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Effects of Cationic Hydroxyethyl Cellulose on Dyslipidemia in Hamsters

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ABSTRACT: Cationic hydroxyethyl cellulose (cHEC) was supplemented in a high-fat diet to determine if this new soluble fiber had an effect on hypercholesterolemia and dyslipidemia associated with cardiovascular disease using Golden Syrian hamster as an animal model. Supplementation of 3−5% cHEC in a high-fat diet for 4 weeks led to significant weight gain reduction in hamsters. In addition, significant decreases in adipose and liver weights, concentrations of plasma total, VLDL, and LDL cholesterol, and hepatic lipids were shown. No significant improvements in glucose and insulin levels were observed with cHEC; however, a significant increase in plasma adiponectin and a decrease in leptin were observed. As compared with controls, 8% cHEC-fed hamsters had greater levels of mRNA for *CYP7A1* (cytochrome P450 7A1; 2-fold of control; $P < 0.05$), *CYP51* (lanosterol 14α-demethylase; 6-fold of control; $P < 0.05$), and *LDLR* (LDL receptor; 1.5-fold of control) in the liver. These findings suggest the possibility of the use of cHEC for cholesterol reduction and beneficial effects on the cardiovascular risk factors.

KEYWORDS: *hamsters, soluble dietary fiber, hypocholesterolemic effect, dyslipidemia, cardiovascular disease*

INTRODUCTION

Dyslipidemia is defined as a disorder of lipoprotein metabolism, whose manifestation includes elevated levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, triglyceride concentrations, and a decrease in the high-density lipoprotein (HDL) cholesterol concentration in the blood.[1] Dyslipidemia is considered one of the top five major risk factors leading to cardiovascular disease (CVD) and is also closely associated with type II diabetes.[2,3] The current worldwide prevalence and increase of these common metabolic disorders is alarming and as a consequence may be predictive of an epidemic of type II diabetes.[4] Therefore, there is a need for low-cost, widely available effective alternatives to pharmacological medicines by the general public, in addition to the recommended combination of healthier lifestyle changes and diets.

Dietary intake of soluble viscous fibers such as β-glucan, guar gum, pectin, and psyllium has been shown to lower plasma cholesterol, a risk factor for CVD, and also has shown a decrease in postprandial glycemia.[5,6] Previous efforts in characterizing the cholesterol-lowering effect of these natural soluble fibers had led to several different proposed mechanisms in which the viscosity and/or fermentability of the dietary fibers can play an important role in interfering with cholesterol absorption and/or inhibiting cholesterol synthesis.[6−9] In addition to fermentable natural fibers, hydroxypropyl methylcellulose (HPMC), a nonfermentable, viscous semisynthetic cellulose derivative, has also been shown to have hypocholesterolemic and hypoglycemic effects in both animal models and human studies.[10−14] While HPMC and other soluble dietary fibers are effective, there is an interest in developing new dietary fibers that have better efficacy to reduce fat absorption as well as improve cholesterol lowering.

Recently, we reported that a new water-soluble polymeric quaternary ammonium hydroxyethyl cellulosic fiber (polymer JR-30M, Figure 1), or cationic hydroxyethyl cellulose (cHEC),

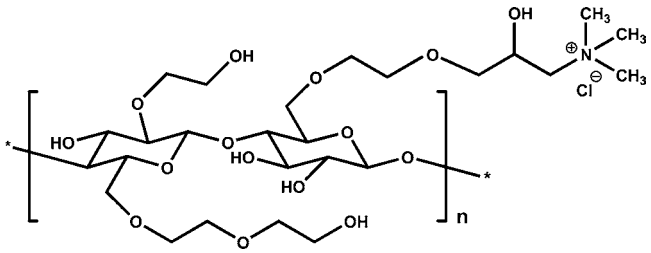

Figure 1. Schematic illustration of the structure of cHEC.

was highly effective on weight loss and metabolic disorders associated with obesity in a high-fat diet-induced obese (DIO) mouse model.[15] This very high molecular weight cationic polymer currently does not have generally recognized as safe (GRAS) status. In a previous study, the new soluble fiber cHEC was reported to induce significant body weight loss in obese B6 mice and normalize glucose homeostasis and insulin resistance when cHEC was supplemented in a high-fat diet. While the DIO mice are ideal for developing humanlike type II diabetes

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and metabolic syndrome symptoms and for elucidating the underlying mechanism of obesity,[16−20] the hamster model has been extensively used for studying the effects of diet on plasma lipid levels and the associated metabolic mechanisms.[21,22] In this study, we aim to investigate the cholesterol metabolism and lipid-lowering effects of cHEC in the hamster model to better understand the possibility of the use of cHEC for cholesterol reduction and beneficial effects on the cardiovascular risk factors.

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC)-grade isopropyl alcohol, methanol, acetonitrile, hexane, ethyl acetate, and water were obtained from Fisher (Somerville, NJ). Sodium hydroxide, hydrochloric acid, EDTA, glutaraldehyde, formaldehyde, and phosphate buffer (pH 7.4, 0.2 M) were also purchased from Fisher. Osmium tetroxide and propylene oxide were purchased from Electron Microscopy Sciences (Hatfield, PA). All reference standards were purchased from Steraloids, Inc. (Newport, CT). Deoxycholic acid (DCA) and lithocholic acid (LCA) were used as bile acid standards, and coprostanol, cholesterol, sitosterol, and stigmasanol were used as sterol standards.

