not been identified. The oil-rich microalgal species *Schizochytrium* has been investigated and found to have very high contents of long-chain polyunsaturated fatty acids in its lipid.[12,13] It has been affirmed as a Generally Recognized as Safe (GRAS) item by the U.S. FDA for food application.[14] The present study aimed to adopt hamsters as a model to (i) investigate if the alga of *Schizochytrium* species possessed plasma cholesterol-lowering activity; (ii) examine the effect of dietary alga on the gene expression of intestinal NPC1L1, ABCG 5/8, ACAT2, and MTP; and (iii) characterize the interaction of dietary alga with hepatic SREBP-2, LXRα, HMG-CoA reductase, LDLR, and CYP7A1.

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Table 1. Composition (Grams per Kilogram of Diet) of the Control Diet (CTL) and Three Experimental Diets Supplemented with 2% Whole Algal Powder (AP), 1% Algal Lipids (AL), and 1% Algal Residue (AR), Respectively

	CTL	AP	AL	AR
cornstarch	508	508	508	508
casein	242	242	242	242
sucrose	119	119	119	119
lard	50	50	50	50
mineral mix	40	40	40	40
vitamin mix	20	20	20	20
DL-methionine	1	1	1	1
whole algae cell power	0	20	0	0
algal lipids	0	0	10	0
algal residues		0		10

MATERIALS AND METHODS

Diets. The four diets were prepared as previously described.[15] All diet ingredients were purchased from Harlan Teklad (Madison, WI) except for lard, which was obtained from the local market. DL-Methionine and cholesterol were purchased from Sigma Chemical (St. Louis, MO). The control diet (CTL) was prepared by mixing the following ingredients (g/kg diet): cornstarch, 508; casein, 242; lard, 50; sucrose, 119; mineral mix, 40; vitamin mix, 20; DL-methionine, 1; cholesterol, 1 (Table 1). The other three experimental diets were prepared by adding 2% whole algal powder (AP, freeze-dried algal cell of *Schizochytrium* sp., a gift from Runke Bioengineering, Guangdong, China), 1% algal lipids (AL), and 1% algal residue (AR) by weight, respectively, into the control diet. AR was the substances left after lipid extraction of AP. It was found that AL extracted from AP accounted for 49.8% (by weight) of AP. AP, AL, and AR were stored at −20 °C for further use. The powdered diets were mixed with a gelatin solution (20 g/L) in a ratio of 200 g of diet per liter. Once the gelatin had set, the diets were cut into pieces of approximately 10 g cubes and stored frozen at −20 °C.

Animals. Forty-eight hamsters (*Mesocricetus auratus*; 120−130 g) were randomly divided into four groups (n = 12) and housed in wire-bottom cages at 23 °C in an animal room with a 12 h light−dark cycle. All hamsters were maintained on their respective diets for 6 weeks. Diets and water were given ad libitum, with any uneaten food being weighed and replaced with fresh food daily. Body weights were measured, and the fecal samples per cage were collected weekly. Blood (0.5 mL) was obtained from the retro-orbital sinus into a heparinized capillary tube at the end of weeks 0, 3, and 6 following food deprivation for 14 h overnight and light anesthesia, using a mixture of ketamine, xylazine, and saline (v/v/v, 4:1:5).[15] After the blood had been centrifuged at 1000g for 10 min, the plasma was collected and stored at −20 °C until analysis. Following the last blood sample collection at week 6, all of the hamsters were killed by carbon dioxide suffocation. The livers were removed, washed in saline, and weighed. The first 10 cm of duodenum was discarded, and the next 30 cm of the small intestine was kept. All tissue samples were flash frozen in liquid nitrogen and stored at −80 °C until analysis. Experiments were conducted following the approval of and in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong [09-180 DH/HA&P/8/2/1 pt.5].

Fatty Acid Analysis. The fatty acid composition of the lipid was determined as previously described.[13] The lipid was transesterified, and the resultant fatty acid methyl esters (FAMEs) were analyzed in a HP 6890 capillary gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a J&W Scientific Innowax capillary column (30 m × 0.25 mm). Nonadecanoic acid (C19:0) was used as an internal standard to quantify each fatty acid. The individual FAMEs were identified by chromatographic comparison with authentic standards (Sigma Chemical). The fatty acid composition of the algal lipids is shown in Table 2.

