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Altered Hepatic Gene Expression Profiles Associated with Improved Fatty Liver, Insulin Resistance, and Intestinal Permeability after Hydroxypropyl Methylcellulose (HPMC) Supplementation in Diet-Induced Obese Mice

Hyunsook Kim,*[,†,‡] Glenn E. Bartley,[‡] Scott A. Young,[§] Kun-Ho Seo,[#] and Wallace Yokoyama[‡]

[†]Department of Nutrition, University of California, Davis, California, United States

[‡]Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California, United States

[§]Dow Wolff Cellulosics, Midland, Michigan, United States

[#]Department of Public Health, College of Veterinary Medicine, Konkuk University, Seoul, South Korea

Supporting Information

ABSTRACT: The effect of hydroxypropyl methylcellulose (HPMC) on hepatic gene expression was analyzed by exon microarray and real-time PCR from livers of diet-induced obese (DIO) mice fed a high-fat (HF) diet supplemented with either 6% HPMC or 6% microcrystalline cellulose (MCC). HPMC-fed mice exhibited significantly reduced body weight gain (55% lower compared to MCC), liver weight (13%), plasma LDL-cholesterol concentration (45%), and HF diet-increased intestinal permeability (48%). HPMC significantly reduced areas under the curve for 2 h insulin and glucose responses, indicating enhanced insulin sensitivity and glucose metabolism. HPMC up-regulated hepatic genes related to fatty acid oxidation, cholesterol and bile acid synthesis, and cellular activation of glucocorticoid (bile acid recycling) and down-regulated genes related to oxidative stress, triglyceride synthesis, and polyunsaturated fatty acid elongation. In conclusion, HPMC consumption ameliorates the effects of a HF diet on intestinal permeability, insulin resistance, hepatic lipid accumulation, glucocorticoid-related bile acid recycling, oxidative stress, and weight gain in DIO mice.

KEYWORDS: *steatosis, lipid metabolism, insulin sensitivity, intestinal permeability, soluble dietary fiber*

INTRODUCTION

Over 75% of obese individuals are diagnosed with nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome.[1,2] NAFLD ranges from steatosis (simple fatty liver) to nonalcoholic steatohepatitis (NASH), a condition that increases liver-related morbidity and mortality.[3] Gut-mediated lipopolysaccharides (LPS, a major outer membrane component of Gram-negative bacteria) have been shown to be involved in the transition from simple steatosis to steatohepatitis.[4] LPS entry into the circulation from the intestinal lumen occurs not only via paracellular transit but also via transcellular pathways accompanying micellar absorption of fat and chylomicron transport. Recent studies have suggested that circulating LPS levels are modified by intestinal permeability and the population of intestinal microbiota.[5] LPS has been found to regulate the pro-inflammatory pathway of TNF-α production via binding to Toll-like receptor 4 (TLR4) in liver,[6−8] suggesting a role for LPS in hepatic inflammation and NAFLD.

Diet composition affects development of NAFLD. Consumption of high-fat (HF) or high-fructose diets induces hepatic steatosis in animal models[9,10] and HF may also be implicated in humans.[11] Hepatic inflammatory response to LPS is enhanced by dietary saturated fatty acid and cholesterol as well as leptin-mediated signaling.[8,12] On the other hand, diets rich in dietary fiber (DF) including soluble dietary fiber (SDF) have been shown to improve hepatic steatosis in animals.[13−16] Furthermore, DF reduced biomarkers of systemic inflammation and modulated circulating LPS levels in humans and animals.[17−21] The reduction of circulating LPS levels by fermentable SDF may be related to increased expression of intestinal tight-junction proteins, resulting in reduced intestinal permeability to LPS as shown in HF diet-induced obese (DIO) mice.[18,19]

Hydroxypropyl methylcellulose (HPMC) is a modified cellulose that has been shown to have the same physiological properties as other SDF such as psyllium and β-glucan, but is not fermentable. Animal and human studies have shown that HPMC and other viscous SDF have beneficial health effects such as hypocholesterolemic and postprandial hypoglycemic effects, adipocytokine alteration, and body weight reduction.[22−24] A mechanism of cholesterol lowering by HPMC and other highly viscous SDF may be increased fecal excretion of bile acids and cholesterol.[25] Recently, we reported that hamsters fed a HF diet supplemented with HPMC had altered hepatic expression of genes related to bile acid, cholesterol, and fatty acid metabolism that was strongly correlated with improved plasma lipid profiles.[26] HPMC is not absorbed by the host and is not fermented by the microbiota. However, in a

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recent mouse study HPMC was shown to modulate fecal and cecum microbiota, lower total bacteria numbers, and increase the ratio of bacteroidetes to firmicutes.[27] HPMC may reduce circulating LPS levels via modulation of intestinal microbiota as well as by reducing intestinal permeability to LPS.

In this study, to determine whether HPMC modulates intestinal barrier function and hepatic response to gut-derived LPS, we evaluated in vivo intestinal permeability, insulin tolerance, and global gene expression analysis using exon microarray in livers obtained from HF-induced obese mice fed either 6% HPMC or 6% microcrystalline cellulose (MCC).