Animals and Diets. Male Golden Syrian hamsters (∼80 g, LVG strain, Charles River, Wilmington, MA) were acclimated and given water and a 5001 rodent diet (LabDiet, PMI International, Redwood, CA; protein 239 g/kg; fat, 50 g/kg; non-nitrogenous substances, 487 g/kg; crude fiber, 51 g/kg; ash, 70 g/kg; energy, 17 MJ/kg; and sufficient amounts of minerals and vitamins for healthy maintenance) ad libitum for 1 week prior to the initiation of the experimental diets. Hamsters were housed individually in wire mesh cages at 20−22 °C, 60% relative humidity, and a 12 h alternating light cycle. In study 1, hamsters were weighed and randomized into groups of 10 hamsters each and were fed high-fat diets ad libitum containing 3 or 5% (by weight) cHEC (The Dow Chemical Company, Midland, MI) or 5% microcrystalline cellulose (MCC) (Dyets) for 4 weeks (Table 1).

Table 1. Diet Composition

	g/kg				
	study 1			study 2	
ingredients/diet type	5% MCC	3% cHEC	5% cHEC	8% MCC	8% cHEC
butter fat	140	140	140	140	140
corn oil	50	50	50	50	50
fish oil	10	10	10	10	10
cholesterol	1	1	1	1	1
MCC	50	20	0	80	0
cHEC	0	30	50	0	80
casein	200	200	200	200	200
corn starch	498	498	498	468	468
DL methionine	3	3	3	3	3
choline bitartrate	3	3	3	3	3
AIN-93 mineral mix	35	35	35	35	35
AIN-93VX vitamin mix	10	10	10	10	10

MCC, an insoluble fiber that has little effect on sterol metabolism, was used as the control fiber.[23] Diets consisted of 18% of energy as protein, 43% as carbohydrate, and 39% as fat supplemented with 0.1% cholesterol (Table 1). In study 2, the MCC and cHEC contents were increased to 8% at the expense of cornstarch (Table 1), resulting in a slight reduction of caloric density from 4.6 in study 1 to 4.5 kcal/g diet. Hamsters in study 2 were fed the high-fat diet for 5 weeks to increase body weights to ∼130 g prior to the treatments. These hamsters were randomized into two groups and fed either the 8% MCC or 8% cHEC diets for 7 weeks (Table 2). Body weights were recorded weekly, and food intake was monitored twice per week. The study was approved by the Animal Care and Use Committee, Western Regional Research Center, ARS, U.S. Department of Agriculture (Albany, CA).

Fecal, Plasma, and Tissue Collection. Hamster feces were collected for 2 consecutive days immediately prior to the end of study 1. Fecal samples were lyophilized, milled, and stored at −20 °C. Bile acids and sterols were determined by HPLC as described previously.[24] Hamsters were feed deprived for 12 h and anesthetized with isoflurane. Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solutions (15 wt %:v), and plasma was separated after centrifugation at 2000*g* for 30 min at 4 °C and were kept at −80 °C until analysis. Livers were collected, weighed, and immediately frozen in liquid nitrogen for analysis.

Plasma Biomarker Analysis. TC, free cholesterol (FC), and triacylglycerides (TAG) in plasma were analyzed by enzymatic colorimetric assays using a Hitachi 914 Clinical Analyzer, Roche Diagnostics (Indianapolis, IN) with assay kits from Roche Diagnostics and Diagnostic Chemicals, Ltd. (Oxford, CT). The concentrations of plasma lipoproteins, LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C) were determined using L-type LDL-C and L-type HDL-C assay kits from Roche Diagnostics and Wako Chemicals (Richmond, VA), respectively. The plasma VLDL-cholesterol (VLDL-C) levels were calculated by subtracting HDL-C and LDL-C from TC levels. Plasma concentrations of adiponectin, leptin, and insulin of feed-deprived hamsters were determined using mouse adiponectin (B-Bridge International, Sunnyvale, CA), leptin (Assay Designs, Ann Arbor, MI), and insulin (Mercodia Inc., Winston Salem, NC) immunoassay kits, as previously described.[14] Fasting glucose levels were measured by collecting blood from each hamster by the tail-prick approach. A drop of blood collected by a sterile needle was analyzed using a OneTouchUltra meter with FastDraw test strips (Johnson and Johnson, Milpitas, CA).

Hepatic Lipid Analysis. Lipids from lyophilized liver samples from study 1 were extracted using an accelerated solvent extractor (Dionex ASE, Sunnyvale, CA) at 100 °C, ∼13.8 MPa, with 75/25 hexane/2-propanol. The extracted solutions were dried and weighed to determine the percentage of total lipids in liver. The sample extract was analyzed on a Hitachi 914 clinical analyzer to determine hepatic TC, FC, and TAG levels using the kits described above.

