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Corn husk oil lowers plasma LDL cholesterol concentrations by decreasing cholesterol absorption and altering hepatic cholesterol metabolism in guinea pigs

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To test the hypocholesterolemic mechanisms of corn husk oil (CoHO), male Hartley guinea pigs were fed diets containing increasing doses of CoHO, either 0 (control), 5, 10, or 15 g/100 g, and 0.25 g/100 g cholesterol. A positive control group (LC) with low dietary cholesterol (0.04 g/100 g) was also included. Fat was adjusted to 15 g/100 g in all diets by the addition of regular corn oil. Plasma low density lipoprotein (LDL) cholesterol concentrations were 32, 55, and 57% ($P < 0.0005$) lower with increasing doses of CoHO. In addition, intake of CoHO resulted in 32 to 43% lower hepatic total and esterified cholesterol and 55 to 60% lower triacylglycerol concentrations compared with the control group ($P < 0.01$). CoHO intake resulted in plasma and hepatic cholesterol concentrations similar to those in guinea pigs from the LC group. The number of cholesteryl ester and free cholesterol molecules was higher in LDL from the control group than in LDL from the CoHO or the LC groups. Hepatic β-hydroxy-β-methylglutaryl-coenzyme A reductase activity was not modified by CoHO intake whereas cholesterol 7α-hydroxylase was up-regulated by 45 to 49% ($P < 0.01$) in the 10 and 15 g/100 g CoHO groups. Hepatic acyl coenzyme A cholesterol acyltransferase activity was down-regulated in a dose-dependent manner by 54, 58, and 63% with increasing doses of CoHO. CoHO intake resulted in increased fecal cholesterol excretion by 40 to 55% compared with the control and LC groups. Total fecal neutral sterol excretion was enhanced 42 to 55% by CoHO compared with the control group and by 59 to 68% compared with the LC group. The data from these studies suggest that CoHO has its hypocholesterolemic effect by decreasing cholesterol absorption and increasing bile acid output. These alterations in the intestinal lumen alter hepatic cholesterol metabolism and may affect the synthesis and catabolism of lipoproteins. (J. Nutr. Biochem. 11:358–366, 2000)

Keywords: corn husk oil; LDL cholesterol; bile acids; neutral sterols; guinea pigs

Introduction

Elevated levels of plasma cholesterol pose a major risk factor for cardiovascular disease.[1,2] Numerous animal[3–7] and human studies[8–10] have documented the hypocholesterolemic effects of dietary soluble fiber and the mechanisms of action have been partly elucidated.[11] Though there are numerous well-documented studies about the protective effect of fiber in lowering plasma cholesterol concentrations, our knowledge is limited regarding the use of corn husks.[12] Lime-treated corn husks have been shown to lower plasma low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) in guinea pigs by decreasing microsomal free cholesterol,

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which alters the regulatory enzymes of cholesterol homeostasis and up-regulates hepatic LDL receptors.[13] Human studies with lime-treated maize husks have also shown plasma LDL-C lowering in normal and hypercholesterolemic adult men.[14]

Corn husk oil (CoHO) is obtained from the husk of corn. The fatty acid profile of CoHO is similar to that of corn oil; however, the sterol composition of CoHO is different from that of corn oil. CoHO contains approximately 13.9% phytosterols, 6.7% as ferulate esters, 5.9% as steryl esters, and 1.3% as free sterols. Numerous studies have documented the protective effect of phytosterols on plasma lipids[15–18]; however, the mechanisms of action are not well understood. Several mechanisms such as competitively blocking cholesterol absorption,[19,20] increasing bile salt excretion,[21] hindering cholesterol esterification,[22] and displacement of cholesterol from bile salt micelles[23] have been proposed to account for the action of phytosterols on lipid metabolism.

The main objectives of this study were (1) to assess the plasma cholesterol-lowering properties of CoHO, (2) to determine whether there is a dose response associated with CoHO intake, and (3) to evaluate some of the mechanisms involved in the plasma cholesterol-lowering response. Guinea pigs were chosen as the animal model because their lipoprotein profiles are similar to those of humans. Guinea pigs have a high LDL:high density lipoprotein (HDL) ratio,[24] a hepatic cholesterol pool consisting of more free than esterified cholesterol,[25] similar tissue distribution of cholesterol synthesis, with liver contributing less than 20%, and similar responses to diet.[26]

Materials and methods

Materials

Reagents were obtained from the following sources: enzymatic cholesterol and triacylglycerol kits, cholesterol oxidase, cholesterol esterase, and peroxidase from Boehringer-Mannheim (Indianapolis, IN USA). Phospholipid and free cholesterol enzymatic kits were obtained from Wako Pure Chemical (Osaka, Japan). Quick-seal ultracentrifuge tubes were from Beckman (Palo Alto, CA USA) and halothane from Halocarbon (Hackensack, NJ USA). DL-hydroxy-[3-^{14}C] methylglutaryl-coenzyme A (1.81 GBq/mmol), DL-[5-^{3}H] mevalonic acid (370 GBq/mmol), cholesteryl-[1,2,6,7-^{3}H] oleate (370 GBq/mmol), Aquasol, Liquiflour (toluene concentrate), and [^{14}C] cholesterol were purchased from Dupont NEN (Boston, MA USA). Oleoyl-[1-^{14}C] coenzyme A (1.8 GBq/mmol) and DL-β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) were bought from Amersham (Clearbrook, IL USA). Cholesteryl oleate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate (NADP), ethylenediamine-tetraacetic acid (EDTA), NaF, Triton X-100, bovine albumin, petroleum ether, methylene chloride, and sucrose were obtained from Sigma Chemical Co. (St. Louis, MO USA). Aluminum and glass silica gel plates were purchased from EM Science (Gibbstown, NJ USA). 5-α Cholestane and 7α- and 7β-hydroxycholesterol were obtained from Steraloids Inc. (Wilton, NH USA). CoHO was provided by Monsanto Company (St. Louis, MO USA).