Table 2. Fatty Acid Composition of Algal Lipids Extracted from *Schizochytrium* sp.[a]

fatty acid	% total	fatty acid	% total
12:0	0.16 ± 0.01	20:0	0.08 ± 0.00
14:0	3.57 ± 0.03	20:3(n-6)	0.19 ± 0.00
14:1	0.51 ± 0.01	20:4(n-6)	1.14 ± 0.02
15:0	1.57 ± 0.02	20:3(n-3)	0.04 ± 0.00
16:0	40.49 ± 0.06	20:5(n-3)	0.64 ± 0.02
16:1	0.24 ± 0.01	22:0	0.06 ± 0.00
17:0	0.62 ± 0.00	23:0	0.21 ± 0.02
18:0	1.22 ± 0.01	22:5(n-3)	8.79 ± 0.05
18:1(n-9)	0.41 ± 0.03	24:0	0.05 ± 0.02
18:1(n-7)	0.27 ± 0.00	22:6(n-3)	38.56 ± 0.04
18:2(n-6)	0.94 ± 0.01		
18:3(n-3)	0.25 ± 0.01		

[a] Each fatty acid is expressed as a percentage of the total fatty acids. Values are the mean ± SD, n = 3.

Measurement of Plasma Lipoproteins. Plasma TC and triacylglycerols (TG) were measured using the commercially available enzymatic kits from Infinity (Waltham, MA) and Stanbio Laboratories (Boerne, TX), respectively.[16] To quantify plasma HDL-C, very low-density lipoprotein (VLDL) and LDL were first precipitated with phosphotungstic acid and magnesium chloride using a commercial kit (Stanbio Laboratories). HDL-C in the supernatant was quantified similarly as it was for TC. Non-HDL-C was calculated by deducting HDL-C from TC.

Quantification of Liver Cholesterol. Hepatic cholesterol was determined using a method as previously described.[17,18] In brief, 5α-cholestane as an internal standard was added into the 300 homogenized liver samples. The total lipids were extracted into a methanol/chloroform mixture (2:1, v/v). Following the solvent evaporation, the liver lipids were mildly saponified, and the cholesterol was converted into its trimethylsilyl ether (TMS) derivative before GC analysis.

Quantification of Total Fecal Sterols. Total sterols in the feces were analyzed as we described previously.[16] Before the sample was saponified, 5α-cholestane and hyodeoxycholic acid were added into the fecal samples as internal standards for quantification of fecal neutral and acidic sterols, respectively. The fecal neutral sterols were extracted into cyclohexane and were converted into their TMS derivatives. The acid sterols in the bottom aqueous layer were saponified, extracted, and converted into their TMS derivatives. The analyses of individual neutral and acidic sterol TMS derivatives were performed in a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco, Bellefonte, PA) using a Shimadzu GC-14 B gas−liquid chromatograph equipped with a flame ionization detector (Kyoto, Japan). Total sterol excretion was a sum of acidic and neutral sterols.

Western Blotting Analysis of Hepatic SREBP-2, LDLR, HMG-CoA Reductase, LXRα, and CYP7A1. Western blotting analyses of hepatic SREBP-2, LDLR, HMG-CoA reductase, LXRα, and CYP7A1 were carried out as previously described.[15,16] In brief, the total protein was extracted from the frozen liver samples.[19] After the total protein was centrifuged at 35000 rpm, the supernatant was removed and the pellet saved. The target proteins were separated on a 7% SDS-PAGE gel and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA) using a semidry transfer system. Membranes were then blocked in 5% nonfat milk Tris-buffered saline with Tween-20 for 1 h and overnight at 4 °C in the same solution

Table 3. Real-Time PCR Primers Used To Measure Intestinal and Hepatic RNA Levels

gene	accession no.	forward primer 5′−3′	reverse primer 5′−3′	probe
GAPDH	DQ403055	GAACATCATCCCTGCATCCA	CCAGTGAGCTTCCCGTTCA	CTTGCCCACAGCCTTGGCAGC
CYP7A1	L04690	GGTAGTGTGCTGTTGTATATGGGTTA	ACAGCCCAGGTATGGAATCAAC	CACCTGCTTTCCTTCTCC
HMG-CoA reductase	X00494	CGAAGGGTTTGCAGTGATAAAGGA	GCCATAGTCACATGAAGCTTCTGTA	ACGTGCGAATCTGCT
LDLR	M94387	GCCGGGACTGGTCAG ATG	ACAGCCACCATTGTTGTCCA	GCACTCATTGGTCCTGCAGTCCTT
SREBP-2	U12330	GGACTTGGTCATGGGAACAGATG	TGTAATCAATGGCCTTCCTCAGAAC	CCAAGATGCACAAATC
NPC1L1	DQ897680	CCTGACCTTTATAGAACTCACCACAGA	GGGCCAAAATGCTCGTCAT	
ABCG5	AF312713	TGATTGGCAGCTATAATTTTGGG	GTTGGGCTGCGATGGAAA	
ABCG8	AF324495	TGCTGGCCATCATAGGGAG	TCCTGATTTCATCTTGCCACC	
ACAT2	ref 21	CCGAGATGCTTCGATTTGGA	GTGCGGTAGTAGTTGGAGAAGGA	
MTP	X59657	GTCAGGAAGCTGTGTCAGAATG	CTCCTTTTTCTCTGGCTTTTCA	
18S	M33069	TAAGTCCCTGCCCTTTGTACACA	GATCCGAGGGCCTCACTAAAC	