Histology. Fresh hamster liver from study 2, less than 3 mm thick, was fixed in 2% glutaraldehyde and 2% formaldehyde in phosphate buffer (pH 7.4, 0.2 M) under refrigeration. The tissues were then cut into 1 mm cubes, rinsed in phosphate buffer, postfixed in 1% osmium tetroxide, and dehydrated in graded ethanols and propylene oxide followed by infiltration and embedding in epoxy resin. Ultrathin, 70−90 Å sections were obtained using an Ultracut E microtome (Reichert-Jung, Wetzlar, Germany) equipped with a diamond knife, placed on 200 mesh copper grids, and subsequently stained with uranyl acetate and lead citrate. The sections were examined, and images were acquired using an accelerating voltage of 80 Kv in a JEOL 1230 TEM (Tokyo, Japan) equipped with a Multiscan CCD camera (Gatan Inc., Oxford, England).

Fecal Lipid Analysis. Fecal lipids were extracted on a Dionex ASE system using a 2% acetic acid solution in hexane:2-propanol (3:2, v/v) at 15 MPa and 60 °C for 30 min. The monoacylglycerides/free fatty acids (MAG/FA), diacylglycerides (DAG), TAG, total bile acids, and sterols in the extract samples were analyzed using a modified chromatography method.[24]

Real-Time PCR. Total RNA from hamster livers from study 2 was extracted using TRIzol plus RNA purification kit (Invitrogen, Grand Island, NY), and cDNA was synthesized using GeneAmp RNA PCR kit (Applied Biosystems, Carlsbad, CA) per the manufacturer's protocol. Approximately 1 μL of diluted cDNA (1:10) was used in each real-time RT-PCR using SYBR Green Supermix (Bio-Rad) with an Mx3000P instrument (Stratagene, Cedar Creek, TX). The cycle conditions were as follows: 5 min at 95 °C followed by 20−35 cycles of incubation at 94 °C for 15 s, then 55−60 °C for 1 min, and 72 °C for 30 s. Used were the following primer combinations: 5′-GCCACCTGGCTGGTGAACAGTG-3′ and 5′-GGTGGTAGT-TGTGGAAGCCCTCG-3′ for stearoyl-CoA desaturase-1 (*SCD-1*);

Table 2. Morphometrics, Plasma Lipid, and Hepatic Lipid Concentrations in Hamsters[a]

	study 1			study 2	
	5% MCC	3% cHEC	5% cHEC	8% MCC	8% cHEC
length of study (weeks)	4	4	4	7[b]	7[b]
morphometric data					
daily food intake (g)	5.0 ± 0.4 a	5.6 ± 1.0 a	5.9 ± 1.1 a	7.2 ± 0.7	7.3 ± 0.7
daily energy intake (kcal)	23.0 ± 1.7 a	25.5 ± 4.7 a	27.2 ± 5.1 a	31.8 ± 3.3	32.3 ± 3.2
initial body weight (g)	70 ± 4.2	70.3 ± 2.8	71.4 ± 3.1	131.1 ± 13.3	127.3 ± 7.2
final body weight (g)	116.6 ± 11.1	109.4 ± 7.1	107.0 ± 7.1	133.9 ± 13.9	113.1 ± 7.1[b]
weight gain (g)	46.6 ± 11.8 a	39.1 ± 6.7 ab	35.6 ± 6.0 b	2.8 ± 8.0	−14.2 ± 7.6[b]
liver weight (g)	5.99 ± 0.76 a	4.10 ± 0.47 b	3.47 ± 0.43 b	7.14 ± 1.4	3.75 ± 0.54[b]
mesenteric adipose (g)	3.45 ± 1.13 a	2.76 ± 0.20 ab	2.60 ± 0.55 b	NA	NA
retroperitoneal-adipose (g)	1.72 ± 0.43 a	1.30 ± 0.33 b	1.31 ± 0.16 b	0.81 ± 0.28	0.86 ± 0.30
kidney (g)	0.44 ± 0.04	0.44 ± 0.02	0.43 ± 0.03	0.51 ± 0.10	0.54 ± 0.04
plasma lipids					
triglycerides (mg/dL)	325 ± 146 a	199 ± 98 b	150 ± 36 b	144.9 ± 14.7	86.0 ± 55.3[b]
TC (mg/dL)	404 ± 43 a	298 ± 84 b	210 ± 48 c	330.1 ± 52.2	131.4 ± 23.2[b]
FC (mg/dL)	88 ± 13 a	65 ± 24 b	49 ± 14 b	90.3 ± 15.1	34.4 ± 6.2[b]
hepatic lipids					
% lipid	20.7 ± 3.0 a	16.7 ± 1.3 b	16.2 ± 2.6 b	30.9 ± 2.4	17.6 ± 1.2[b]
triglycerides (mg/g)	22.3 ± 3.5 a	18.3 ± 2.8 a	22.2 ± 9.3 a	23.8 ± 3.1	23.2 ± 6.1
FC (mg/g)	10.4 ± 2.0 a	10.3 ± 1.2 a	8.4 ± 1.4 b	13.7 ± 1.4	6.7 ± 1.0[b]
TC (mg/g)	33.5 ± 5.5 a	18.4 ± 5.2 b	12.6 ± 3.0 c	57.7 ± 5.5	10.8 ± 2.1[b]

[a]Data are means ± SDs, n = 10 hamsters. Different letters indicate significant differences between treatments ($P < 0.05$). [b]All hamsters were fed 8% MCC diets for 5 weeks. After 5 weeks, half were placed on 8% cHEC diets, and hamsters on both diets were fed for another 7 weeks.