Table 1 Composition of experimental diets

Nutrient	Nutrient (g/100 g)				
Dietary groups	I	II	III	IV	V
Protein[1]	22.3	22.3	22.3	22.3	22.3
Corn oil	15	15	10	5	0
Corn husk oil	0	0	5	10	15
Starch	30	30	30	30	30
Sucrose	10.6	10.6	10.6	10.6	10.6
Vitamins[2]	1.1	1.1	1.1	1.1	1.1
Minerals[2]	8.2	8.2	8.2	8.2	8.2
Fiber[3]	12.5	12.5	12.5	12.5	12.5
Kcal/g	3.87	3.87	3.80	3.73	3.66
Nutrient caloric density (g/1,000 kcal)					
Fiber	32.2	32.2	32.9	33.5	34.2
Minerals	21.1	22.1	21.6	21.9	22.1
Vitamins	2.83	2.83	2.89	2.95	3.0
Protein	57.4	57.4	58.7	59.8	60.9
Cholesterol	0.64	0.10	0.66	0.67	0.69

Diet I is the negative control, Diet II is the positive control, and Diets II, IV, and V have increasing concentrations of corn husk oil.
[1]Protein is soybean protein.
[2]Vitamins and minerals were formulated to meet National Research Council (NRC) specified requirements for guinea pigs.
[3]Fiber is a mixture of 10 g/100 g cellulose and 2.5 g/100 g guar gum.

Diets

Diets were prepared and pelleted by Research Diets Inc. (New Brunswick, NJ USA). Formulation of the five diets is presented in *Table 1*. All the diets were identical in composition except for the type of fat and the amount of dietary cholesterol. Diets I, III, IV, and V contained 0.25 g/100 g cholesterol and increasing concentrations of CoHO replacing corn oil: 0 (control diet), 5, 10, and 15 g/100 g. Diet II was a positive control, low cholesterol diet (LC) and contained 15 g/100 g regular corn oil and 0.04 g/100 g cholesterol, which is equivalent to 300 mg/day in humans.[27] The nutrient caloric density was adjusted for all diets (*Table 1*). The fatty acid composition of the corn oil and CoHO and the phytosterol composition of CoHO are presented in *Table 2*.

Animals

Fifty male Hartley guinea pigs (Harlan Sprague-Dawley, Indianapolis, IN USA), weighing between 300 and 400 g, were randomly assigned to one of the five dietary groups (10 per group).

Table 2 Fatty acid composition of corn oil and corn husk oil

Fatty acids	Corn oil (%)	Corn husk oil[1] (%)
16:0	10.0	7.5
18:0	1.8	3.5
18:1	22.2	48
18:2	54.7	40.5
20:0	6.0	0.5
Other	5.3	—

[1] Corn husk oil contains 13.9% phytoesterols and 86.1% fatty acids. Phytoesterols are composed of 6.7% ferulate esters, 5.9% steryl esters, and 1.3% free sterols. Sterol esters are compasterol, campestanol, stigmasterol, sitostanol, and sitosterol. Free sterols are stigmasterol, sitosterol, and sitostanol. Ferulate esters are compesterol, campestanol, sitosterol, and sitostanol. Sitostanol constituted more than 80% total phytoesterols.

Two guinea pigs were kept per metal cage and were housed in a light cycle room (light from 7:00 AM to 7:00 PM) with a temperature of 23°C and free access to diet and water. Guinea pigs were fed the experimental diets for a period of 4 weeks. Nonfasted animals were sacrificed by cardiac puncture after halothane anesthesia. Blood (approximately 15 mL per guinea pig) was collected to analyze plasma lipids and to isolate lipoproteins for further characterization. Liver was harvested to determine hepatic lipids and to isolate microsomes to measure the activities of regulatory enzymes of cholesterol homeostasis. To measure the fecal neutral sterols, six animals from each group were housed individually for 3 days and food intake was monitored and feces were collected. Animal studies were conducted in accordance with U.S. Public Health Service guidelines. Experimental protocols were approved by the University of Connecticut Institutional Care and Use Committee.