MATERIALS AND METHODS

Chemicals. Bovine cholesterol lipoprotein standards and fluorescein isocyanate labeled dextran polymer (FITC-dextran) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isoflurane was purchased from Phoenix Pharmaceutical (St. Joseph, MO, USA). Sodium hydroxide and hydrochloric acid used for hydrolysis were purchased from Fisher (Somerville, NJ, USA). HPLC grade methanol, isopropyl alcohol, acetonitrile, hexane, ethyl acetate, EDTA, and water were also obtained from Fisher. HPMC supplied by The Dow Chemical Co. (Midland, MI, USA) was of food grade, as defined by the U.S. Pharmacopoeia (USP 27 NF22 S1 for Hypromellose), with viscosity of 100,000 cP. MCC) was obtained from Dyets (Bethlehem, PA, USA).

Animals and Diets. Male C57BL/6J mice were purchased from Charles River Laboratories (Wilmington, MA, USA) and housed individually in an environmentally controlled room (20−22 °C, 60% relative humidity, 12 h alternating light/dark cycle). The mice were acclimated and given ad libitum access to water and mouse chow diet (LabDiet 5015, PMI International, Redwood, CA, USA) for 1 week prior to initiation of the experimental diets. Mice were weighed and randomized into two groups of 30 mice each. Mice were fed ad libitum either mouse chow diet or high-fat (HF) diets containing 17% of energy as protein, 37% as carbohydrate, and 47% as fat with 0.1% cholesterol. After 5 weeks, mice were weighed, and obese mice were identified as those having gained significantly greater weight compared to the mice fed chow diet. The obese mice were then randomized into two groups (n = 10 each) and fed ad libitum for 5 weeks HF diets containing either 6% HPMC or 6% MCC, an insoluble fiber with little effect on sterol metabolism[28] as a control diet (Supporting Information Table 1). Body weights were recorded weekly, and food intake was monitored twice per week. The study protocol, P-04-02, was approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA, USA.

Plasma and Liver Collection. Mice were feed-deprived for 12 h and anesthetized with isoflurane. Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% w/v). The plasma was separated after centrifugation at 2000g for 30 min at 4 °C. Livers were collected, weighed, and immediately frozen in liquid nitrogen for analysis.

After freeze-drying, the livers were ground to a powder, weighed, and combined with 2 mL of $CHCl_3$/MeOH (2:1), sonicated for 5 min, and then extracted overnight. The samples were centrifuged for 10 min × 1000 rpm, and the supernatant was removed with a glass pipet. Another 2 mL of $CHCl_3$/MeOH was added, sonicated, and allowed to stand overnight to extract. The solvent of the combined extracts was removed under nitrogen.[29]

Plasma Glucose and Lipid Analysis. Blood glucose concentrations in feed-deprived mice were measured in tail vein samples using a OneTouch Ultrameter (LifeScan Inc., Milpitas, CA, USA). Plasma lipoprotein cholesterol was determined by size exclusion chromatography as previously described.[30] Briefly, an Agilent 1100 chromatograph was employed with a postcolumn derivatization reactor, consisting of a mixing coil (1615-50 Bodman, Aston, PA, USA) in a temperature-controlled water jacket (Aura Industrials, Staten, NY, USA). Fifteen microliters of plasma was injected onto a Superose 6HR HPLC column (Pharmacia LKB Biotechnology, Piscataway, NJ, USA). The lipoprotein fractions were eluted with a pH 7.0 solution containing 0.15 M NaCl and 0.02% sodium azide at a flow rate of 0.5 mL/min. A Hewlett-Packard (Agilent, Palo Alto, CA, USA) HPLC pump 79851-A was used to deliver cholesterol reagent (Roche Diagnostics, Indianapolis, IN, USA) at a flow rate of 0.2 mL/min. Bovine cholesterol lipoprotein standards were used to calibrate the signal on the basis of peak areas.

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT). Both tests were done after 5 weeks of diet treatment. After 3 h of fasting, mice were injected intraperitoneally with glucose (2 g/kg body weight), and tail vein blood glucose levels were determined at 0, 15, 30, 60, and 120 min after glucose injection using a OneTouch Ultrameter (LifeScan Inc.). ITT was performed with mice injected intraperitoneally with insulin (0.5 U/kg body weight) after 3 h of fasting. Blood glucose levels were determined from tail vein blood at 0, 30, and 60 min after insulin injection using a OneTouch Ultrameter (LifeScan Inc.).