5′-ACTGCTAAGGAGGATTTCACTCT-3′ and 5′-CTCATCCAGGTATCGATCATATT-3′ for cytochrome P450 7A1 (*CYP7A1*); 5′-GAGAGGAAGTTTGCCTATGTGCC-3′ and 5′-TGTAACGGATTACTGGGTTTTCT-3′ for lanosterol 14α-demethylase (*CYPS1*); 5′-TGAGGAACATCAACAGCATAAAC-3′ and 5′-ATCCTCCAGGCTGACCATCTGT-3′ for LDL receptor (*LDLR*); and 5′-TGAGGAACATCAACAGCATAAAC-3′ and 5′-ATCCTCCAGGCTGACCATCTGT-3′ for β-actin. The primers were validated by size and sequencing of PCR products. No accumulation of nonspecific products and primer dimers was observed in a gel electrophoresis test of the PCR products. The results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalizing to β-actin expression.

Statistical Analysis. All data are presented as means ± SDs. One-way analysis of variance followed by Tukey−Kramer HSD (honestly significant difference) test was used for multiple comparisons. The body weight profiles of the diet groups were also analyzed by repeated-measure analysis. Significance was defined at $P < 0.05$. The Pearson correlation coefficient was determined for investigating the relationship between various biomarkers and parameters. Regression using both caloric intake and dose level as variables was performed to determine dose−response effect of end points of MCC control and 3% and 5% cHEC diet groups after treatment. JMP 8.0.2 and SAS 9.2 (SAS Institute Inc., Cary, NC) were used for the statistical analysis.

RESULTS

cHEC Attenuates Weight Gain in Hamsters. The effects of cHEC-supplemented diet on hamster body weight after 4 consecutive weeks of feeding are summarized in Table 2. The weekly body weight gain of hamsters over the 4 weeks study is shown in Figure 2. In the 5% cHEC diet group, weight gain was reduced significantly even after 2 weeks of treatment, despite the similar food intake between the 5% cHEC and 5% MCC groups (Table 2). Consumption of the 3% cHEC diet for 4 weeks did not significantly reduce weight gain as compared with that observed in the 5% MCC control group.

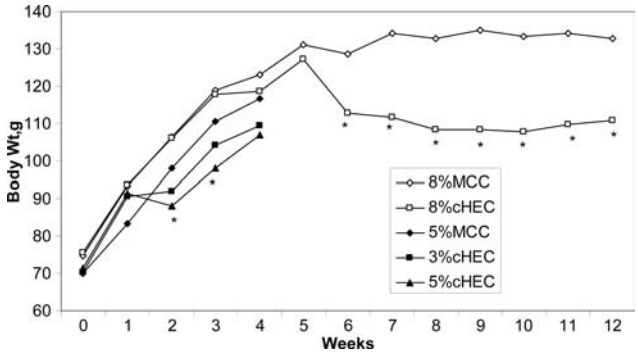

Figure 2. Body weight of hamsters in each diet group. Values are means ± SEMs, n = 10 hamsters. Means not sharing the same letter at each time point are significantly different ($P < 0.05$).

Fatty Liver and Fat Accumulations in Adipose Tissue Are Improved by cHEC. Relative liver weights expressed as percentages of body weights were 5.14, 3.75, and 3.24% for the 5% MCC and 3% and 5% cHEC diet groups, respectively. Similarly, relative liver weights of the 8% MCC and 8% cHEC groups were observed at 5.33 and 3.32%, respectively. Liver weights were significantly reduced in the 3 and 5% cHEC treatment groups than in the 5% MCC group ($P < 0.05$) (Table 2) by 32 and 42%, respectively. The regression analyses revealed a significant dose effect for the reductions in liver ($P < 0.0001$) tissue weight by cHEC, while the caloric intake effect was not significant. Significant liver weight reduction was also observed for the 8% cHEC treatment group in study 2. The effect of cHEC on fat accumulation based on the weights of two fat pads, mesenteric and retroperitoneal adipose tissues, was investigated. Relative mesenteric adipose weights (organ weight as a percentage of body weight) were 2.96, 2.52, and 2.43% for 5% MCC, 3% cHEC, and 5% cHEC groups, respectively. Additionally, relative retroperitoneal adipose weights were 1.48,

1.19, and 1.22% for 5% MCC, 3% cHEC, and 5% cHEC diet groups, respectively. While mesenteric adipose weight was significantly reduced only in hamsters supplemented with 5% cHEC ($P < 0.05$) as compared to the controls, retroperitoneal adipose weight was significantly reduced by 24% in both the 3 and the 5% cHEC treatment groups than in the control group ($P < 0.05$).

Plasma Lipid Levels and Lipoproteins Are Improved by cHEC. Plasma TC was 26 and 48% significantly lower in hamsters supplemented with 3 and 5% cHEC, respectively, as compared with control group ($P < 0.05$). The plasma TAG concentrations were 39 and 54% significantly lower ($P < 0.05$) in the 3 and 5% cHEC groups, respectively, when compared with the hamsters fed the MCC control diet. In study 2, similar improvement in plasma lipid levels was observed for TC, FC, and TAG (Table 2), indicating the normalization effect of cHEC supplemented in high-fat diet on the plasma cholesterols and triglycerides concentrations after 7 weeks of treatment.