Lipoprotein isolation

Plasma samples were obtained from blood collected by cardiac puncture from guinea pigs under halothane anesthesia, with EDTA (1.5 mg/mL) as an anticoagulant. Five hundred microliters of plasma from each sample was stored at 4°C for further analysis and the rest was used for lipoprotein isolation. A mixture of aprotinin (0.5 mL/100 mL), phenyl methyl sulfonyl fluoride (PMSF; 0.1 mL/100 mL), and sodium azide (0.1 mL/100 mL) were added to the samples to prevent changes in lipoprotein concentration during isolation.[6,7]

Lipoprotein isolation was done by sequential ultracentrifugation[28] in a LE-8M Ultracentrifuge (Beckman). VLDL was isolated at a density of 1.006 g/mL at 125,000 g at 15°C for 19 hr in a Ti-50 rotor. LDL was isolated in a density range of 1.019 to 1.09 g/mL in quick-seal tubes at 200,000 g at 15°C for 3 hr in a vertical Ti-65.2 rotor.[29]

Plasma and hepatic lipids

Plasma samples were analyzed for cholesterol and triglycerides by enzymatic methods.[30] Plasma HDL cholesterol was determined using the precipitation method of Warnick et al.[31] using 2 M Mg-dextran sulfate as a modification.[29] Hepatic total and free cholesterol and triglycerides were determined according to Carr et al.[32] after following the extraction of hepatic lipids with chloroform-methanol 2:1. Cholesteryl ester concentrations were calculated by subtracting free cholesterol from total cholesterol.

Lipoprotein characterization

VLDL and LDL composition was calculated by determining free and esterified cholesterol,[30] protein was determined by a modified Lowry procedure,[33] and triacylglycerol (TAG) and phospholipids were analyzed by enzymatic kits. The number of constituent molecules of LDL was calculated on the basis of one apo B per LDL particle with a molecular mass of 412,000 kD.[34] The numbers of molecules of TAG, free cholesterol, esterified cholesterol, and phospholipids were calculated using molecular weights of 885.4, 386.6, 645, and 734, respectively.[35] Apo B in VLDL was precipitated overnight at room temperature with an equal volume of 70% isopropyl alcohol[36] to determine apo B concentration in VLDL to calculate the number of molecules of all the other components. LDL diameter was calculated according to the method of Van Heek and Zilversmit.[37]

Hepatic microsome isolation

Liver tissues were pressed through a tissue grinder into 1:2.5 (wt/v) cold homogenization buffer (50 mmol/L KH_2PO_4, 0.1 mol/L sucrose, 50 mmol/L KCI, 50 mmol/L NaCl, 30 mmol/L EDTA, and 2 μmol/L dithiothreitol, pH 7.2) and mixed using a Potter-Elvehjem homogenizer (Eberbach Corp., Ann Arbor, MI USA). A microsomal fraction was isolated after two 15-min centrifugations at 100,000 g followed by ultracentrifugation at 100,000 g in a Ti-50 rotor at 4°C. Microsomes were resuspended in the homogenization buffer and centrifuged for 1 additional hour at 100,000 g. After centrifugation, microsomal pellets were homogenized and stored at −70°C. The protein content of microsomes was measured by the method reported by Markwell et al.[33]

Hepatic HMG-CoA reductase assay

Hepatic microsomes were used to measure the activity of HMG-CoA reductase as described by Shapiro et al.[38] Two hundred micrograms of the microsomal protein was briefly incubated with 7.5 nmol (0.33 GBq/nmol) [3-^{14}C] HMG-CoA, 4.5 mol glucose-6-phosphate, 3.6 μmol EDTA, 0.45 μmol NADP, and 0.3 IU glucose-6-phosphate dehydrogenase, and 0.024 Gbq [^{3}H] mevalonic acid was added as an internal recovery standard. The reaction was stopped after 15 min with 10 M HCl and 1.2 Kg/L of unlabeled mevalonate was added to increase the recovery. The samples were further incubated at 37°C for 30 min, the microsomal protein was precipitated by centrifugation for 1 min, and an aliquot of the supernatant (0.1 mL) was applied to the aluminium silica plates. Plates were developed in acetone-benzene 1:1 and the area containing the mevalonate (R_f 0.6−0.9) was scraped and mixed with 5 mL of aquasol to measure the radioactivity in a liquid scintillation counter. HMG-CoA reductase activity was expressed as pmol of [^{14}C] mevalonate produced per minute per milligram of microsomal protein and the recovery was between 70 ± 8%.

Hepatic acyl coenzyme A cholesterol acyltransferase assay

Hepatic acyl coenzyme A cholesterol acyltransferase (ACAT; E.C.2.3.1.26) activity was assayed by the method of Smith et al.[39] The isolated hepatic microsomes (0.8–1 mg protein per assay) were preincubated with albumin (84 mg/mL) and buffer (50 mmol KH_2PO_4/L, 1 mol sucrose/L, 50 mmol KCl/L, 30 mmol EDTA/L, and 50 mmol NaF/L) to a final volume of 0.18 mL. After 5 min at 37°C, 20 μL (500 μmol/L) of oleoyl-[1-^{14}C] coenzyme (0.15 GBq/pmol) was added, and the reaction was continued for 15 min at the same temperature. The reaction was stopped by adding 2.5 mL of chloroform-methanol 2:1 and [^{3}H] cholesterol oleate (0.045 GBq/assay) was added as an internal recovery standard. The samples were mixed and allowed to stand overnight at room temperature. The aqueous phase was removed and the organic phase was dried under nitrogen. The samples were resuspended in 0.150 mL of chloroform containing 30 μg of unlabeled cholesteryl oleate. Samples were applied to glass silica gel thin layer chromatography (TLC) plates and developed in hexane-diethyl ether 9:1. Cholesteryl esters were visualized with iodine vapors, scraped from the TLC plates, and counted in a scintillation counter. Recoveries of the [^{3}H] cholesteryl oleate ranged from 82 ± 14%.