Table 4. Changes in Hepatic Cholesterol and Plasma Total Cholesterol (TC), Total Triacylglycerols (TG), High-Density Lipoprotein Cholesterol (HDL-C), Non-HDL Cholesterol (Non-HDL-C), and TC/HDL-C Ratio in Hamsters Fed the Control (CTL) Diet and Three Experimental Diets Supplemented with 2% Whole Algal Powder (AP), 1% Algal Lipids (AL), and 1% Algal Residue (AR), Respectively[a]

	CTL	AP	AL	AR	*P*
week 0					
TC (mg/dL)	98.0 ± 7.5	99.4 ± 7.1	98.0 ± 8.8	100.1 ± 8.7	0.90
TG (mg/dL)	41.2 ± 6.9	47.6 ± 10.9	46.4 ± 8.7	45.1 ± 12.4	0.40
HDL-C (mg/dL)	46.0 ± 13.5	41.9 ± 1.5	48.0 ± 11.7	50.4 ± 12.6	0.29
non-HDL-C (mg/dL)	52.0 ± 13.7	57.6 ± 6.2	50.0 ± 11.2	49.7 ± 11.4	0.28
non-HDL-C/HDL-C	1.3 ± 0.5	1.4 ± 0.1	1.1 ± 0.3	1.1 ± 0.4	0.20
TC/HDL-C	2.3 ± 0.5	2.4 ± 0.1	2.1 ± 0.3	2.1 ± 0.4	0.20
week 3					
TC (mg/dL)	248.1 ± 22.7 a	215.7 ± 22.6 b	228.9 ± 19.9 b	230.2 ± 14.2 b	<0.01
TG (mg/dL)	95.2 ± 29.9 b	136.9 ± 34.7 a	130.7 ± 40.4 a	101 3 ± 25.9 b	<0.01
HDL-C (mg/dL)	42.5 ± 3.5 a	35.2 ± 4.5 b	37.8 ± 3.5 b	42.8 ± 7.4 a	<0.01
non-HDL-C (mg/dL)	202.1 ± 19.3 a	181.4 ± 19.3 b	191.4 ± 20.0 ab	185.7 ± 14.5 ab	<0.10
non-HDL-C/HDL-C	4.9 ± 0.6 ab	5.3 ± 0.9 a	5.1 ± 0.8 ab	4.4 ± 0.92 b	<0.01
TC/HDL-C	5.9 ± 0.6 ab	6.3 ± 0.2 a	6.1 ± 0.8 ab	5.4 ± 0.9 b	<0.01
week 6					
TC (mg/dL)	221.6 ± 14.8 a	176.4 ± 13.5 b	176.0 ± 18.9 b	216.5 ± 14.4 a	<0.01
TG (mg/dL)	132.5 ± 48.0	140.7 ± 41.8	134.0 ± 28.5	130.1 ± 37.4	0.94
HDL-C (mg/dL)	48.4 ± 8.3 a	40.2 ± 5.5 b	41.1 ± 4.5 b	44.5 ± 7.7 ab	<0.02
non-HDL-C (mg/dL)	174.2 ± 8.1 a	134.7 ± 11.6 b	135.6 ± 21.3 b	173.0 ± 11.5 a	<0.01
non-HDL-C/HDL-C	3.6 ± 0.6 ab	3.3 ± 0.4 b	3.4 ± 0.8 ab	4.0 ± 0.9 a	0.16
TC/HDL-C	4.6 ± 0.6 c	4.3 ± 0.4 c	4.4 ± 0.8 b	5.0 ± 0.9 a	<0.16
liver cholesterol (mg/g)	45.8 ± 7.3 a	35.0 ± 7.1 b	42.4 ± 8.7 a	45.9 ± 3.8 a	0.04

[a] Values are the mean ± SD, $n = 12$. Means in a row for a given week with different letters differ significantly, $P < 0.05$.

containing anti-LDLR antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-HMG-CoA reductase (Upstate USA Inc., Lake Placid, NY), anti-CYP7A1 (Santa Cruz Biotechnology), anti-LXRα, or anti-SREBP-2 antibody (Santa Cruz Biotechnology).[15] The membrane was then incubated for 1 h at 4 °C in diluted horseradish peroxidase-linked goat anti-rabbit IgG, donkey anti-rabbit IgG, or goat anti-mouse IgG. The membranes were developed with ECL enhanced chemiluminescence agent and subjected to autoradiography on SuperRX medical X-ray film. Densitometry was quantified using the Bio-Rad Quantity 1 software. Data on the abundance of SREBP-2, LDLR, HMG-CoA reductase, LXRα, and CYP7A1 were normalized with β-actin.[15]