The 5% cHEC treatment group in hamsters showed significant reduction of LDL-C by 42% and VLDL-C by 65% ($P < 0.05$) as compared to controls (Figure 3). No differences

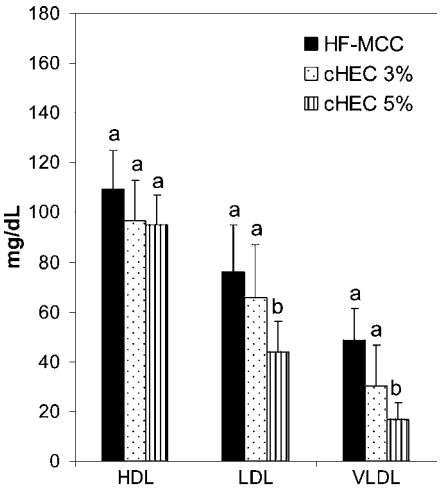

Figure 3. Lipoprotein cholesterol concentrations in hamsters in each diet group after 4 weeks of treatment. Different letters indicate significant differences ($P < 0.05$).

in HDL-C were observed between the cHEC treatments and the HF groups. Similar to the dose−response effect of cHEC on TC reduction, cHEC also reduced VLDL-C and LDL-C in a dose−response manner (VLDL, $P = 0.0007$; LDL, $P = 0.0116$). Concentrations of HDL-C did not show a dose-dependent response ($P = 0.2323$).

Effects of cHEC on Changes in Plasma Glucose and Plasma Insulin. Fasting plasma glucose and insulin levels were not different between cHEC and controls after 4 weeks of feeding Figure 4. Insulin sensitivity of these diet groups was also evaluated by the QUICKI index based on fasting glucose and insulin concentrations. The 5% cHEC group showed a significant improved sensitivity ($P < 0.05$) as compared to the 5% MCC control group. The dose-dependent effects of cHEC on plasma glucose reduction ($P = 0.0303$) and increased insulin sensitivity based on QUICKI ($P = 0.0024$) were significant. However, no significant dose effect of cHEC was observed for the plasma insulin level ($P = 0.1036$).

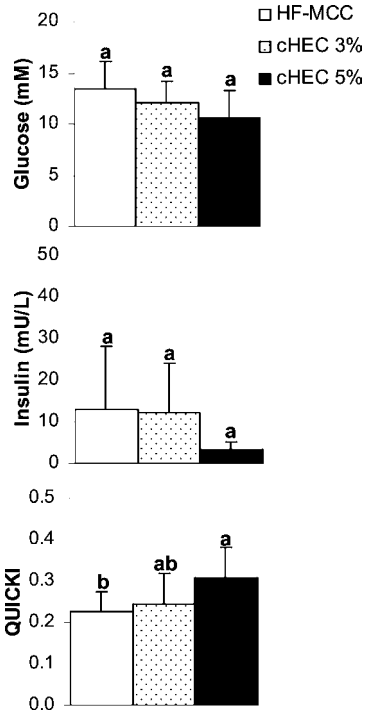

Figure 4. Effect of dietary supplementation with either cation hydroxyethyl cellulose or MCC on fasting glucose, plasma insulin, and the quantitative insulin-sensitivity check index (QUICKI) for hamsters in each diet group. QUICKI = 1/[log(fasting insulin μU/mL) + log(fasting glucose mg/dL)]. Data are expressed as means ± SDs, $n = 10$ hamsters. Different letters indicate significant differences ($P < 0.05$).

Effects of cHEC on Plasma Adiponectin and Leptin Concentrations. The circulating leptin levels were significantly reduced ($P < 0.05$) in both 3 and 5% cHEC groups by 45 and 59%, respectively, as compared with levels in the control group (Figure 5). Significant increases in adiponectin levels were observed for both 3 and 5% cHEC groups 33 and 35%, respectively, as compared with levels in the control group ($P < 0.05$). Moreover, both leptin and adiponectin levels were modulated by cHEC in a dose-dependent manner (leptin, $P = 0.0064$; adiponectin, $P = 0.0008$).

Fecal Lipid Excretion Is Increased by cHEC. Despite similar caloric intakes among the diet groups, significant body weight reductions of the cHEC supplemented hamsters were observed. This suggests that cHEC supplementation was able to alter energy absorption or expenditure. To determine the effect of cHEC on fat absorption, fecal lipid contents were analyzed (Table 3). Excretion of total bile acids, sterols, DAG, and TAG in hamsters supplemented with either 3 or 5% cHEC group did not show a significant difference when compared to control group. However, the excretion of the sum of MAG and FA was significantly increased by 3.3- and 4.4-fold upon 3 and 5% cHEC supplementation, respectively ($P < 0.05$). Additionally, the increased excretion of MAG/FA ($P < 0.0001$) and total percent lipids ($P < 0.0001$) by cHEC was significantly dependent on the level of cHEC present in the supplement.