Hepatic cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase activity was assayed by the method modified by Jelinek et al.[40] using [^{14}C] cholesterol (3.6 μmol per assay) as a substrate. Cholesterol was delivered as cholesterol: phosphatidylcholine liposomes (1:8 by weight) prepared by sonication and a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose-6-phosphate) was included in the assay. Glucose-6-phosphate dehydrogenase (0.3 IU) was added and the samples were incubated for an additional 30 min. The reaction was stopped by adding 5 mL of chloroform-methanol 2:1 and 1 mL of

Table 3 Plasma TC, VLDL, LDL, and HDL cholesterol and triacylglycerol concentrations of guinea pigs fed increasing doses of corn husk oil (CHO) and guinea pigs fed a low cholesterol (LC) diet

	Plasma lipids (mmol/L)*				
Groups	TC	VLDL-C	LDL-C	HDL-C	TAG
0 g/100 g CHO	5.39 ± 1.62[a]	0.36 ± 0.10[a]	4.68 ± 1.60[a]	0.36 ± 0.10	0.74 ± 0.44
5 g/100 g CHO	380 ± 1.44[b]	0.18 ± 0.08[b]	3.21 ± 1.55[b]	0.38 ± 0.15	0.68 ± 0.32
10 g/100 g CHO	2.79 ± 1.27[b]	0.21 ± 0.08[b]	2.12 ± 1.22[b]	0.47 ± 0.13	0.75 ± 0.42
15 g/100 g CHO	2.56 ± 0.49[b]	0.16 ± 0.08[b]	2.02 ± 0.62[b]	0.39 ± 0.23	0.54 ± 0.23
LC	2.79 ± 1.27[b]	0.31 ± 0.21[a,b]	1.84 ± 0.75[b]	0.46 ± 0.10	ND

*Values are presented as mean ± SD for 10 guinea pigs per dietary treatment. Numbers in a column with different superscripts are significantly different as determined by one-way analysis of variance and Newman-Keuls as post-hoc test ($P < 0.01$).
TC—total cholesterol. VLDL-C—very low density lipoprotein cholesterol. LDL-C—low density lipoprotein cholesterol. HDL–high density lipoprotein cholesterol. TAG—triacylglycerol. ND—not determined.

acidified water (5% sulfuric acid). The tubes were thoroughly mixed and then the top layer was discarded and the samples dried under nitrogen. Samples and 7α- and 7β-hydroxycholesterol standards each were dissolved in 100 μL of chloroform, applied to glass silica gel TLC plates, and developed in ethyl acetate-toluene 3:2. The plates were exposed to iodine vapors to mark the 7α- and 7β-hydroxycholesterol standards and then placed on XAR-5 film with intensifying screen overnight. Using the film as a reference, the [^{14}C] 7α-hydroxycholesterol spots were determined and scraped. Five milliliters of liquiflour was added and the radioactivity was counted in a scintillation counter.

Fecal neutral sterols measurement

Fecal neutral sterols were measured by the method of Daggy et al.[41] Two hundred fifty milligrams of dry feces were saponified with 3 mL of 0.3 M KOH and placed in a Reacti-Therm heating block for 1 hr at 70°C. Twenty-five microliters of 5-α cholestane was added to each sample as an internal standard. After 1 hr the samples were removed from the heating blocks, cooled at room temperature, and poured into Whatman Autovial Syringeless Filter Device. Twenty milliliters of petroleum ether and 2 mL of DI water were added to the filtrate. The samples were mixed and 14 mL was transferred to another screw top tube and mixed again after the addition of 10 mL of petroleum ether. Eight milliliters of this extract was taken, dried under nitrogen, resuspended in 500 μL of methylene chloride, transferred to a polypropylene-footed glass GC vial, and analyzed by capillary gas chromatography in a HP-5 ultra 2 column, 50 meters, 0.33 film thickness, 0.32 ID 1:100 split mode. Cholesterol, coprostanol, sitosterol, sitostanol, and stigmasterol were quantified by the use of appropriate standards.

Statistical analysis

One-way analysis of variance was used to determine differences in total cholesterol, LDL-C, VLDL-C, hepatic lipids, hepatic enzyme activities, and neutral sterols among the five dietary groups. The Newman-Keuls test was used as post-hoc test to test the differences among groups. Data are presented as mean ± SD, and differences were considered significant at a *P*-value of less than 0.05.

