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Lime-treated corn husks lower plasma LDL cholesterol in guinea pigs by altering hepatic cholesterol metabolism

Reyna Luz Vidal-Quintanar, Laura Hernandez,* Karin Conde,* Marcela Vergara-Jimenez,* and Maria Luz Fernandez*

*Departamento de Investigacion y Postgrado, University of Sonora, Hermosillo Sonora Mexico and *Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269*

Lime-treated corn husks (LTCH) containing 69% of fiber (5.0% as soluble fiber) were evaluated on their hypolipidemic properties in guinea pigs. Animals were fed two doses of corn husks replacing dietary fiber: 7.5% and 10% (w/w) and LTCH effects on hepatic cholesterol metabolism and lipoprotein levels were compared to a control diet containing cellulose. LTCH significantly lowered plasma LDL cholesterol by 25% ($P < 0.01$) and VLDL cholesterol by 37% ($P < 0.05$), although a dose effect was not observed in this parameter. Plasma triacylglycerol (TAG) concentrations and HDL cholesterol levels were not affected by LTCH intake. The number of cholesteryl ester, TAG and phospholipid molecules were lower in VLDL from animals fed LTCH diets compared to control ($P < 0.01$) indicating modifications on VLDL size that were confirmed by the smaller calculated VLDL diameter of guinea pigs treated with LTCH. In addition, LTCH intake resulted in a smaller, cholesteryl ester depleted LDL particle compared with LDL derived from control animals. Hepatic cholesterol and TAG were not affected by LTCH, however, microsomal-free cholesterol was lower in animals fed LTCH compared with control. In agreement with the observed reductions on microsomal cholesterol, HMG-CoA reductase activity was upregulated 87% and 100%, ACAT downregulated 54% and 65% and cholesterol 7α-hydroxylase activity upregulated 120% and 180% by LTCH diets in a dose dependent manner ($P < 0.01$). In addition, LDL binding to hepatic membranes (B_{max}) was higher in animals fed LTCH diets. These results indicate that the lower plasma VLDL and LDL cholesterol concentrations induced by LTCH intake are associated with decreases in microsomal cholesterol that alter the regulatory enzymes of cholesterol homeostasis and upregulate LDL receptors. (J. Nutr. Biochem. 8:479–486, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Plasma lipid disorders are characterized by increased serum concentrations of cholesterol, triacylglycerol (TAG) and apolipoprotein (apo) B and by a reduced concentration of high-density lipoprotein cholesterol (HDL-C). Epidemiological studies indicate that coronary heart disease (CHD) results from a variety of risk factors. Heredity plays a significant role and a family history of premature CHD is considered a major risk factor.[1] Life style, including cigarette smoking, high blood pressure, high blood cholesterol concentrations, glucose intolerance, obesity, and physical inactivity, constitute important risk factors for CHD which are modifiable.[2] In addition, a number of dietary factors alter lipoprotein profiles and have a significant effect on CHD.[1]

Numerous studies have demonstrated that a high intake of dietary fiber lowers plasma cholesterol concentrations and therefore the associated risk of CHD.[3] It has been demonstrated that a fiber intake of 20 to 50 gm/day can significantly lower plasma LDL cholesterol concentrations without affecting HDL cholesterol levels resulting in an improved lipoprotein profile.[3] The mechanisms by which dietary soluble fiber lowers plasma cholesterol have been thoroughly discussed.[4] Binding to bile acids and interrupting the enterohepatic circulation of bile acids or inhibiting

Address reprint requests to Dr. M.L. Fernandez at Department of Nutritional Sciences, University of Connecticut, Ext U-17, 3624 Horsebarn Road, Storrs, CT 06269 USA.
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655 Avenue of the Americas, New York, NY 10010

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cholesterol and lipid absorption are the most commonly accepted actions of dietary soluble fiber.[5] The outcome of these proposed mechanisms is an alteration of hepatic cholesterol homeostasis, which has a significant influence on plasma lipid levels by affecting lipoprotein secretion and catabolism.[6]

Although there are many clinical and animals studies on the effect of several types of dietary soluble fiber including pectin, guar gum, psyllium, and oat bran on lipid metabolism,[6–11] very little information exists on the use of corn fiber[12] and no studies to our knowledge have been conducted on the fiber from lime-treated corn husks (LTCH).