In Vivo Intestinal Permeability. HPMC and MCC diet effects on intestinal permeability to 4000 Da MW FITC-dextran were measured.[18] The mice were fasted for 6 h before FITC-dextran (600 mg/kg body weight, 125 mg/mL) was administered by gavage. After 1 h, 120 μL of blood was collected from the tail vein and centrifuged at 4 °C, 12000g, for 5 min. Fifty microliters of serum was diluted with an equal volume of PBS (pH 7.4), and the serum FITC-dextran concentration was analyzed with a fluorescence spectrophotometer (HTS-7000 plus-plate-reader; Perkin-Elmer, Wellesley, MA, USA) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. A standard curve of FITC-dextran diluted in nontreated plasma diluted with PBS (1:2 v/v) was used to determine FITC-dextran concentration.

Gene Expression and Exon Microarray Analysis. Total RNA from livers was extracted using a TRIzolplus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA, USA) from three biological replicates of epididymal adipose tissues per each treatment. The quality of total RNA was determined using a 2100 Bioanalyzer instrument and RNA 6000 Nano LabChip assay (Agilent Technologies, Palo Alto, CA, USA), and 10 μg of total RNA was then used to synthesize one-cycle cDNA (first-strand and second-strand cDNA synthesis) followed by cleanup of double-stranded cDNA and biotin-labeled cRNA synthesis. The biotin-labeled cRNA was used for fragmentation for target preparation using One-Cycle Target Labeling and Control reagents (Affymetrix, Santa Clara, CA, USA). Fragmented cRNA samples were hybridized to an Affymetrix GeneChip Mouse exon 1.0 ST array, an expression and exon splicing array containing 1.2 million probesets representing 80000 genes. The hybridization signals were acquired and analyzed using the GeneChip Scanner 3000 High-Resolution Scanner (Affymetrix) and the Affymetrix GeneChip Operating Software (GCOS). Analysis of both gene expression and exon alternative splicing from the microarray data was performed using a GeneSpring GX version 11.0 program (Agilent Technologies, Santa Clara, CA). Gene expression was determined to be significant when the fold change value was >1.5. The splice index was defined as the log of the ratio of exon-level expression over gene-level expression. A fold change in splice index value ≥2 between treatment and control groups was considered to be differentially spliced.[31] Transcripts with at least one differentially spliced exon were considered to be differentially regulated splicing.[31]

Real-Time RT-PCR. Total RNA from livers was extracted using a TRIzolplus RNA purification kit (Invitrogen, Life Technologies), and cDNA was synthesized using a GeneAmpRNA PCR kit (Applied Biosystems, Foster City, CA) per the manufacturer's protocol. One microliter of diluted cDNA (1:10) was used in each real-time RT-PCR using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX, USA). The cycle conditions were as follows: 5 min at 95 °C followed by 40 cycles of incubation at 94 °C for 15 s, then 55−60 °C for 1 min, and 72 °C for 30 s. The sequences of the primers used for this study are shown in Table 2 of the Supporting Information. 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 normalization to expression of *36B4* mRNA using the ΔΔCT method.[32]

Statistical Analysis. All data are expressed as means ± SE. Analysis of variance (ANOVA) was performed to examine the effect of treatment on plasma lipid levels, body and tissue weights, and energy intake using the JMP7 statistical program (SAS Institute, Cary, NC, USA). Significance was defined at $P < 0.05$. Statistical analysis of differences in microarray analysis data using the GeneSpring GX 11.0 program was performed by using an unpaired Student's t test. Ingenuity Pathway Analysis (IPA, Ingenuity Systems, Redwood City, CA, USA) was used to identify the biological functions, canonical pathway, and network pathway that were most significant to the data set. Right-tailed Fisher's exact testing was used to calculate a P value determining the probability. The probability that each biological function for data set, biological function and/or disease assigned to that network for that data set, association between genes in the data set, and the canonical pathway was explained by change alone.

RESULTS

Metabolic Effect and in Vivo Intestinal Permeability by HPMC Supplementation. Body weight gain and total energy intake were significantly less in the HPMC group compared with the control group, resulting in a 60% lower energy efficiency ratio in the HPMC group (Table 1). Liver, epididymal, and subcutaneous adipose weights of the HPMC group were 13, 49, and 51% lower, respectively, than those of the control group ($P < 0.05$) (Table 1). Plasma LDL-cholesterol concentration was 45% lower than that of the control group ($P < 0.05$) (Table 1), but plasma VLDL-, HDL-, and total-cholesterol concentrations did not differ between the control and HPMC groups (Table 1). To investigate whether HPMC supplementation alters HF-induced intestinal permeability, in vivo intestinal permeability was determined by using dextran-4000-FITC after HPMC feeding. Intestinal permeability was 48% lower in the HPMC group than in the control group ($P < 0.05$) (Table 1).