Results

Effect of CoHO on plasma lipids

There were no significant differences in body weights among the guinea pigs fed the different diets. The daily weight gain was 6 ± 2, 9 ± 1, 6 ± 1, 7 ± 2, and 6 ± 2 g/day, respectively, for the control, 5, 10, and 15 g/100 g, and LC groups. In addition, guinea pigs consumed similar

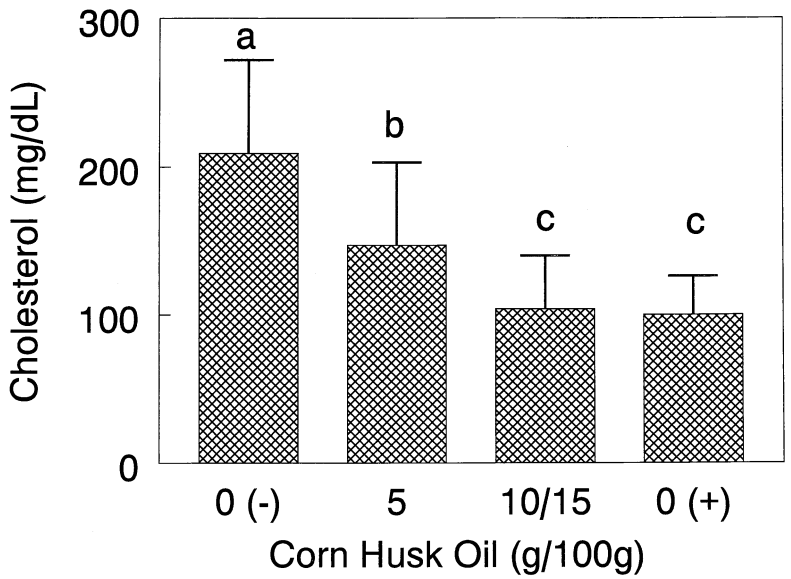

Figure 1 Plasma total cholesterol dose response in guinea pigs fed increasing doses of corn husk oil. The LC group (+) was fed 0.04 g/100 g cholesterol. a, b, and c indicate significant differences.

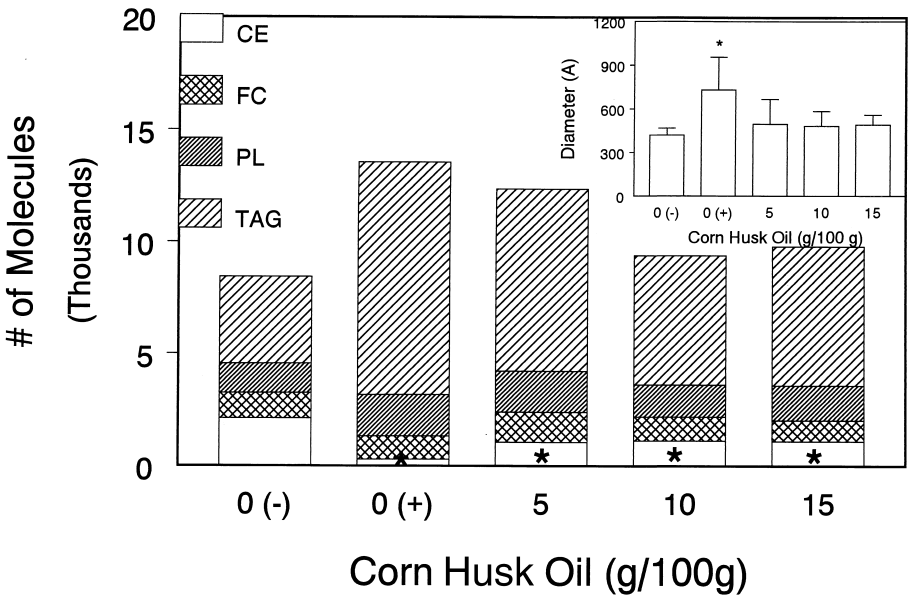

Figure 2 Number of cholesteryl ester (CE), free cholesterol (FC), phospholipids (PL), and triacylglycerol (TAG) molecules in very low density lipoprotein (VLDL) from guinea pigs fed 0 g, 5, 10, and 15 g/100 g corn husk oil, with 0.25 g/100 g cholesterol and control diet 0 (+) with 0.04 g/100 g cholesterol. The inset represents VLDL diameters. *Significantly different from the 0 g/100 g.

amounts of diet and the mean food intake was 26.23 ± 7.6 g/day. CoHO intake resulted in lower plasma cholesterol by 30% in the 5 g/100 g CoHO group and by 49% and 53% in the 10 and 15 g/100 g CoHO groups compared with the control ($P < 0.0005$; *Table 3*). Total plasma cholesterol exhibited a dose-dependent response when the 10 and 15 g/100 g CoHO samples were pooled together. The cholesterol lowering in guinea pigs fed 10 and 15 g/100 g CoHO was comparable to the results obtained in guinea pigs fed low cholesterol (0.04 g/100 g; *Figure 1*). The lowering of LDL-C followed a similar trend: With increasing doses of CoHO, LDL-C was 32, 55, and 57% lower and VLDL-C was 44 to 57% lower than the control ($P < 0.0005$). CoHO intake for all the three groups resulted in plasma total, LDL-C, and VLDL-C values similar to those in guinea pigs fed relatively low levels of dietary cholesterol (LC). Plasma HDL concentrations were unaffected by CoHO treatment or by dietary cholesterol. Plasma TAG concentrations were also unaffected by CoHO intake (*Table 3*).