Real-Time PCR Analysis of mRNA of Liver SREBP-2, LDLR, HMG-CoA Reductase, LXRα, CYP7A1, ABCG5, ABCG8, MTP, and Small Intestine NPC1L1, ABCG5, ABCG8, ACAT2, and MTP. Real-time PCR analysis of mRNA was conducted as previously described.[15] In brief, the liver mRNA was extracted and isolated using Tizol reagent (Invitrogen, Carslbad, CA), followed by conversion of total RNA from the liver and intestine to its cDNA (cDNA) using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Reverse transcription was carried out in a thermocycler (Gene Amp PCR system 9700, Applied Biosystems), with program set as initiation for 10 min at 25 °C, followed by incubation at 50 °C for

Table 5. Fecal Excretion of Total Neutral Sterols and Total Acidic Sterols (Milligrams per Day) in Hamsters Fed the Control (CTL) Diet and Three Experimental Diets Supplemented with 2% Whole Algal Powder (AP), 1% Algal Lipids (AL), and 1% Algal Residue (AR), Respectively, at Week 6[a]

	CTL	AP	AL	AR	*P*
fecal neutral sterols					
coprostanol	0.31 ± 0.08 b	0.22 ± 0.09 b	0.49 ± 0.06 a	0.25 ± 0.06 b	<0.01
coprostanone	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.31
cholesterol	0.42 ± 0.10 b	0.61 ± 0.12 a	0.43 ± 0.08 b	0.48 ± 0.05 ab	0.03
dihydrocholesterol	0.18 ± 0.04 b	0.16 ± 0.04 b	0.24 ± 0.02 a	0.16 ± 0.03 b	0.01
campesterol	0.04 ± 0.01 b	0.05 ± 0.01 ab	0.06 ± 0.01 a	0.04 ± 0.01 b	0.12
total	0.99 ± 0.21 b	1.08 ± 0.25 ab	1.26 ± 0.09 a	0.97 ± 0.10 b	0.12
fecal acidic sterols					
lithocholic	0.19 ± 0.08 b	2.10 ± 0.25 a	1.73 ± 0.97 a	2.40 ± 0.57 a	<0.01
deoxycholic	0.27 ± 0.23	0.11 ± 0.01	0.09 ± 0.03	0.10 ± 0.05	0.42
chenodeoxycholic	0.11 ± 0.05	0.50 ± 0.40	0.52 ± 0.60	0.33 ± 0.17	0.39
cholic acid	0.06 ± 0.04	0.19 ± 0.19	0.23 ± 0.18	0.12 ± 0.13	0.27
ursodeoxycholic	0.04 ± 0.02 b	0.06 ± 0.01 ab	0.08 ± 0.04 a	0.08 ± 0.01 a	0.06
total	0.66 ± 0.24 b	2.96 ± 0.28 a	2.64 ± 0.95 a	2.99 ± 0.55 a	<0.01

[a] Values are expressed as the mean ± SD, $n = 10$. Means in the same row with different letters differ significantly, $P < 0.05$.

Table 6. Total Cholesterol Intake, Fecal Excretion of Total Sterols, and Cholesterol Balance in Hamsters Fed the Control (CTL) Diet and Three Experimental Diets Supplemented with 2% Whole Algal Powder (AP), 1% Algal Lipids (AL), and 1% Algal Residue (AR), Respectively[a]

	CTL	AP	AL	AR
week 1				
cholesterol intake (mg)	10.32 ± 0.28 b	11.25 ± 0.67 a	10.79 ± 0.69 ab	11.25 ± 0.62 a
total sterol excretion (mg)	2.43 ± 0.59	2.09 ± 0.29	1.92 ± 0.57	2.74 ± 0.55
apparent cholesterol retention[b] (mg)	7.89 ± 0.67 b	9.15 ± 0.52 a	8.86 ± 0.99 a	8.51 ± 0.38 ab
apparent cholesterol absorption[c] (%)	76.45 ± 5.71	81.39 ± 1.21	82.40 ± 5.14	75.73 ± 3.19
week 3				
cholesterol intake (mg)	11.93 ± 1.31	12.77 ± 0.79	11.86 ± 0.64	13.0 ± 0.60
total sterol excretion (mg)	3.15 ± 0.63	3.47 ± 1.63	3.41 ± 0.87	3.69 ± 0.95
apparent cholesterol retention (mg)	8.75 ± 0.86	9.29 ± 0.74	8.44 ± 0.51	9.30 ± 0.97
apparent cholesterol absorption (%)	73.70 ± 1.85	73.11 ± 8.61	71.38 ± 5.79	71.49 ± 5.67
week 6				
cholesterol intake (mg)	9.88 ± 0.80 ab	10.62 ± 0.20 ab	9.55 ± 0.35 b	10.70 ± 0.84 a
total sterol excretion (mg)	1.65 ± 0.45 b	4.03 ± 0.53 a	3.90 ± 1.05 a	3.96 ± 0.65 a
apparent cholesterol retention (mg)	8.23 ± 0.53 a	6.59 ± 0.20 b	5.65 ± 1.32 b	6.74 ± 1.01 b
apparent cholesterol absorption (%)	83.30 ± 2.54 a	62.05 ± 2.96 b	59.16 ± 1.67 b	62.99 ± 6.26 b