Table 1. Anthropometrics and Plasma Lipid Concentrations in Mice Fed 6% MCC or HPMC Diet for 5 Weeks[a]

	6% MCC	6% HPMC[b]
anthropometric data		
body wt gain (g)	10.3 ± 1.58	4.61 ± 0.55*
total energy intake (kcal)	419 ± 9.13	356 ± 9.83*
liver wt (g)	1.15 ± 0.05	0.94 ± 0.02*
epididymal adipose tissue wt (g)	1.97 ± 0.14	1.01 ± 0.15*
subcutaneous adipose tissue wt (g)	1.11 ± 0.11	0.54 ± 0.09*
blood glucose (mg/dL)	222 ± 18.2	196 ± 12.7
plasma lipid (mg/dL)		
VLDL cholesterol	4.36 ± 0.52	3.98 ± 0.46
LDL cholesterol	13.7 ± 2.13	7.60 ± 1.32*
HDL cholesterol	73.4 ± 3.75	74.2 ± 2.93
total cholesterol	91.4 ± 5.51	85.8 ± 4.15
intestinal permeability		
plasma dextran-4000-FITC (μg/mL)	0.62 ± 0.12	0.32 ± 0.05*

[a]Values are means ± SE, $n = 10$. [b]*, different from MCC, $P < 0.05$.

Effect of HPMC Supplementation on Insulin and Glucose Response. Dietary HPMC supplementation significantly lowered insulin response at 30 and 60 min ($P < 0.05$) and area under the curve during a 2 h insulin response (Figure 1A,B). HPMC supplementation also resulted in a marked reduction of peak glucose response at 30 min ($P < 0.05$) and area under the curve during a 2 h glucose response (Figure 1C,D).

Effect of HPMC Supplementation on Hepatic Gene Expression Related to Bile Acid and Lipid Metabolism. The mRNA level of hepatic gene involved in bile acid synthesis, CYP7A1, was up-regulated by 1.9-fold in the HPMC group relative to the control group (Figure 2). The levels of gene expression of SREBP-1c and SCD-1 were down-regulated by 2- and 5-fold, respectively, in the HPMC group relative to the control group (Figure 2).

Microarray Analysis of Hepatic Gene Expression Profiles. The comprehensive expression of hepatic genes of DIO mice fed HF diets supplemented with either 6% MCC or 6% HPMC was assessed by exon microarray analysis. A total of 35 genes including one unknown gene were differentially expressed in mice fed 6% HPMC compared to 6% MCC ($P < 0.05$, fold change >1.5) (Table 2). Among these genes, 13 were down-regulated and 22 up-regulated. Table 2 shows the genes differentially down- and up-regulated genes by HPMC based on biological process of gene ontology descriptions. The gene for glucocorticoid binding globulin protein involved in the glucocorticoid (GC) metabolic process, serine (or cysteine) peptidase inhibitor clade A member 6 (Serpina6; fold change −2.2), was down-regulated in mice fed HPMC compared to mice fed MCC. Genes encoding enzymes for C21-steroid hormone biosynthesis including hydroxy-δ-5-steroid dehydrogenase 3 β- and steroid δ-isomerase 2 (Hsd3b2; fold change −1.9), hydroxy-δ-5-steroid dehydrogenase 3 β- and steroid δ-isomerase 3 (Hsd3b3; fold change −1.7) were down-regulated. The expression level of aquaporin 8 (Aqp8; fold change −1.8), encoding a protein related to canalicular water transport during bile formation,[33] was also down-regulated. On the other hand, genes involved in cholesterol metabolism, including hydroxysteroid (17-β) dehydrogenase 7 (Hsd17b7; fold change 1.8), NAD(P)-dependent steroid dehydrogenase-like (Nsdhl; fold change 2.1), transmembrane 7 superfamily member 2 (Tm7sf2; fold change 2.4), cytochrome P450 family 51 (Cyp51; fold change 2.8), and squalene epoxidase (Sqle; fold change 3.2), were up-regulated following HPMC supplementation. Genes involved in fatty acid β-oxidation, such as enoyl-CoenzymeA hydratase (Ehhadh; fold change 2.0) and acetyl-coenzyme A carboxylase β (Acacb; fold change 2.3), were also up-regulated. The expression levels of elongation of very long chain fatty acid-like 2 (Elovl2; fold change 1.5) and sterol-C4-methyl oxidase-like (Sc4 mol; fold change 2.3), coding genes related to polyunsaturated fatty acid elongation were up-regulated. Patatin-like phospholipase domain containing 2 (Pnpla2; fold change 1.5), which is involved in triglyceride breakdown by regulating adipose triglyceride lipase, was up-regulated. Retinol dehydrogenase 11 (Rdh11; fold change 1.6), which is related to oxidation−reduction during lipid metabolic process, was up-regulated. Flavin containing monooxygenase2 (Fmo2; fold change 1.6), a gene related to reduction of oxidative stress, was also up-regulated. RT-PCR analysis confirmed that the expression level of Serpina6, encoding a protein involved in glucocorticoid metabolic process, was down-regulated by HPMC supplementation (Figure 3). The expression levels of fatty acid desaturase 1 (Fads1) and elongation of very long chain fatty acid-like 2 (Elovl2), which is related to polyunsaturated fatty acid elongation processes, were up-regulated (Figure 3). Expression of acetyl-coenzyme A carboxylase β (Acacb), the gene encoding a protein involved