VLDL from the control group contained a higher proportion of cholesteryl ester molecules than did VLDL from the CoHO or LC diets (*Figure 2*). Treatment with CoHO resulted in a decrease in the number of cholesteryl ester molecules by 33 to 51% compared with the control. No differences were observed in the number of free cholesterol or phospholipid molecules among groups. CoHO intake resulted in a 41 to 44% higher number of TAG compared with the control. VLDL from guinea pigs fed low cholesterol (LC diet) had a larger diameter compared with that of the other four dietary groups (*Figure 2*).

LDL composition was modified by CoHO treatment. The number of molecules of cholesteryl ester was higher in the control group ($P < 0.01$) than in the CoHO or the LC groups (*Table 4*). The number of free cholesterol molecules was 30 to 40% lower in LDL from the CoHO groups compared with the control and similar in number to LDL from guinea pigs fed the low cholesterol diet (*Table 4*).

Effect of CoHO on hepatic cholesterol concentrations

CoHO intake resulted in 32 to 43% lower hepatic total cholesterol compared with the control group ($P < 0.001$). Guinea pigs fed the low cholesterol diet had even lower hepatic total cholesterol concentrations (*Table 5*). Hepatic free cholesterol did not change with increasing doses of CoHO whereas hepatic cholesteryl esters were 47, 70, and 72% lower in the CoHO groups compared with controls. In

Table 4 Number of cholesteryl ester (CE), free cholesterol (FC), triacylglycerol (TAG), and phospolipids (PL) in low density lipoprotein (LDL) of guinea pigs fed increasing doses of corn husk oil (CHO) and guinea pigs fed a low cholesterol (LC) diet

	LDL number of molecules[1]			
Groups	CE	FC	TAG	PL
0 g/100 g CHO	1081 ± 462^{a}	277 ± 97^{a}	118 ± 38^{b}	169 ± 122^{a}
5 g/100 g CHO	740 ± 154^{b}	187 ± 31^{b}	93 ± 21^{b}	112 ± 74^{a}
10 g/100 g CHO	734 ± 154^{b}	197 ± 61^{b}	95 ± 23^{b}	90 ± 25^{b}
15 g/100 g CHO	734 ± 122^{b}	166 ± 41^{b}	100 ± 20^{b}	109 ± 64^{a,b}
LC	522 ± 104^{b}	103 ± 84^{b}	205 ± 72^{a}	185 ± 47^{a}

[1]Values are presented as mean ± SD for 10 guinea pigs per dietary treatment. Numbers in a column with different superscripts are significantly different as determined by one-way analysis of variance and Newman-Keuls as post-hoc test ($P < 0.01$).

Table 5 Hepatic total cholesterol (TC), free cholesterol (FC), esterified cholesterol (CE), and triacylglycerol (TAG) of guinea pigs fed increasing doses of corn husk oil (CHO) and guinea pigs fed a low cholesterol (LC) diet

	Hepatic lipids[1] (μmol/g)			
Groups	TC	FC	CE	TAG
0 g/100 g CHO	6.21 ± 1.69[a]	4.53 ± 1.22[a]	1.71 ± 0.80[a]	21.6 ± 6.35[a]
5 g/100 g CHO	3.57 ± 1.24[b]	2.69 ± 1.27[b]	0.90 ± 0.54[b]	9.53 ± 4.42[b]
10 g/100 g CHO	4.11 ± 1.54[b]	3.65 ± 1.58[a,b]	0.52 ± 0.28[b]	9.42 ± 4.42[b]
15 g/100 g CHO	4.29 ± 1.75[b]	3.91 ± 1.71[a,b]	0.49 ± 0.28[b]	8.16 ± 4.08[b]
LC	1.75 ± 0.59[c]	1.58 ± 0.54[c]	0.23 ± 0.18[c]	4.20 ± 1.47[b]

[1]Values are presented as mean ± SD for 10 guinea pigs per dietary treatment. Numbers in a column with different superscripts are significantly different as determined by one-way analysis of variance and Newman-Keuls as post-hoc test ($P < 0.01$).

addition, the 5 and 10 g/100 g CoHO groups had 57% and the 15 g/100 g CoHO group 63% lower hepatic TAG compared with the control (*Table 5*). Similar to the results for hepatic cholesterol, TAG concentrations were considerably lower in animals fed low dietary cholesterol compared with the control group. CoHO treatment resulted in general in a hepatic profile similar to guinea pigs fed low levels of cholesterol (*Table 4*).

Effect of CoHO on hepatic enzyme activities

Guinea pigs from all groups had similar HMG-CoA reductase activity, indicating no effects on this enzyme by CoHO intake (*Table 6*). In contrast, CoHO intake resulted in higher hepatic cholesterol 7α-hydroxylase activity compared with the control group. Intake of 10 and 15 g/100 g CoHO up-regulated cholesterol 7α-hydroxylase activity by 45 to 49% ($P < 0.01$; *Table 6*). In addition, CoHO intake down-regulated hepatic ACAT activity in a dose-dependent manner by 54, 58, and 63% in the 5, 10, and 15 g/100 g CoHO groups compared with the control group (*Table 6*).