[a] Values are the mean ± SD, $n = 12$. Means in a row for a given week with different letters differ significantly, $P < 0.05$. [b] Apparent cholesterol retention = [cholesterol intake − excretion of fecal total sterols]. [c] Apparent cholesterol absorption = [cholesterol intake − excretion of fecal total sterols] ÷ [cholesterol intake] × 100.

90 min and at 85 °C for an additional 5 min. The cDNA synthesized was stored at −20 °C. Real-time PCR analysis was carried out on a Fast Real-time PCR System 7500 (Applied Biosystems). Primers and TaqMan probes were used for real-time PCR analysis of liver GAPDH, CYP7A1, HMG-CoA reductase, LDL-R, SREBP-2 and LXRα.[15] For intestine NPC1L1, ABCG5, ABCG8, ACAT2, MTP, and 18S, SYBR green was used as fluorophore[20−22] (Table 3). The reaction mixture was subjected to thermal cycling under the following conditions: heating to 95 °C in 20 s, followed by 40 cycles at 95 °C for 3 s and 60 °C for 30 s. Data were analyzed using Sequence Detection Software version 1.3.1.21 (Applied Biosystems). Gene expressions were calculated according to the comparative threshold cycle (C_T) method (Applied Biosystems).

Statistics. All values are expressed as the mean ± standard deviation (SD). Following the two-way analysis of variance (ANOVA), a post hoc LSD test was carried out to detect the differences among the group means using SigmaStat Advisory Statistical software (SigmaStat version 14.0, SPSS Inc., Chicago, IL). Significance was defined as a *P* value of <0.05.

RESULTS

Food Intake and Body and Organ Weight. The AL group had a food intake of 11.12 g per day, which was significantly lower than those of the AP (11.99 g) and AR (12.16 g) hamsters; however, this did not differ from the control (11.45 g). There were no significant differences in the final body weights among the four groups. The weights of kidney, heart, liver, and adipose