In Mexico, the incidence of coronary heart disease has increased over the past three decades and has become the leading cause of death in this country.[13] There are significant differences in prevalence across regions and the population from the Northern part of the country is characterized by elevated plasma cholesterol and TAG concentrations.[13]

The present studies were performed to determine whether LTCH, a by product of tortilla making containing 69% of fiber, would lower plasma cholesterol concentrations to define its potential use as a hypocholesterolemic agent when incorporated into food commodities to be consumed in the Northern part of Mexico. Two doses of LTCH, 7.5 and 10% (w/w) corresponding to 5.2% and 6.9% of dietary fiber were tested to assess first whether LTCH lowers plasma cholesterol concentrations in a dose dependent manner, and if that is the case, which are the possible mechanisms of hypocholesterolemia. Guinea pigs were used as the animal model for these studies because they are similar to humans in their lipid profile, their distribution of hepatic cholesterol pools[14] and their responses to conventional sources of dietary fiber.[6,10]

Methods and materials

Materials

DL-Hydroxyl-[3-^{14}C]methyl glutaryl 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 and Liquifluor (toluene concentrate) were purchased from Du Pont NEN (Boston, MA). Oleoyl-[1-^{14}C]coenzyme A (1.8 GBq/mmol) and DL-3-hydroxy-3 methylglutaryl coenzyme A were purchased from Amersham (Clearbrook, IL USA); cholesteryl oleate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP and bovine albumin were from Sigma Chemical (St. Louis, MO USA). Enzymatic cholesterol assay kits, cholesterol oxidase, cholesterol esterase, and hydroperoxidase were purchased from Boehringer Mannheim (Indianapolis, IN USA) and halothane from Halocarbon (Hackensack, NJ USA). Lime-treated corn husks are a by product of tortilla industry in Mexico. The lime treated corn husks for these studies was obtained from Maseca. S.A. (Cd. Obregon, Sonora, Mexico) and had the following composition: 68.5% total fiber (5% soluble fiber), 8.7% protein, 2.9% fat, 1.6% ash, and 18.2% carbohydrate.

Diets

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ USA). All diets had identical composition except for the partial replacement of cellulose by 7.5 and 10% LTCH (*Table 1*). Fat was given at a concentration of 15.1% and represented 35% of total energy. The fat used was palm oil and the composition of the fatty acids was: C16:0 43.3%, C18:0 4.1%, C18:1 39.8%, C18:2 9.7%. Cholesterol was added to a final concentration of 0.04% (w/w) which is equivalent to 300 mg/day in humans.[15]

Table 1 Composition of experimental diets

Component	Diets (gm/100 gm diet)		
	Control	7.5% LTCH[a]	10% LTCH
Soybean protein	22.4	22.4	22.4
Fat (palm oil)	15.1	15.1	15.1
Sucrose/starch[b]	39.6	39.6	39.6
Mineral mix[c]	8.2	8.2	8.2
Vitamin mix[c]	1.1	1.1	1.1
Cellulose	12.5	5.0	2.5
Corn husks	0.0	7.5	10.0
Cholesterol	0.04	0.04	0.04

[a]Lime-treated corn husks.
[b]Sucrose:Starch (1.43:1).
[c]Composition of mineral and vitamin mixes have been reported previously.[44]

Animals

Male Hartley guinea pigs (Sasco Sprague Dawley, Omaha, NE USA), weighing between 250 and 300 gm, were randomly assigned to one of three dietary groups. They were housed in a room with a controlled light cycle (light 0700 to 1900 hr) and provided with free access to the semipurified diets and water. After 4 weeks, nonfasted animals were killed by anesthetization with halothane and heart puncture to obtain liver and plasma for isolation of hepatic microsomes and plasma lipoproteins. Preliminary studies have shown that this time (4 weeks) is sufficient to establish a constant plasma cholesterol concentration and a metabolic steady state.[10] All animal experiments were conducted in accordance with U.S. Public Health Service and U.S. Department of Agriculture guidelines, and experimental protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee.