Effect of CoHO on fecal neutral sterols

CoHO treatment resulted in increased fecal cholesterol excretion by 40 to 55% compared with the control group and with guinea pigs fed low dietary cholesterol (positive control *Table 7*). Fecal coprostanol remained unaffected by intervention with CoHO or dietary cholesterol. Fecal sitostanol was almost negligible in both the negative and positive control groups and increased significantly in a dose-dependent manner by 98 and 99% with increasing doses of CoHO (*Table 7*). A dose of 15 g/100 g CoHO intake enhanced fecal stigmasterol excretion by 93% and fecal sitosterol by 75 to 80% compared with the control group and with the low cholesterol group, respectively (*Table 7*).

Fecal sitostanol excretion was much higher in the CoHO fed guinea pigs than in control groups. A dose-dependent increase in sitostanol excretion was observed following intake of increasing doses of CoHO. Values for fecal sitostanol were 0, 21.16 ± 6.1, 26.34 ± 13.4, and 38.77 ± 25.1 for the control and 5, 10, and 15 g/100 g CoHO groups, respectively. CoHO intake enhanced total neutral sterol excretion by 42 to 55% compared with the control group and by 59 to 68% compared with the guinea pigs fed low cholesterol diets (*Table 7*).

Table 6 HMG-CoA reductase (HMG-CoA R), cholesterol 7α-hydroxylase (C7H), acylcoenzyme A cholesterol acyltransferase (ACAT) activities of guinea pigs fed increasing doses of corn husk oil (CHO)

	Activity (pmol/min/mg)[1]		
Groups	HMG-CoA R	C7H	ACAT
0 g/100 g CHO	5.98 ± 1.89	3.49 ± 1.22[b]	113 ± 58[a]
5 g/100 g CHO	5.39 ± 2.26	3.76 ± 1.71[b]	53 ± 18[b]
10 g/100 g CHO	6.58 ± 2.30	6.81 ± 3.38[a]	48 ± 27[b]
15 g/100 g CHO	6.47 ± 1.95	6.29 ± 2.98[a]	42 ± 20[b]

[1]Values are presented as mean ± SD for 10 guinea pigs per dietary treatment. Numbers in a column with different superscripts are significantly different as determined by one-way analysis of variance and Newman-Keuls as post-hoc test ($P < 0.01$).
HMG-CoA—β-hydroxy-β-methylglutaryl-coenzyme A.

Discussion

CoHO effects on neutral sterol excretion

Numerous studies have documented that phytosterols decrease cholesterol absorption and several mechanisms such as competitively blocking cholesterol absorption,[19,20] increasing bile salt excretion,[21] hindering cholesterol esterification,[22] and displacement of cholesterol from bile salt micelles[23] have been postulated for its action. Phytosterols and cholesterol share similarities in structure and this in part could also be responsible for the hypocholesterolemic effect of CoHO. In addition, phytosterols are bulkier molecules than is cholesterol and could affect sterol metabolism by modifications in binding to enzymes and transport proteins.[42]

Rice bran oil has been associated with significant cholesterol-lowering effects,[43] and the hypocholesterolemic properties of rice bran oil have been attributed to the nonsaponifiables present in the oil.[44] Studies have indicated that nearly 20% of the nonsaponifiables in rice bran oil have been accounted for by oryzanol, a mixture of ferulic acid esters of plant sterols and triterpene alcohols.[45] Studies have also indicated that the nonsaponifiables inhibit dietary cholesterol absorption by increasing the fecal neutral sterol excretion and this prevents the diet-induced hypercholesterolemia.[45]

Sterol analysis of CoHO indicated that ferulate ester is the major component and accounts for 48% of the phytosterols fraction of CoHO. The steryl esters contribute to 42%

Table 7 Fecal neutral sterols and total neutral sterols concentrations of guinea pigs fed increasing doses of corn husk oil (CHO) and guinea pigs fed a low cholesterol (LC) diet

	Fecal neutral sterols[1] (mg/kg/day)				
Group	Cholesterol	Coprostanol	Sitostanol	Stigmasterol	Sitosterol
0 g/100 g CHO	3.80 ± 2.98[b]	5.14 ± 2.7	0 ± 0[b]	0 ± 0[b]	3.54 ± 2.45[b]
5 g/100 g CHO	8.33 ± 3.16[a]	9.26 ± 5.60	21.16 ± 6.10[a]	0.59 ± 0.71[a]	7.57 ± 3.47[a]
10 g/100 g CHO	6.27 ± 3.82[a]	5.14 ± 1.99	26.34 ± 13.40[a]	1.24 ± 1.14[a]	9.81 ± 6.51[a]
15 g/100 g CHO	7.29 ± 4.5[a]	5.23 ± 4.47	38.77 ± 25.10[a]	2.43 ± 1.59[a]	14.32 ± 8.53[a]
LC	2.55 ± 2.72[b]	3.57 ± 3.70	0.35 ± 0.57[b]	0.17 ± 0.43[b]	2.94 ± 1.92[b]

[1]Values are presented as mean ± SD for six guinea pigs per dietary treatment. Numbers in a column with different superscripts are significantly different as determined by one-way analysis of variance and Newman-Keuls as post-hoc test ($P < 0.01$).

of the phytosterol fraction and the remaining 10% is accounted by free sterols. Sitostanol is the major component of the sterol fraction. In the present study a significant increase in total fecal neutral sterols has been observed with 5, 10, and 15 g/100 g CoHO intake, which could be due to the presence of sitostanol or the ferulate esters.[45] This indicates that CoHO has a profound effect on cholesterol metabolism and that the phytosterols present in CoHO may be responsible for the decrease in cholesterol absorption.