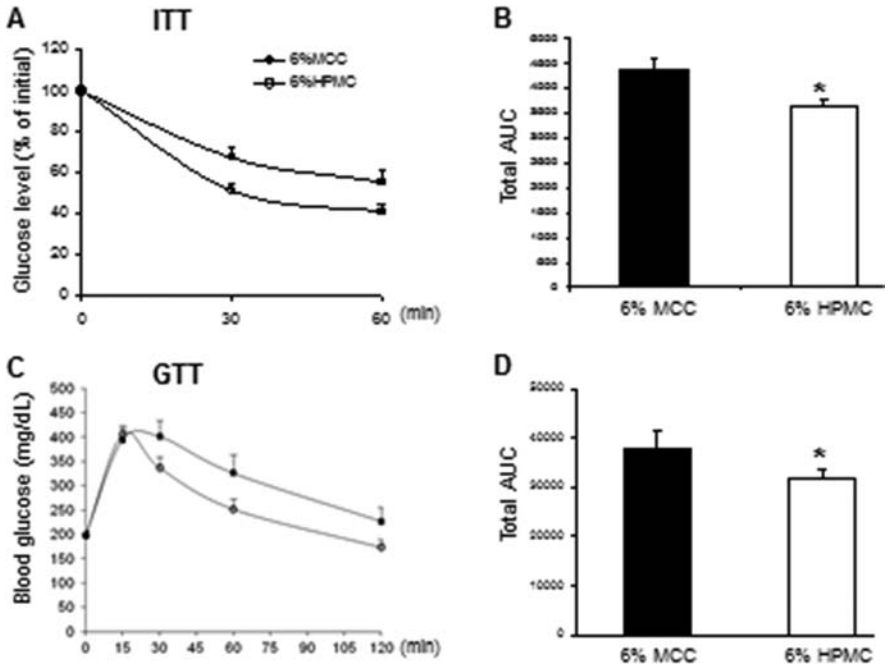

Figure 1. Insulin tolerance and glucose tolerance in obese mice fed HF-diet supplemented with either 6% HPMC or 6% MCC for 5 weeks: (A) insulin tolerance tests (ITT) performed in the fasting state; (B) area under the curve values in ITT; (C) glucose tolerance tests (GTT) performed in the fasting state; (D) area under the curve values in GTT. Data are expressed as the mean ± SE n = 8−9/group. ∗ indicates significant difference at P < 0.05.

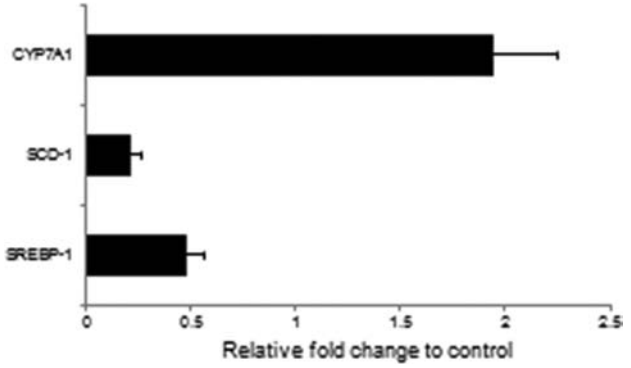

Figure 2. Relative heptic expression of CYP7A1, SCD-1, and SREBP-1c genes in obese mice fed HF-diet supplemented with either 6% HPMC or 6% MCC for 5 weeks. Data are expressed as the mean ± SE n = 6/group.

in fatty acid β-oxidation, was up-regulated following HPMC treatment (Figure 3).

To further analyze the role of differentially expressed genes by HPMC supplementation, we conducted a pathway analysis using the IPA System and identified several biological functions and canonical gene pathways differentially regulated by HPMC supplementation (Table 3). Genes related to biological functions of lipid metabolism and cardiovascular diseases were significantly expressed at different levels. Canonical pathways of androgen and estrogen metabolism, C21-steroid hormone metabolism, and LXR/RXR activation were affected by the HPMC supplementation. In addition, the networks involving lipid metabolism were affected. Networks involving sterol regulatory element binding transcription factor 1 (SREBF1), which is a transcription factor that regulates lipid homeostasis, were affected (data not shown).

Analysis of exon microarray data using the GeneSpring GX 11.0 program resulted in the identification of 10 genes with >1.0 of splicing index (SI) regulated by HPMC supplementation. However, there were no transcripts with SI ≥2, and RT-PCR analysis did not detect any alternative splicing in these 10 genes (data not shown).

DISCUSSION

HPMC and other SDFs have been previously shown to regulate expression of hepatic genes related to bile acid and cholesterol metabolism as well as lipid metabolism. In this study, we explored HPMC effects for novel functions in the liver using exon microarray analysis. We found that in addition to cholesterol and bile acid metabolism, dietary HPMC supplementation significantly affected expression of hepatic genes for glucocorticoid metabolic processes, enzymes for C21-steroid metabolism, androgen and estrogen hormone synthesis, and polyunsaturated fatty acid elongation processes. HPMC markedly up-regulated the expression of hepatic genes related to fatty acid β-oxidation (ACACB), polyunsaturated fatty acid elongation (Fads1, ELOVL2, SC4MOL), and bile acid and cholesterol synthesis (CYP7A1 and CYP51), whereas it significantly down-regulated the expression of gene related to glucocorticoid transport (Serpina6) in the blood. The alterations of hepatic gene expression were accompanied by reduced liver and body weight and energy intake as compared to the control group.