Plasma and liver lipids

Total plasma and lipoprotein cholesterol concentrations were determined by enzymatic analysis.[16] VLDL, IDL, LDL and HDL were separated by sequential ultracentrifugation in a L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA USA) at 125,000 × g at 15°C for 19 hr in a Ti-50 rotor. Separation was based on the following density fractions: VLDL; d = 1.006kg/L, IDL d = 1.006–1.019 kg/L and LDL; d = 1.019–1.090 kg/L. HDL cholesterol was determined using the precipitation method of Warnick et al.[17]

Hepatic total and free cholesterol and TAG were determined according to Carr et al.[18] after extraction of hepatic lipids with chloroform/methanol (2:1). Hepatic cholesteryl ester concentrations were estimated by subtracting hepatic free from total cholesterol.

VLDL and LDL characterization

VLDL and LDL composition were calculated by determining free and esterified cholesterol;[18] protein was determined by a modified Lowry procedure,[19] triacylglycerol and phospholipids as described previously.[9,10] VLDL apo B was selectively precipitated with isopropanol[20] and the amount of protein corresponding to apo B was calculated by subtracting the supernatant from the total protein concentration. The number of constituent molecules of VLDL and

LDL was calculated on the basis of one apo B per LDL with a molecular mass of 412,000 kD.[21] The molecular weights were: 885.4, 386.6, 646, and 734 for triacylglycerol, free and esterified cholesterol and phospholipids as reported previously.[10] VLDL and LDL diameters were calculated according to the model of Van Heek and Zilversmit.[22]

Microsome isolation

Guinea pigs were killed at the nadir of the diurnal rhythm, livers were removed, and hepatic microsomes were isolated by pressing liver tissues through a tissue grinder into homogenization buffer (50 μmol/L KH_2PO_4, 0.1 mol/L sucrose, 50 μmol/L KCl, 50 μmol/L NaCL, 30 μmol/L EDTA and 2 μmol/L dithiothreitol, pH 7.2) in a 1:3 ratio (liver:buffer). This preparation was further homogenized with a Potter-Elvenjhem homogenizer. A microsomal fraction was isolated by two 15-min centrifugations at 10,000 × g (JA-20 rotor, J2-21) followed by 1 hr centrifugation at 100,000 × g in a Ti-50 rotor at 4°C. Microsomes were resuspended in the homogenization buffer and centrifuged an additional hour at 1000,000 × g. After centrifugations microsomal pellets were homogenized and stored at −70°C. Microsomal protein was determined according to the method of Markwell et al.[19]

Hepatic microsome lipids

Free cholesterol was assayed in hepatic microsomes isolated from guinea pigs fed the different diets. Microsomes (2 to 3 mg protein) were treated with 20 volumes of chloroform:methanol (2:1) according to Folch.[23] Samples were dried under nitrogen and lipids were solubilized with 1 mL of water with Triton 100 × (1%). Free cholesterol was determined by enzymatic assay.[16]