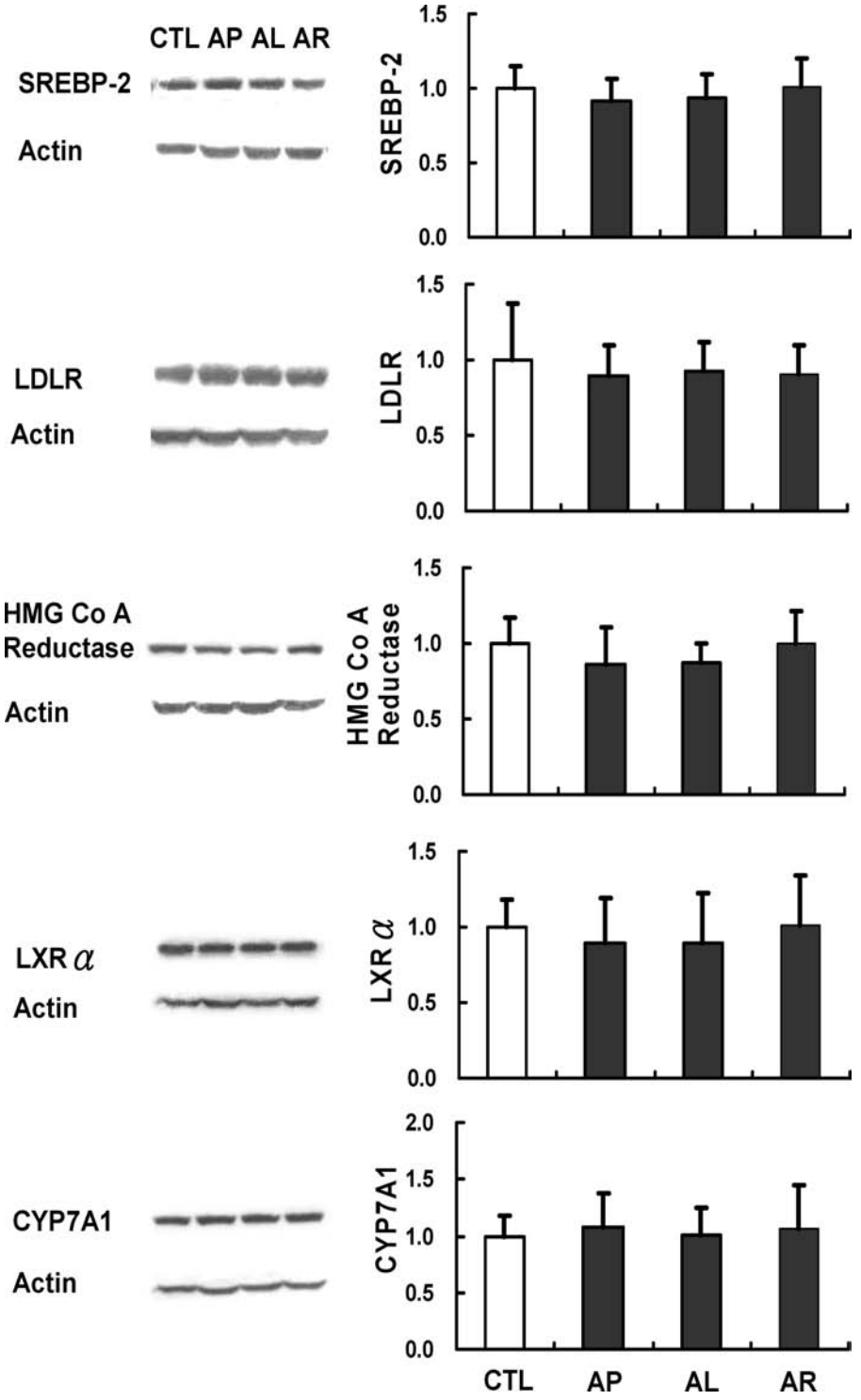

Figure 1. Effect of dietary algal power (AP), algal lipids (AL), and algal residue (AR) on the relative immunoreactive mass of hepatic sterol regulatory element-binding protein-2 (SREBP-2), liver X receptor (LXRα), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, LDL receptor (LDLR), and cholesterol-7α-hydroxylase (CYP7A1) in hamsters fed a high-cholesterol diet. Data are normalized with β-actin. Values are expressed as the mean ± SD (n = 12), with those for the control being arbitrarily taken as 1.

tissues (epididymal and perirenal pads) were similar among the four groups.

Plasma TC, HDL-C, TG, and Non-HDL-C/HDL-C. Four groups of hamsters had similar levels of plasma TC, HDL-C, and TG at the beginning of the experiment (week 0). At the end of week 3, plasma TC, HDL, and non-HDL in the AP and AL groups were significantly decreased compared with those in the control (Table 4). However, no significant difference was seen between AR and the control hamsters. When the experiment reached the end of week 6, AP and AL hamsters had plasma TC, HDL-C, and

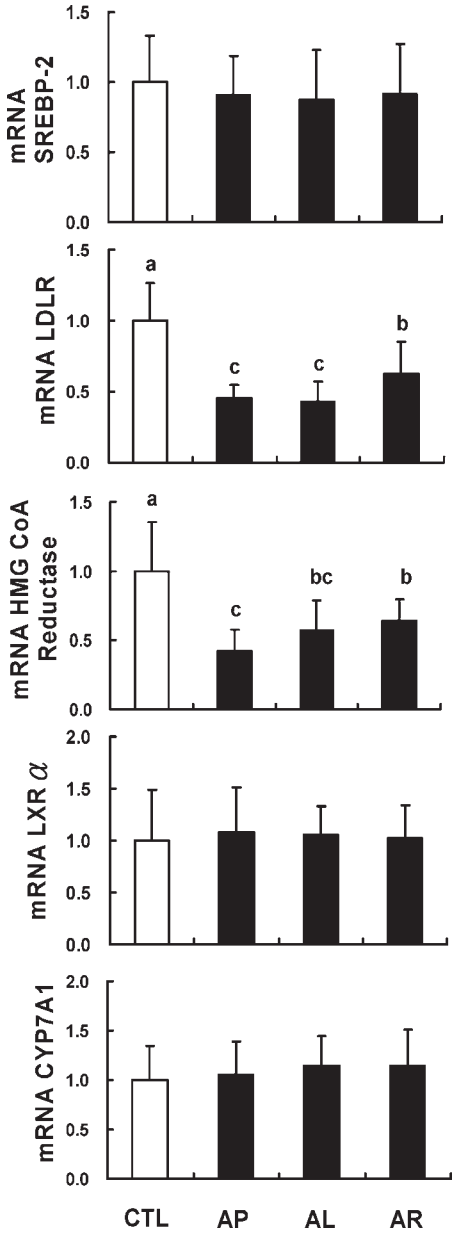

Figure 2. Effect of dietary algal power (AP), algal lipids (AL), and algal residue (AR) on mRNA levels of hepatic sterol regulatory element-binding protein-2 (SREBP-2), liver X receptor α (LXRα), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, LDL receptor (LDLR), and cholesterol-7α-hydroxylase (CYP7A1) in hamsters fed a high cholesterol diet. Data are normalized with GAPDH. Values are expressed as the mean ± SD (n = 12), with those for the control group being arbitrarily taken as 1. [a—c]Means with different letters differ significantly, $P < 0.05$.