On the basis of studies implicating LPS absorption with hepatic inflammation,[5,8,10] we hypothesized that reduced intestinal permeability following HPMC supplementation would down-regulate hepatic expression of genes related to inflammation, thus ameliorating HF-induced NAFLD. In the current study, HPMC supplementation significantly reduced HF diet-induced in vivo intestinal permeability by approximately 50% compared to control diet. Unexpectedly,

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Table 2. Summary of Selected Hepatic Genes Significantly Down- or Up-regulated in Mice Fed HF Diet Supplemented with HPMC (Fold Change ≥1.5)

biological process (GO)[a]	symbol	name	fold change	gene ID
down-regulated				
bile water transport	Aqp8	aquaporin 8	−1.8	AF018952
C21-steroid hormone biosynthesis	Hsd3b2	hydroxy-δ-5-steroid dehydrogenase 3 β- and steroid δ-isomerase 2	−1.9	BC040397
	Hsd3b3	hydroxy-δ-5-steroid dehydrogenase 3 β- and steroid δ-isomerase 3	−1.7	M77015
oxidation−reduction process	Cyp2a4	cytochrome P450 family 2 subfamily a polypeptide 4	−2.2	BC063778
glucocorticoid metabolic process	Serpina6	serine (or cysteine) peptidase inhibitor clade A member 6	−2.2	BC013632
metabolic process (methylation)	Hnmt	histamine *N*-methyltransferase	−1.7	AB070524
up-regulated				
acyl-CoA metabolic process, fatty acid β-oxidation	Ehhadh	enoyl-coenzyme A hydratase	2.0	BC016899
acyl-CoA metabolic process, fatty acid β-oxidation	Acacb	acetyl-coenzyme A carboxylase β	2.3	AY451394
cholesterol biosynthetic process	Hsd17b7	hydroxysteroid (17-β) dehydrogenase 7	1.8	BC011464
	Nsdhl	NAD(P) dependent steroid dehydrogenase-like	2.1	AF100198
	Tm7sf2	transmembrane 7 superfamily member 2	2.4	BC014769
	Cyp51	cytochrome P450 family 51	2.8	BC031813
cholesterol metabolic process	Sqle	squalene epoxidase	3.2	BC056361
fatty acid elongation, polyunsaturated fatty acid	Elovl2	elongation of very long chain fatty acids (FEN1/Elo2 SUR4/Elo3 yeast)-like 2	1.5	AF170908
	Fads1	fatty acid desaturase 1	1.5	BC026831
	Sc4mol	sterol-C4-methyl oxidase-like	2.3	BC006802
lipid catabolic process	Pnpla2	patatin-like phospholipase domain containing 2	1.5	BC019188
lipid metabolic process (oxidation−reduction process)	Rdh11	retinol dehydrogenase 11	1.6	AF474027
NADP metabolic process (oxidative stress-reduction process)	Fmo2	flavin containing monooxygenase 2	1.6	AF184981

[a]Genes were classified into categories depending on the putative biological process in which they are involved according to the classification used by the Gene Ontology Consortium (GO).

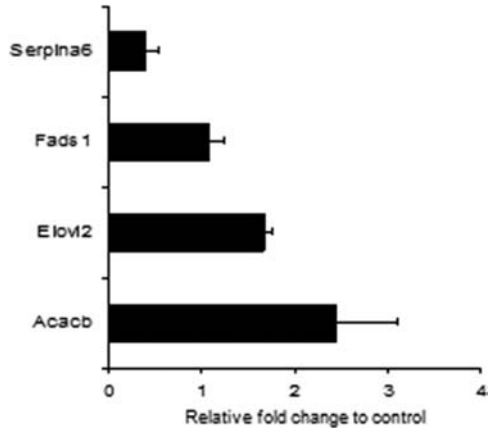

Figure 3. Relative hepatic expression of Serpina6, Fads1, Elovl2, and Acacb genes in obese mice fed HF-diet supplemented with either 6% HPMC or 6% MCC for 5 weeks. Data are expressed as the mean ± SE n = 5/group.