Hepatic HMG-CoA reductase assay

Microsomal HMG-CoA reductase (E.C.1.1.1.34) activity was measured by the radioisotopic method of Shapiro et al.[24] Briefly, 200 μg of microsomal protein was incubated with 7.5 nmol (0.33 GBq/nmol) [3-^{14}C]HMG-CoA, 4.5 μmol glucose-6-phosphate, 3.6 μmol EDTA, 0.45 μmol NADP, and 0.3 IU glucose-6 phosphate dehydrogenase for 15 min at 37°C to a final volume of 0.05 mL. [^{3}H]Mevalonic acid was used as an internal recovery standard (0.024 GBq per assay), HCl (0.025 mL) was added to stop the reaction, and samples were further incubated at 37°C for 30 min. After incubation, microsomal protein was precipitated by microfugation for 1 min, and an aliquot of the supernatant was applied to silica gel TLC plates (Alltech, Deerfield, IL USA). Plates were developed in acetone-benzene (1:1), and the area containing the mevalonate (Rf 0.6 to 0.9) was scraped and mixed with 5 mL of Aquasol. Radioactivity was measured using a scintillation counter. HMG-CoA reductase activity was expressed as picomoles of [^{14}C]mevalonate produced per minute per milligram of microsomal protein. Recoveries of [^{3}H]mevalonate were between 60% and 80%.

Hepatic ACAT assay

The optimal conditions for assay of guinea pig hepatic microsomal ACAT (E.C.2.3.1.26) were determined for protein dependence and time course. The assay was linear for 15 min with protein concentration ranging from 2 to 6 gm/L. The assay was as follows: microsomal protein (0.8 to 1 mg per assay) was preincubated with 84 g/L albumin, and amount of albumin equivalent to the molar ratio of the substrate (1:1, albumin:[^{14}C]oleoyl-CoA),[25] and buffer (50 μmol/L KH_2PO_4, 0.1mol/L sucrose, 50 μmol/L KCl, 30 μmol/L EDTA and 50 μmol/L NaF) 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 A (0.15 GBq/pmol) was added, and the reaction proceeded for 15 min at the same temperature. The reaction was stopped by the addition of 2.5 mL of chloroform-methanol (2:1). The [^{3}H]cholesteryl oleate recovery standard (0.045 GBq per assay) was added with an additional 2.5 mL of chloroform: methanol and 1 mL of water containing 0.5% H_2SO_4. Tubes were mixed and allowed to stand overnight at room temperature. The aqueous phase was removed, and after evaporation of the organic phase to dryness, samples were resuspended in 150 μL of chloroform containing 30 μg of unlabeled cholesteryl oleate. Samples were applied to 20 × 20 cm glass silica gel TLC plates (Alltech) and developed in hexane-diethyl ether (9:1, v/v). Cholesteryl oleate was visualized with iodine vapors and scraped from the TLC plates, 5 mL of Liquifluor was added, and radioactivity was counted in a scintillation counter. Recoveries of the [^{3}H]cholesteryl oleate ranged from 75 to 90%.

Hepatic cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed according to Jelinek et al.[26] using [^{14}C]cholesterol as substrate, except the cholesterol was delivered as cholesterol:phosphotidylcholine liposomes (1:8 by weight) prepared by sonication and an NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose-6-phosphate) was included in the assay as a source of NADPH. After addition of glucose-6-phosphate dehydrogenase (0.3 I.U.), samples were incubated for an additional 30 min. The reaction was stopped by addition of 5 mL of chloroform:methanol 2:1 and 1 mL acidified water (5% sulfuric acid). Tubes were mixed, the top layer was discarded, and samples were dried under nitrogen. Samples and 7α and 7β-hydroxycholesterol standards each were dissolved in 100 μL of chloroform, applied to silica gel TLC plates, and developed with ethyl acetate-toluene 3:2. The plate was placed in iodine vapors to mark the 7α and 7β-hydroxycholesterol standards and positioned on XAR-5 film with intensifying screen overnight. Using the film as a guide, the location of the [^{14}C]7α-hydroxycholesterol spots was determined, scraped from the plate, and counted in a scintillation counter.