Hepatic Lipid Levels Are Reduced by cHEC. The total lipid levels in hamster liver were significantly reduced in 3 and 5% cHEC groups by 19 and 22% ($P < 0.05$) as shown in Table 2. In addition, a significant reduction in total hepatic cholesterol levels was observed in both 3 and 5% cHEC groups by 45 and

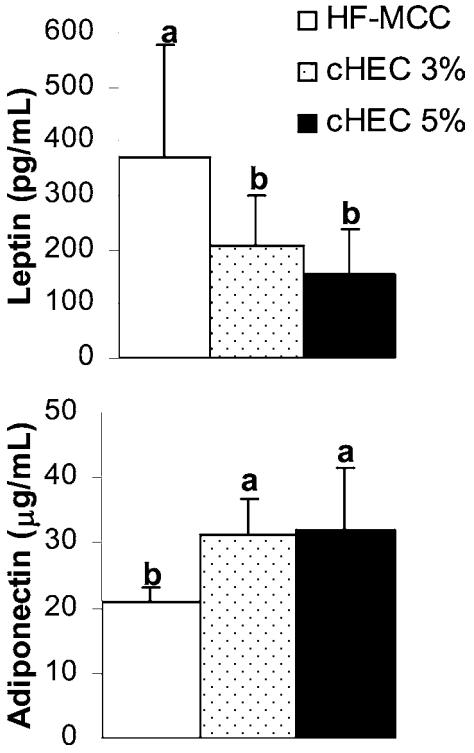

Figure 5. Effect of dietary supplementation with either cHEC or microcrystalline on plasma concentrations of leptin and adiponectin for hamsters in each diet group. Data are expressed as means ± SDs, n = 10 hamsters. Different letters indicate significant differences (P < 0.05).

Table 3. Fecal Bile Acids, Sterols, MAG/FA, DAG, and TAG Excretion in Hamsters[a]

	study 1		
	MCC	3% cHEC	5% cHEC
bile acids (mg/g)	0.7 ± 0.2 a	0.8 ± 0.3 a	1.0 ± 0.4 a
MAG/FA (mg/g)	2.9 ± 0.6 b	9.7 ± 2.7 a	12.9 ± 3.9 a
sterol (mg/g)	4.2 ± 0.6 a	4.8 ± 0.8 a	5.1 ± 1.0 a
DAG (mg/g)	1.1 ± 0.3 a	1.3 ± 0.5 a	1.5 ± 0.4 a
TAG (mg/g)	0.3 ± 0.1 a	0.5 ± 0.2 a	0.4 ± 0.1 a

[a]Values are means ± SDs, n = 10 hamsters. Different letters indicate significant differences between treatments (P < 0.05).

62%, respectively (P < 0.05). However, no significant reduction in TAG levels was observed. Dose-dependent decreases of cHEC in hepatic total lipid levels (P = 0.0021) and liver TC (P < 0.0001) with increasing cHEC supplementation were observed. The effect of 8% cHEC supplemented high-fat diet on the liver lipids concentrations in study 2 was similar to the results observed in study 1, where significant reductions of cholesterols and FCs were observed while there were minimal changes for TAG levels (Table 2).

Expression of Hepatic Genes Related to Bile Acids, Cholesterol, and Fatty Acid Metabolism. To understand the molecular mechanisms underlying the metabolic changes by 8% cHEC-supplemented high-fat diet on normalizing the metabolic disorders associated with high-fat-induced dislipidemia in hamsters, the expression profiles of selected genes related to bile acid synthesis, cholesterol synthesis, and fatty acid metabolism in the liver were examined in a second animal study, in which the level of cHEC supplemented in the diet was at 8%. The expression of mRNA of *CYP7A1*, the rate-limiting enzyme of the cholesterol catabolic pathway, *CYP51* (lanosterol 14α-demethylase), and *LDLR* (LDL receptor) were significantly upregulated in the 8% cHEC group as compared with the 8% MCC control group (P < 0.05). The expression levels of a key enzyme in fatty acid synthesis, *SCD-1* (stearoyl-CoA desaturase-1), was significantly lower in the 8% cHEC group as compared with the 8% MCC control group.

Furthermore, a histological assessment of livers from hamsters fed high-fat diets supplemented with either 8% cHEC or 8% MCC control group were examined (Figure 6A,B). The hamsters fed the high-fat diet supplemented with