non-HDL-C significantly lower than the control group (Table 4). No differences in plasma TC, HDL-C, and non-HDL-C were detected between the control and AR group at the end of week 6 (Table 4).

Liver Cholesterol. The hepatic cholesterol level in the AP group was significantly lower than in the other three groups. However, no significant differences were seen among the control, AL, and AR hamsters (Table 4).

Fecal Total Sterol Excretion and Apparent Cholesterol Balance. Greater excretion of fecal cholesterol was seen in the AP group compared with the control hamsters (Table 5). AL hamsters had greater excretion of coprostanol compared with the other three groups. With regard to the fecal individual acidic sterols, the three experimental groups had greater excretion of lithocholic acid compared with the control hamsters (Table 5). It was apparent that the total sterol excretion (sum of neutral and acidic) was increased in the three experimental groups compared with the control hamsters (Table 6). Because excessive cholesterol was mainly eliminated in forms of either fecal neutral or acidic sterols, apparent cholesterol retention (mg/hamster/day) could be calculated by difference between the cholesterol intake and excretion of fecal total sterols. When the apparent cholesterol absorption was calculated as apparent cholesterol retention/cholesterol intake, results showed the apparent cholesterol absorption decreased in the AP, AL, and AR groups at week 6.

Western Blotting and mRNA Analysis of SREBP-2, HMG-CoA Reductase, LDLR, LXRα, and CYP7A1. Immunoreactive mass of liver SREBP-2, HMG-CoA reductase, LDLR, LXRα, and CYP7A1 was measured using Western blotting (Figure 1). No significant differences in these proteins among the four groups were seen, although the three experimental groups had a decreasing trend in LDLR protein mass. In contrast, the three experimental groups had mRNA LDLR significantly less than the control. It appeared that the AP and AL groups had decreased mRNA LDLR levels compared with AR hamsters (Figure 2). Compared with the control hamsters, the three experimental groups had decreased expression of HMG-CoA reductase, with AP being the most effective.

Real-Time PCR mRNA Analysis of Intestinal NPC1L1, ABCG5, ABCG8, ACAT2, and MTP. RT-PCR analysis demonstrated that AP and AL groups down-regulated the mRNA NPC1L1 level compared with the control (Figure 3). However, no differences in mRNA levels of MTP, ABCG5/8, and ACAT2 were seen among the four groups.

DISCUSSION

The present study found that the whole powder of microalga *Schizochytrium* sp. possessed a hypocholesterolemic activity in hamsters fed a high-cholesterol diet. This result was in agreement with that of Cheng and Shih,[11] who reported that the spray-dried alga from *C. pyrenoidosa* could prevent a rise in cholesterol level in both rats and hamsters after a chronic high-fat diet treatment. The present study demonstrated further that the algal lipids were the active ingredients, because the cholesterol-lowering activity was associated only with the whole powder and lipids extract but not with the algal residues. In this regard, the target alga *Schizochytrium* sp. is unique in its lipid composition. Table 1 shows that the dried microalga had almost 50% lipids which contained the four major fatty acids, namely, lauric acid (14:0), palmitic acid (16:0), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic (DHA, 22:6n-3) (Table 2), accounting for 3.57, 40.49, 8.79, and 38.56%, respectively. It is known that lauric acid and palmitic acid raise plasma cholesterol by down-regulation of LDLR activity.[23] Information regarding the effect of pure DHA on blood cholesterol is very scarce. Some evidence has suggested that DHA can inhibit HMG-CoA reductase activity, reduce the cholesterol synthesis, and therefore lower plasma cholesterol level.[24] However, no specific study has investigated the effect of the isolated DPA alone. If the algal lipids were the active ingredients for the observed hypocholesterolemic activity,