LDL binding assays

Pooled samples of guinea pig LDL were radioiodinated with ^{125}I by the iodine monochloride method[27] to give a specific activity between 100–300 cpm/ng. Hepatic microsomes from animals fed the homologous diet were incubated with the radiolabeled LDL over a concentration range of 10 to 80 μg/mL in the presence or absence of 1 mg/mL of unlabeled human LDL, an effective competitor at 37°C[9] to determine total and nonspecific binding. Receptor mediated binding was determined by difference. After incubation for 2 hr at 37°C, microsomes were pelleted by ultracentrifugation in a Ti 42.2 rotor at 100,000 × g for 1 hr, followed by a washing of 30 min. Tubes were sliced at the bottom and counted in a gamma counter. K_d and B_{max} were determined from Woolf plots.[28]

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant differences in plasma lipids, lipoprotein cholesterol, LDL and VLDL composition, hepatic free, esterified and microsomal cholesterol and TAG, HMG-CoA reductase, ACAT, cholesterol 7α-hydroxylase and LDL binding to hepatic membranes of animals from the three dietary groups. The Newman Keuls was used as post hoc test to determine differences among means.

Table 2 Plasma total and lipoprotein cholesterol and triacylglycerol of guinea pigs fed semipurified diets containing control (0), 7.5, or 10% (w/w) lime-treated corn husks (LTCH)*

Plasma lipids (mmo/L)	Diets		
	Control	7.5% LTCH	10% LTCH
Total cholesterol	2.07 ± 0.46[a]	1.49 ± 0.33[b]	1.55 ± 0.41[b]
Triacylglycerol	1.41 ± 0.87	1.23 ± 0.52	1.09 ± 0.33
VLDL cholesterol	0.09 ± 0.04[a]	0.06 ± 0.01[b]	0.05 ± 0.02[b]
LDL cholesterol	1.62 ± 0.52[a]	1.07 ± 0.19[b]	1.08 ± 0.38[b]
HDL cholesterol	0.32 ± 0.08	0.23 ± 0.09	0.35 ± 0.16

*Values are presented as mean ± S.D. for Control (n = 10); 7.5% (n = 6) and 10% (n = 5) LTCH fed animals. Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman-Keuls as post-hoc test.

Results

Lime treated corn husks and plasma lipids and lipoproteins

There were no significant differences in body weight gain (7.5 ± 2.2, 7.4 ± 2.9, and 7.6 ± 2.5 g/day) or final weights in animals fed the three dietary treatments for 4 weeks, indicating that guinea pigs consumed similar amounts of nutrients. Final body weights were: 558 ± 25, 527 ± 53, and 549 ± 18 for animals fed control, 7.5% and 10% LTCH respectively. Plasma cholesterol concentrations were 23% lower ($P < 0.05$) in animals fed LTCH diets (*Table 2*). This plasma cholesterol lowering resulted from a 35% and 29% reduction in VLDL and LDL cholesterol respectively (*Table 2*). TAG and HDL cholesterol were not affected by LTCH intake (*Table 2*). No dose response was observed in plasma lipid concentrations.

LTCH significantly altered VLDL and LDL composition number of component molecules and size. The number of cholesteryl ester, TAG and phospholipid molecules were lower in VLDL isolated from LTCH treated guinea pigs (*Table 3*) ($P < 0.01$) indicating that intake of the tested source of corn fiber resulted in smaller VLDL particles as confirmed by the smaller calculated diameter. The percentages of the VLDL components were for free cholesterol: 3 ± 2, 4 ± 3, and 2 ± 1, for cholesteryl ester: 4 ± 1, 1 ± 1, and 2 ± 1; for TAG: 70 ± 4, 69 ± 6, and 70 ± 5; for PL: 12 ± 3, 14 ± 5, and 14 ± 3 and for total protein 12 ± 3, 14 ± 5, and 14 ± 3 for VLDL from control, 7.5% LTCH and 10% LTCH, respectively. Apo B represented 33% of total protein in control and 64% of total protein in LTCH groups.