expression levels of hepatic CRP, TLR-4, and LBP genes were unchanged following HPMC supplementation confirmed by RT-PCR analysis (data not shown). Although microarray data analysis did not show significant changes in the expression of genes related to inflammatory processes, HPMC down-regulated expression of a hepatic gene (FMO2)[34] related to reduction of oxidative stress (FMO2). Other studies have shown that energy intake affects NAFLD,[35] and in this study15% less total energy intake following HPMC supplementation may have contributed to reduced hepatic lipid content and oxidative stress. Decreased energy intake, reduced hepatic lipid content, and oxidative stress may have improved insulin sensitivity. These results suggest that HPMC supplementation prevents progression of NAFLD by reducing hepatic oxidative stress and total energy intake. The DIO C57BL/6J mouse is considered to be an appropriate animal model for the study of NAFLD. This animal model was recently used to define early markers for NASH.[36] After 21 weeks of chronic HF feeding (45% energy as fat), the severity of NASH could be segregated into four groups; there was a greater expression of hepatic genes such as CD68 and TLR2 by the overt NASH responder group. In another study,[37] C57BL/6J mice fed a HF diet (60% of energy as fat) developed hepatic steatosis and inflammation after 24 weeks. In the present study we selected obese responder mice by feeding HF diet for 5 weeks and fed the selected DIO mice HF diets supplemented with either HPMC or MCC for an additional 5 weeks. The 10 weeks of HF diet feeding induced hepatic steatosis and oxidative stress that was improved by HPMC, but HPMC feeding did not affect markers of inflammation in liver. The lack of inflammatory response may be due to the duration of the study and the level of dietary fat; 10 weeks of HF diet (47% of energy as fat) feeding may not have been long enough to induce significant inflammatory response.

With respect to intestinal permeability, in addition to the reduced number of total bacteria and microbiota population changes,[27] the increased excretion of bile acid in response to HPMC[22] may be involved in the improvement of the intestinal barrier function seen in the DIO mice fed HPMC. Consistent with our study, bile acids increased intestinal permeability to LPS by down-regulation of tight junction protein expression,[38,39] whereas bile acid binding resins such as cholestyr-

Table 3. Top 10 Biological Functions (A), Top 12 Canonical Pathways (B), and Top 5 Network Pathways (C) of Hepatic Genes That Were Significantly Up- or Down-regulated by HPMC (Fold Change ≥1.5)[a]

(A) Biological Functions		
	P value	no. of genes differentially expressed
lipid metabolism	2.89×10^{-8}–4.56×10^{-2}	26
small molecule biochemistry	2.89×10^{-8}–4.56×10^{-2}	32
vitamin and mineral metabolism	1.51×10^{-6}–4.08×10^{-2}	9
molecular transport	9.64×10^{-6}–4.48×10^{-2}	17
cell cycle	8.53×10^{-5}–2.74×10^{-2}	4
carbohydrate metabolism	2.06×10^{-4}–4.86×10^{-2}	7
endocrine system development and function	3.51×10^{-4}–4.48×10^{-2}	7
cardiovascular disease	6×10^{-4}–3.87×10^{-2}	5
genetic disorder	6×10^{-4}–4.88×10^{-2}	15
metabolic disease	6×10^{-4}–4.76×10^{-2}	9

(B) Ingenuity Canonical Pathways		
	P value	ratio
androgen and estrogen metabolism	3.29×10^{-6}	5/142 (0.035)
C21-steroid hormone metabolism	7.34×10^{-5}	3/72 (0.042)
tryptophan metabolism	9.61×10^{-4}	4/253 (0.016)
alanine and aspartate metabolism	1.08×10^{-2}	2/88 (0.023)
galactose metabolism	1.25×10^{-2}	2/115 (0.017)
histidine metabolism	1.51×10^{-2}	(0.017)
fructose and mannose metabolism	1.78×10^{-2}	(0.014)
propanoate metabolism	2.14×10^{-2}	(0.015)
starch and sucrose metabolism	2.24×10^{-2}	(0.010)
inositol metabolism	2.45×10^{-2}	(0.053)
linoleic acid metabolism	2.57×10^{-2}	(0.016)
LXR/RXR activation	3.34×10^{-2}	(0.022)

(C) Network Pathways		
	score	focus molecules
gene expression, lipid metabolism, molecular transport	25	12
lipid metabolism, small molecule biochemistry, molecular transport	24	12
lipid metabolism, molecular transport, small molecule biochemistry	23	12
drug metabolism, lipid metabolism, small molecule biochemistry	22	11
drug metabolism, molecular transport, small molecule biochemistry	10	6

[a]The functions, canonical, and network pathways that were most significant to the data set were identified by Ingenuity Pathway Analysis (Ingenuity Systems).

amine reduce intestinal permeability.[40] Diets including HF diet[5] and fermentable soluble fiber[19] also modulated intestinal tight junction integrity through altered protein expression (ZO-1 and occluding).