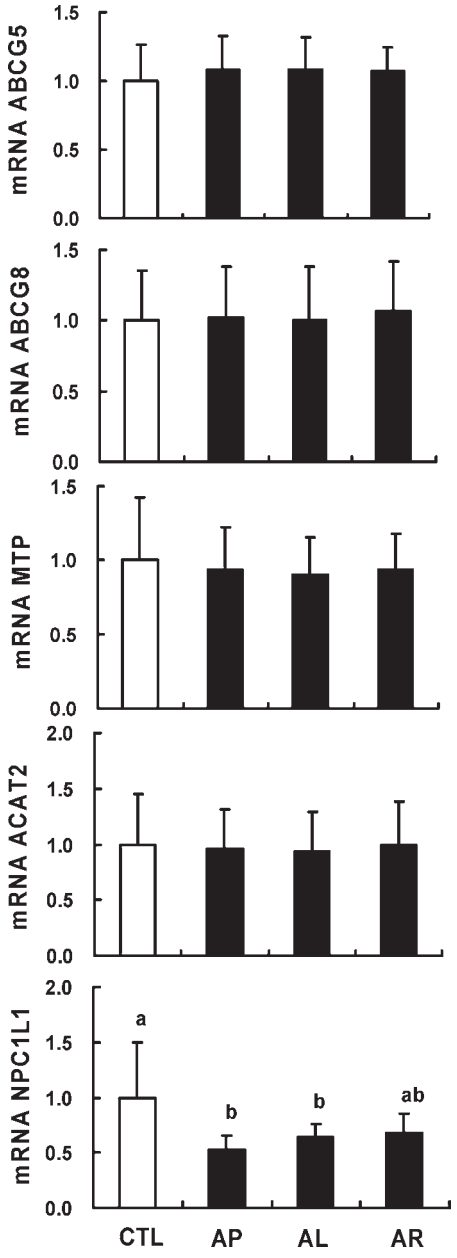

Figure 3. Effect of dietary algal power (AP), algal lipids (AL), and algal residue (AR) on mRNA levels of intestinal Niemann-Pick C1-like 1 (NPC1L1), acyl coenzyme A:cholesterol acyltransferase 2 (ACAT2), microsomal triacylglycerol transport protein (MTP), ATP binding cassette transporters (ABCG5 and ABCG8) in hamsters fed a high-cholesterol diet. Data are normalized with cyclophilin. Values are expressed as the mean ± SD (n = 12), with those for the control group being arbitrarily taken as 1. [a,b]Means with different letters differ significantly, $P < 0.05$.

it was most likely that either DHA or DPA or a combination of the two was accountable.

The present study was the first to investigate the effect of AP, AL, and AR on gene expression of the proteins, transporters, and enzymes involved in cholesterol absorption and metabolism. The major finding was that supplementation of alga, particularly its lipid extract, in diet could down-regulate NPC1L1, LDLR, and HMG-CoA reductase. NPL1L1 is a sterol transporter that is responsible for transferring the cholesterol from the intestinal lumen into the epithelial cells, where cholesterol is converted to CE for the formation of chylomicrons. Down-regulation of NPC1L1 has been shown to be able to lower plasma cholesterol level.[25] The present result was in agreement with that of Mathur et al.,[26] who have shown that fish oil and DHA can down-regulate the expression of NPC1L1. It is also known that LDL is the major cholesterol carrier in circulation and its removal is mainly LDLR-mediated. Some functional foods are capable of decreasing LDLR at translational level and thereby reduce plasma cholesterol.[6] HMG-CoA reductase is a rate-limiting enzyme in cholesterol biosynthesis, and its inhibition can lead to reduction in plasma cholesterol. Statins, a type of potent cholesterol-lowering medications, is an effective HMG-CoA reductase inhibitor and has been demonstrated to be capable of reducing plasma cholesterol concentration by 10−30% in hypercholesterolemia subjects.[27] The present results suggest that the cholesterol-lowering activity of AP and its AL is largely mediated by down-regulation of NPC1L1, LDLR, and HMG-CoA reductase. However, the Western blotting analysis did not demonstrate a corresponding increase in protein mass of LDLR and HMG-CoA reductase. Perhaps the Western blotting analysis was not sensitive enough to detect the minor changes in protein mass compared with RT-PCR. In fact, the present study was the first to demonstrate that dietary algae could decrease the fecal excretion of total sterols, leading to a reduction in apparent total cholesterol absorption.

In summary, we found that the microalga *Schizochytrium* either as a whole powder or a lipid extract favorably modified plasma lipoprotein profile by decreasing plasma TC, non-HDL-C, and TC/HDL-C ratio. Dietary alga-induced reduction in plasma TC was accompanied by greater excretion of total sterols and down-regulation of intestinal NPC1L1, hepatic LDLR, and HMG-CoA reductase, implying that alga decreased cholesterol absorption, synthesis, and catabolism.

AUTHOR INFORMATION

Corresponding Author

*E-mail: (Y.J.) yjiang@hkbu.edu.hk; (Z.-Y.C.) zhenyuchen@cuhk.edu.hk.

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