LTCH also affected the relative percentage of the LDL components (*Table 4*). The relative percentage of cholesteryl ester was lower and protein was higher ($P < 0.01$). Since LDL contains only one apo B per particle, these results indicate that LTCH intake is associated with smaller LDL. In agreement with these observations, the number of cholesteryl ester molecules and the calculated diameter were smaller for LDL isolated from LTCH-fed guinea pigs (*Table 5*). No dose response was observed in the chemical composition and size characteristics of VLDL and LDL.

Lime treated corn husks, hepatic lipids, enzyme activity and apo B/E receptor

LTCH had no effect on total hepatic lipids. Liver free and esterified cholesterol and TAG were not different among LTCH and control groups (*Table 6*). In contrast, microsomal hepatic free cholesterol was 60% lower in animals fed LTCH (*Table 6*) indicating a reduction in the microsomal pool of free cholesterol and suggesting a decrease in substrate availability for ACAT activity.

In agreement with the decreases in hepatic free cholesterol, hepatic ACAT activity was reduced 54% and 65% in a dose dependent manner by intake of 7.5% and 10% LTCH respectively (*Table 7*). Further, the depletion of hepatic microsomal cholesterol resulted in a dose dependent up-regulation of HMG-CoA reductase activity, the regulatory enzyme of cholesterol synthesis. Animals fed the two doses of LTCH had 87% and 100% higher HMG-CoA reductase activity respectively compared to the control group (*Table 7*). Cholesterol 7α-hydroxylase activity was 120% and 180% higher in animals fed 7.5% and 10% LTCH respectively indicating an up-regulation of the regulatory enzyme of cholesterol catabolism to bile acids by intake of this type of dietary fiber.

In addition to altering the regulatory enzymes of cholesterol synthesis, catabolism, and esterification, LTCH intake resulted in higher number of hepatic apo B/E receptors as determined by LDL binding assays. LDL binding to guinea pig hepatic microsomes was 40% and 68% higher by intake of 7.5% and 10% LTCH (*Table 8*). No significant differences in the dissociation constant (K_d) were observed among the different dietary groups.

Table 3 VLDL triacylglycerol (TAG), cholesteryl ester (CE), phospholipid (PL) and free cholesterol (FC) molecules, Apo B percentage and calculated diameter of VLDL particles isolated from guinea pigs fed semipurified diets containing control (0), 7.5%, or 10% (w/w) lime-treated corn husks (LTCH)*

Diet	VLDL (molecules/per particle)					
	FC	CE	TAG	PL	Apo-B (%)	VLDL diameter (Å)
Control	763 ± 627	911 ± 766[a]	6057 ± 2622[a]	1244 ± 337[a]	6 ± 3[b]	524 ± 112[a]
7.5% LTCF	642 ± 379	50 ± 59[b]	3794 ± 1328[b]	829 ± 259[ab]	9 ± 3[a]	387 ± 58[b]
10% LTCF	317 ± 88	100 ± 66[b]	3428 ± 519[b]	714 ± 140[b]	10 ± 1[a]	427 ± 40[ab]

*Values are presented as mean ± SD for Control (n = 10); 7.5% (n = 6); and 10% (n = 5) LTCF fed animals. Values in the same column with different superscripts are significantly different ($P < 0.01$) as determined by one-way ANOVA and the Newman-Keuls as post-hoc test.

Table 4 Composition of LDL isolated from guinea pigs fed semipurified diets containing 0% (Control), 7.5%, or 10% (w/w) lime-treated corn husks (LTCH)*

Diet type	LDL composition (moles/100 moles)				
	FC	CE	TAG	PL	Protein
Control	2 ± 1	44 ± 6[a]	11 ± 4	13 ± 2	30 ± 7[b]
7.5% LTCH	3 ± 0	39 ± 4[b]	10 ± 3	17 ± 3	32 ± 4[a]
10% LTCH	3 ± 0	42 ± 3[ab]	10 ± 3	13 ± 3	33 ± 2[a]

*Values are presented as mean ± SD for Control (n = 10); 7.5% (n = 6), and 10% (n = 5) LTCH fed animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman Keuls post hoc test.