CoHO effects on plasma lipids and lipoprotein concentrations

In these studies, we demonstrated that CoHO has a significant hypocholesterolemic effect in guinea pigs fed high cholesterol and a dose response trend was observed in this plasma cholesterol lowering as a result of decreased cholesterol absorption. Intake of low levels of dietary cholesterol (0.04 g/100 g) resulted in low levels of plasma cholesterol as expected.[7,46] CoHO intake resulted in a lowering of LDL-C in guinea pigs fed high dietary cholesterol (0.25 g/100 g) to similar levels as guinea pigs fed relatively low levels of dietary cholesterol. Thus, high plasma total and LDL cholesterols, the major risk factors in the pathogenesis of coronary heart disease, were significantly lowered by CoHO intake.

CoHO intake not only affected the plasma lipoprotein concentration but also had an effect on lipoprotein composition. CoHO intake resulted in a larger VLDL molecule depleted of cholesteryl esters and enriched in phospholipids and TAG. The larger VLDL particles are good substrates for the enzyme lipoprotein lipase that converts nascent VLDL to mature VLDL through loss of TAG.[47] VLDL enriched with TAG and depleted of cholesteryl ester molecule favors a slower conversion of VLDL to LDL,[48] possibly due to increased clearance by apo B/E receptor, which also leads to lower plasma LDL-C concentrations.

The reductions in both free and esterified cholesterol in LDL by CoHO resulted in a smaller LDL particle. Small LDL generated by intake of diets high in polyunsaturated fat has been shown to have a faster catabolism than does larger LDL associated with saturated fat intake.[49] It could be that the smaller LDL induced by CoHO might also have increased LDL turnover rates, which was previously observed[27,50] as contributing to the observed plasma LDL cholesterol lowering.

CoHO effects on hepatic cholesterol homeostasis

CoHO intake resulted in decreased concentrations of both cholesterol and TAG in the liver. It has been shown in rats that higher concentrations of hepatic cholesterol increase hepatic TAG concentrations due to a decreased synthesis of carnitine, a situation that promotes fatty acid synthesis rather than directing fatty acids towards β-oxidation.[50] The decreases in hepatic cholesterol due to CoHO intake might have promoted fatty acids β-oxidation rather than TAG synthesis, as has been observed in the rat.[50] The lower concentrations of hepatic cholesterol and TAG due to CoHO treatment may be related to decreased rates of VLDL secretion by the liver.[51,52]

CoHO had a significant effect in altering the key enzymes of hepatic cholesterol homeostasis. HMG-CoA reductase, the key enzyme of cholesterol synthesis, remained unaffected by CoHO treatment. In contrast, the key enzyme of cholesterol catabolism, cholesterol 7α-hydroxylase, was up-regulated at the highest concentrations (10 and 15 g/100 g) of CoHO. These results suggest that CoHO interrupted the enterohepatic circulation of bile acids by increasing fecal bile acid output, which resulted in up-regulation of this enzyme. Although CoHO lowered hepatic cholesterol, the magnitude of lowering was not sufficient to induce cholesterol synthesis. We have observed a similar lack of up-regulation of HMG-CoA reductase activity in guinea pigs fed increasing doses of pectin.[7] Up-regulation of the enzyme occurred only at the highest doses of pectin when there was a substantial depletion of the hepatic cholesterol pool.[7] These results indicate that the hypocholesterolemic effects of CoHO are mediated initially by accelerating the rate of catabolism of cholesterol to bile acids and not by suppressing the rate of cholesterol synthesis.

CoHO also affected hepatic ACAT, the regulatory enzyme of cholesterol esterification. CoHO intake resulted in a dose-dependent decrease in ACAT activity that was consistent with the decrease in hepatic cholesteryl ester concentrations, indicating a decreased esterification in the liver. The up-regulation of 7α-hydroxylase and down-regulation of ACAT observed in our study is consistent with other studies where a reduction in hepatic cholesterol has been observed as a result of dietary fiber intake.[30,46]

The lowering of hepatic cholesterol induced by fiber intake has been correlated to increases in LDL receptor in guinea pigs.[7] Accelerated removal of LDL from plasma due

to increases in LDL receptor expression could have contributed to the hypocholesterolemic effects of CoHO.

Conclusion

From these studies we can conclude that CoHO has a major effect on hepatic cholesterol homeostasis and that plasma LDL-C is reduced as a result of modulated hepatic pools. The major effects of CoHO appear to be a decreased cholesterol absorption, as suggested by the increases in neutral sterol excretion, and an interruption of enterohepatic circulation of bile acids, as suggested by the up-regulation of cholesterol 7α-hydroxylase activity. These two mechanisms led to a depleted hepatic cholesterol pool, which may up-regulate the LDL receptor and remove LDL from plasma. These findings enhance our knowledge about how nontraditional dietary sources such as CoHO may exert its hypocholesterolemic effect thereby decreasing the risk for cardiovascular disease.

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