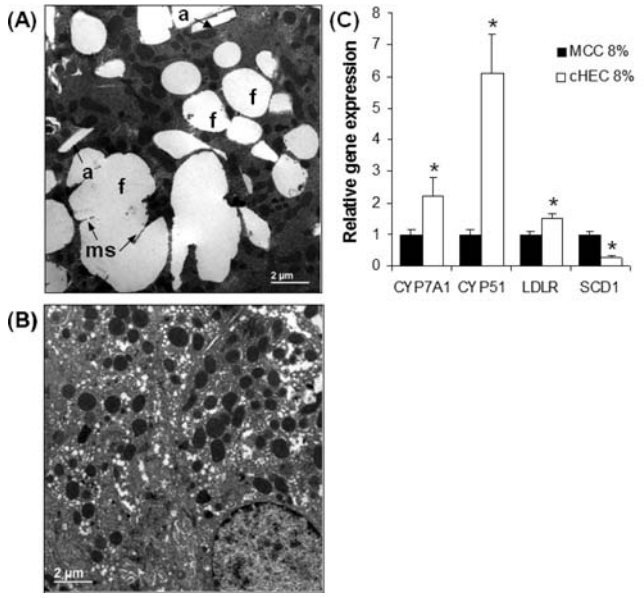

Figure 6. Effect of dietary supplementation with either 8% cHEC or 8% MCC in hamster livers after 4 weeks. (A) A representative TEM micrograph of a section of hamster liver tissue supplemented with 8% MCC showing fat droplets (f), angular lucid inclusions (a), and membranous structure (ms). (B) A representative TEM micrograph of a section of hamster liver tissue supplemented with 8% cHEC. (C) Hepatic mRNA expression of genes related to homeostasis of bile acids, cholesterol, and fatty acid metabolism in male hamsters fed 8% MCC or cHEC diet for 4 weeks. Data are expressed as means ± SEs. The asterisk indicates means significantly different by Student's t test (P < 0.05).

MCC showed substantially more fat droplets and angular lucid inclusions as compared to the hamsters fed 8% cHEC-supplemented high-fat diet.

Correlations of Metabolic Parameters, Adiponectin, Leptin, and Insulin Levels. Correlations between plasma adiponectin, plasma leptin, and metabolic parameter levels were examined to understand their relationships in the hamster model. There was a significant negative correlation between plasma adiponectin and plasma leptin (Table 4). As the sizes of both adipose fat pads are proportional to the body weights, there was also a significant positive correlation between leptin and adipose weights (only retroperitoneal-adipose shown in Table 4). As shown in Table 4, circulating adiponectin is also strongly associated with the improvement of several hypercholesterolemic-related effects, such as reduction in liver TC, plasma TC, and plasma TAG. Similarly, significant correlations between leptin and fasting glucose, fasting insulin, insulin

Table 4. Correlations between Metabolic Parameters and Adiponectin and Leptin Levels[a]

	study 1			
	adiponectin		leptin	
variables	*R*	*P*	*R*	*P*
weight gain (g)	−0.4307	0.0356*	0.7561	<0.0001*
retroperitoneal-adipose weight (g)	−0.3287	0.0942	0.8445	<0.0001*
fasting glucose (mmol/L)	−0.2670	0.1782	0.4858	0.0102*
fasting insulin (mU/L)	−0.1000	0.6196	0.5162	0.0058*
leptin (ng/mL)	−0.4504	0.0184*		
liver TC (mg/g)	−0.4362	0.0229*	0.4301	0.0251*
liver triglycerides (mg/g)	−0.1505	0.4537	−0.1750	0.3827
plasma TC (mg/dL)	−0.4051	0.0361*	0.4652	0.0145*
plasma triglycerides (mg/dL)	−0.4054	0.0359*	0.7730	<0.0001*

[a]Values are Pearson correlations, $n = 10$.

sensitivity, liver TC, plasma TC, and plasma TAG levels were also observed.

DISCUSSION

The cHEC fiber is positively charged under physiological conditions due to quaternary ammonium substituents. These unique properties of cHEC are different from other soluble dietary fibers that have previously shown positive health benefits, including the reduction of plasma cholesterol and postprandial glucose in both animal and human studies.[10−12] In a diet-induced obesity mouse study, cHEC was found to have better efficacy than other soluble dietary fibers, inducing significant body weight loss and normalizing glucose homeostasis and insulin resistance.[15] These results suggested that cHEC supplementation may possibly be used as a novel therapeutic treatment for obesity, insulin resistance, and type II diabetes. Therefore, the effects of cHEC for improving hypercholesterolemia and dyslipidemia were investigated using a hamster model in the present study. Supplementation of 5% cHEC in high-fat diets significantly decreased body weight gain, adipose and liver weights, and concentrations of plasma cholesterol and hepatic lipids of hamsters after 4 weeks of treatment. Therefore, cHEC administration reduced the fat accumulation in the liver and adipose fat affected by a high-fat diet. In addition to weight gain prevention, a weight loss effect of cHEC-supplemented high-fat diet was also observed at a higher dose level (8% w/w of diet) after 7 weeks of treatment. Because the hamsters were on high-fat diet for 5 weeks prior to the start of treatment, metabolic disorders such as fatty liver and high concentrations of liver and plasma lipids were induced. Significant reductions in liver fat accumulations both on lipid levels as well as histological results were achieved by 8% cHEC supplementation even though the dose level was higher than that previously reported in the DIO mouse model.[15] These results were similar to observations in the DIO mice after 5 weeks of cHEC treatment.[15] Many dietary fibers have been suggested to have various degrees of effect on reducing body weight gain and cholesterol by increasing the viscosity of the contents of the small intestine or by reducing bile acids in the intestine.[25] This was evident based on the increased excretion of bile acids and neutral sterols and resulted in reduced intestinal absorption of cholesterol and fat, thus reducing amounts of fat and lipid accumulation in adipose tissue and liver. In the previous DIO mouse study, cHEC enhanced fecal excretion of both bile acids and sterols similar to most of the dietary fibers as well as free fatty acids and fats. However, to our surprise, in hamsters, cHEC supplementation of high-fat diets did not significantly increase fecal excretion of bile acids and neutral sterols. More specifically, cHEC preferentially increased excretion of MAG/FA in a dose-dependent manner. Even though the fecal excretion of bile acids and sterols was not significantly enhanced by cHEC, the levels of liver TCs were observed to be significantly correlated with fecal bile acids ($R = −0.58$; $P = 0.004$) and sterols ($R = −0.55$; $P = 0.006$) output. These observations suggest that cHEC may have different mechanisms in this animal model to lower lipid absorption in the gastrointestinal tract.