Discussion

In these studies we have demonstrated that in addition to the conventional sources of dietary fiber which are known for their hypocholesterolemic properties, lime treated corn husks containing 69% of fiber lower plasma VLDL and LDL cholesterol concentrations by specific actions on hepatic cholesterol homeostasis. The potential use of this product for human consumption becomes important especially in the Northern part of Mexico where there is high prevalence of elevated plasma cholesterol and triacylglycerol among the population.[13]

Lime-treated corn husks and hepatic cholesterol metabolism

Psyllium husks derived from *Plantago ovata* have been shown to produce a depletion of hepatic cholesterol pools both in hamsters[11] and guinea pigs[10] which were associated to upregulation of cholesterol 7α-hydroxylase as a result of fiber binding to bile acids. In addition, upregulation of HMG-CoA reductase activity and decreases in ACAT have been observed by the intake of this soluble fiber.[10] Pectin, another source of soluble fiber lowers hepatic cholesterol concentrations by decreasing cholesterol absorption and enhancing bile acid secretion.[6,8] Oat bran has also been shown to have hypocholesterolemic properties although conflicting results have been obtained by some of the clinical trials.[29,30] Guar gum, a gel-forming galactomannan characterized by high viscosity, lowers plasma cholesterol concentrations possibly by decreasing cholesterol absorption.[9] Similar to the effects of psyllium, pectin, guar gum, or oat bran on plasma lipid levels, LTCH although with a low concentration of soluble fiber (5%) significantly reduced plasma cholesterol by decreasing both VLDL and LDL cholesterol concentrations.

LTCH did not reduce the amount of free or esterified cholesterol in the liver as we have previously observed by intake of pectin, guar gum, or psyllium in guinea pigs,[10,31,32] however, when microsomal-free cholesterol was measured, a significant reduction of this pool of free cholesterol was observed. Significant reductions of microsomal free cholesterol associated with substantial alterations in hepatic enzyme activity have been observed in guinea pigs even in the absence of total hepatic cholesterol modifications.[33] Because this pool of cholesterol (microsomal) presumably is the substrate for ACAT,[34] LTCH is affecting a critical regulatory site for maintaining hepatic cholesterol homeostasis. Studies in African green monkeys have demonstrated that increases in ACAT activity are correlated with increases in LDL cholesterol concentrations and higher risk for development of atherosclerosis.[35] The reductions in ACAT activity by LTCH intake and the associated plasma LDL cholesterol lowering suggest a relationship between hepatic cholesterol esterification and plasma lipoprotein levels and composition.

The up-regulation of HMG-CoA reductase by corn husks is a response to the depletion of hepatic microsomal cholesterol. Studies in guinea pigs[10,31,32] have demonstrated that although soluble fiber intake increases the activity of this regulatory enzyme of cholesterol synthesis, this upregulation does not compensate the depletion of hepatic cholesterol induced by the fiber as is the case in the present investigation.

Although microsomal cholesterol is not considered a major substrate for cholesterol 7α-hydroxylase, LTCH also increased this enzyme activity which suggests that there was

Table 5 Triacylglycerol (TAG), cholesteryl ester (CE), phospholipids and free cholesterol (FC) content of LDL isolated from guinea pigs fed semipurified diets containing 0% (Control), 7.5%, or 10% (w/w) lime-treated corn husks (LTCH)*

Diet type	LDL (molecules per particle)				
	FC	CE	TAG	PL	LDL diameter (A)
Control	103 ± 37[a]	1034 ± 260[a]	163 ± 78	269 ± 73	259 ± 26[a]
7.5% LTCH	65 ± 21[b]	978 ± 121[b]	144 ± 32	257 ± 32	203 ± 20[b]
10% LTCH	101 ± 14[a]	781 ± 146[b]	156 ± 33	287 ± 115	225 ± 16[ab]

*Values are presented as mean ± SD for Control (n = 10); 7.5% (n = 6), and 10% (n = 5) LTCF-fed animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman Keuls post hoc test.