Contrary to our expectation, hepatic expression of Serpina6 was down-regulated by HPMC supplementation. Serpina6 is also known as corticosteroid-binding globulin (CBG) and is dominantly produced by the liver. In plasma 80−90% of GC is bound to CBG and 10−20% by albumin, and only the remaining 5% of free GC is available to bind to intracellular GC receptors in tissues,[41] suggesting plasma CBG concentration is negatively associated with action of local GC. It has been well documented that chronic exposure to increased circulating GC induces obesity-related insulin resistance, diabetes, and fatty liver. In rats chronic administration of cortisone induces type 2 diabetes when they are fed high-fat diets.[42] Circulating GC is elevated in mouse models of dyslipidemia and hepatosteatosis,[43] in insulin-resistant and glucose-intolerant patients, and in fatty liver disease.[44] Chronic activation of GC has been suggested to induce fatty liver by increasing hepatic triglyceride synthesis.[45−47] In the current study, HPMC supplementation improved insulin sensitivity and glucose homeostasis as indicated by ITT and GTT results. Furthermore, HPMC significantly reduced liver weight and lipid content in the current and previous studies.[26] Therefore, HPMC supplementation is expected to up-regulate hepatic expression of CBG gene and lower hepatic action of GC. This discrepancy may be accounted for by hepatic concentration of GC and by the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which increases local GC concentrations by conversion of cortisone to cortisol.[48] However, hepatic expression of 11β-HSD1 gene was unchanged by HPMC supplementation (data not shown), although the activity of hepatic 11β-HSD1 was not determined. On the other hand, glucocorticoid up-regulated ABCB11 mRNA expression in rat hepatocytes and increased the recycling of bile acids.[49,50] Our previous studies[26] suggested that HPMC supplementation reduces enterohepatic BA recycling by increased fecal bile acid excretion. As a compensatory mechanism, hepatic BA synthesis was enhanced by HPMC supplementation. Subsequently, to release enterohepatic BA, hepatic BA excretion was increased as the expression of ABCB11 (a gene encoding a bile acid transporter) was up-regulated. Therefore, we cannot exclude the possibility that HPMC supplementation down-regulates circulating CBG and thus increases delivery GC to liver to up-regulate hepatic ABCB11 expression, resulting in enterohepatic bile acid recycling to compensate for fecal bile acid excretion.

In the current study, HPMC supplementation significantly down-regulated genes related to fatty acid synthesis such as SREBP-1c and SCD-1 and up-regulated expression of PNPLA2 gene, a gene indirectly related to hydrolysis of triglyceride to diacylglycerol.[51] HPMC also up-regulated hepatic genes related to rate-limiting enzymes for fatty acid oxidation such as Acacb and Ehhadh. Taken together, HPMC supplementation improves hepatic steatosis due to reduction of hepatic triglyceride synthesis and increase of fatty acid oxidation.

HPMC supplementation significantly up-regulated expression of genes related to cholesterol and bile acid biosynthetic process such as HSD17b7, NSDH1, TM7SF2, CYP51, SQLE, and CYP7A1 as compared to control, indicating increased hepatic cholesterol and bile acid synthesis to maintain homeostasis of the bile acid pool consistent with our previous study.[26] In Golden Syrian hamsters we found that modulation of fecal bile acid excretion and intestinal cholesterol absorption following HPMC intake contributes to the up-regulation of hepatic genes related to cholesterol and bile acid metabolism.[26]

Impaired glucose tolerance and fasting hyperglycemia both contribute to the development of type 2 diabetes from prediabetic status.[52] Our study revealed that HPMC supplementation significantly improved insulin sensitivity and glucose metabolism as shown by 16% lower AUC during 2 h ITT and GTT compared to the control group. Improved insulin sensitivity may also contribute to lower hepatic lipid synthesis. Modulation of hepatic genes related to lipid synthesis and fatty acid oxidation following HPMC supplementation may

in part contribute to the improved insulin sensitivity. However, HPMC supplementation only tended to lower fasting glucose concentration compared to the control group and did not reach statistical significance.

In summary, we have demonstrated that 6% HPMC intake reduced body weight gain, improved insulin sensitivity, and markedly enhanced glucose tolerance in obese mice fed a HF diet. Consistent with our previous data, HPMC up-regulates genes involved in cholesterol and bile acid synthesis and down-regulates genes involved in steroid biosynthesis, reducing plasma LDL-cholesterol concentration. HPMC's effects appear to involve a decreased hepatic lipid accumulation via the modulation of genes related to triglyceride synthesis and fatty acid oxidation. Whereas the effect of HPMC on hepatic steatosis could be mediated by both reduction of energy intake and increases in fecal lipid excretion, HPMC also altered intestinal barrier function and down-regulated a gene related to an oxidative stress pathway. Genes related to hepatic glucocorticoid activation may be affected by hepatic bile acid recycling to compensate for fecal bile acid excretion. Pathway analysis of microarray data identified lipid metabolism and cardiovascular diseases as being differentially regulated by HPMC. However, changes in alternative splicing regulation were not observed. Collectively, these findings suggest that consumption of a viscous and nonfermentable SDF, HPMC, by reducing hepatic steatosis, oxidative stress, intestinal permeability, and IR in response to a HF meal, may be potentially beneficial for prevention of metabolic diseases such as NAFLD.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(H.K.) Mailing address: Department of Nutrition, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA. E-mail: hyskim@ucdavis.edu. Phone: (530) 752-6305. Fax: (530) 752-8632.

Notes

The authors declare no competing financial interest.

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