In hamsters, the plasma cholesterol lowering induced by cHEC is proportional to the dose of dietary cHEC and dependent on the depletion of hepatic cholesterol induced by the action of the fiber in the intestinal lumen. The cholesterol reduction effect by cHEC was observed to be dose-dependent for plasma LDL-C, free, and TC in hamsters fed a high-fat diet. Furthermore, correlations were observed between the reductions of plasma TC levels and liver TC levels ($R = 0.8467$; $P < 0.0001$), indicating significant improved cholesterol metabolism elicited by cHEC consumption. At a higher dose level of 8% cHEC in study 2, similar correlations between the plasma cholesterol reductions and the decreases of hepatic cholesterols were observed (data not shown).

To understand the changes in hepatic cholesterol metabolism upon cHEC treatment and the potential effect of cHEC to ameliorate the metabolic disorders induced by a high-fat diet, the expression profile of genes encoding the key enzymes and receptor in the bile acid and cholesterol metabolic pathways was examined.

In the 8% cHEC group, the mRNA level of *CYP7A1* was upregulated as compared to the control group. Even though changes in mRNA expression levels are not always a measure of pathway flux, previous studies have shown upregulations of both *CYP7A1* mRNA and CYP7A1 enzyme activity by supplementation of dietary fibers.[23,26−28] The increased expression of *CYP7A1* by cHEC suggests that increased synthesis of bile acids from cholesterol was induced by cHEC intake, leading to reduction of hepatic cholesterol concentrations. This was further supported by the correlation between hepatic cholesterol reduction and increases in fecal bile acids. Moreover, upregulation of *CYP51* and *LDLR* indicated that the hepatic cholesterol reduction was replenished by both de novo cholesterol syntheses through *CYP51* and cholesterol transport by increasing *LDLR* expression for the import of exogenous cholesterol, resulting in lowered plasma cholesterol levels in cHEC diet group. Significant correlations between plasma cholesterol levels with the expression levels of *CYP7A1* ($R = −0.54$, $P = 0.0471$), *CYP51* ($R = −0.70$, $P = 0.0051$), and *LDLR* ($R = −0.62$, $P = 0.0172$) were also observed for supporting the proposed mechanism of cholesterol reduction effect of cHEC. These observations on hepatic gene expressions for cholesterol metabolism were similar to the effects of HPMC on cholesterol reduction in hamsters[13] despite the differences in the fecal output profiles. In addition to the changes in genes involved in cholesterol metabolism, the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids, *SCD-1*, was significantly downregulated by cHEC. Moreover, a significant correlation was observed between the circulating triglycerides and the expression levels of *SCD-1* ($R = 0.70$, $P = 0.0052$). This result may indicate that cHEC influenced hepatic triglycerides

in a similar manner as HPMC; however, the levels of triglycerides in the liver were not significantly reduced by either 3 or 5% cHEC. Even at a higher dose level of cHEC in study 2, reduction of hepatic triglycerides was not achieved after 7 weeks of treatment. Even though there was a lack of decrease in hepatic triglycerides, significant correlations between fecal MAG/FA with hepatic triglycerides (R = −0.51; P = 0.013) and plasma triglycerides (R = −0.57; P = 0.0046) were observed.

The adiposity affects the metabolic and endocrine functions of adipose tissue. Significantly reduced fat accumulation upon dietary intervention can influence the adipocytokines expressed in adipocytes, which are involved in the regulation of glucose and lipid homeostasis. In this study, cHEC supplemented in a high-fat diet in hamsters led to an increase in plasma adiponectin concentrations that were correlated with reductions in plasma cholesterol (R = −0.41; P = 0.04) and triglyceride concentrations (R = −0.41; P = 0.04). Leptin levels were significantly reduced following supplementation of the high-fat diet with cHEC. Furthermore, decreased leptin levels appear to be associated with reductions in adipose tissue, liver weight, and liver cholesterol levels. Even though both leptin and adiponectin have been reported to be involved in glucose and lipid metabolism, only leptin levels were strongly correlated with improved insulin and glucose levels following cHEC supplementation in the present study. Therefore, the results indicate that cHEC supplementation of the high-fat diet improves glucose metabolism by reducing lipid storage in insulin-sensitive tissues via improved leptin regulation.

In conclusion, the present study showed that cHEC, a new soluble fiber, significantly improves dyslipidemia and hypercholesterolemia. These data demonstrate that the improvements in plasma lipid profiles in hamsters fed a high-fat diet supplemented with cHEC are linked to increased fecal lipid excretion and the regulation of bile acid and cholesterol metabolism in the liver. This study further supports the potential dietary use of cHEC for the prevention or management of dyslipidemia-related diseases such as CVD, metabolic syndrome, and possibly obesity. Currently, cHEC is used in the personal care market and does not have appropriate approval for use as an excipient, food, or supplement. Future toxicology studies will be required to establish GRAS status for cHEC as a food supplement in human clinical trials.

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The authors declare no competing financial interest.

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