Table 6 Hepatic cholesterol, triacylglycerol, and microsomal-free cholesterol of guinea pigs fed semipurified diets containing 0% (Control) 7.5%, or 10% (w/w) lime-treated corn husks (LTCH)*

Diet type	Hepatic cholesterol (μmol/g)			
	Free	Esterified	TAG	Microsomal cholesterol (nmol/mg protein)
Control	2.52 ± 0.05	0.44 ± 0.18	3.4 ± 3.4	66 ± 32[a]
7.5% LTCH	2.33 ± 0.03	0.67 ± 0.23	3.4 ± 3.0	26 ± 8[b]
10% LTCH	2.51 ± 0.03	0.47 ± 0.31	3.4 ± 4.5	26 ± 6[b]

*Values are presented as mean ± SD for Control (n = 10); 7.5% (n = 6) and 10% (n = 5) LTCF-fed animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman Keuls post hoc test.

interruption of the enterohepatic circulation of bile acids because of the action of corn husks in the intestinal lumen. It is well documented that soluble fiber main primary actions in the small intestine are related to reductions in lipid absorption attributable to micelle disruption, increased viscosity, or water holding capacity that interrupts nutrient absorption.[36] In addition, the interruption of bile acid absorption in the ileum that leads to mobilization of hepatic cholesterol to compensate for this loss is another theory that has been proven by use of hamsters[37] or other animal models.[5,38] LTCH primary action in the intestine is not known at present but because there was a depletion of microsomal cholesterol, an induction of cholesterol 7α-hydroxylase and other alterations in hepatic cholesterol homeostasis, one can speculate that the soluble fiber from corn husks may bind to bile acids and that cholesterol 7α-hydroxylase is upregulated in response to this phenomenon. However, decreases in cholesterol absorption or specific effects in hormone levels associated with LTCH intake might have also contributed to the alterations in hepatic cholesterol metabolism and the consequent decreases in plasma VLDL and LDL cholesterol concentrations.

The significant reductions in hepatic microsomal cholesterol induced by LTCH intake could also be related to the higher number of hepatic LDL receptors observed in animals fed this soluble fiber. It is well established that LDL receptors are regulated at the molecular level when the levels of microsomal cholesterol are low.[39] This could well be an important secondary mechanism of the action of LTCH in controlling plasma cholesterol concentrations. Further studies will focus in determining whether plasma LDL turnover is increased by this type of fiber.

Lime-treated corn husks and plasma lipoprotein levels and composition

One of the major responses to LTCH intake was the significant decreases on plasma VLDL and LDL cholesterol concentrations, both of which have been associated with decreased risk for cardiovascular disease.[2,40] LTCH intake resulted in a mature VLDL particle smaller in size than those isolated from control animals as indicated by the less number of TAG, phospholipids and cholesteryl ester molecules. In addition, because VLDL contains only one apo B per molecule,[41] the higher percentage of apo B in VLDL from LTCH fed animals suggests the presence of a higher number of VLDL particles smaller in size and diameter.

Cholesteryl-ester enriched VLDL have been correlated with increased risk for cardiovascular disease due to the larger amount of cholesterol that can be delivered to macrophages by means of the apo E receptor.[42] This enrichment results in the formation of foam cells and can lead to the early stages of arteriosclerosis. The reduction in ACAT activity induced by LTCH intake possibly led to the formation of cholesteryl ester depleted VLDL particles which are associated with decreased risk for arteriosclerosis.[39] Because the number of VLDL particles seems to be larger in animals fed LTCH, understanding the fate of these particles in plasma is important to interpret the hypocholesterolemic effects of fiber derived from corn husks.

Table 7 Hepatic 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) reductase and acyl-CoA:cholesteryl acyl transferase (ACAT) activities of guinea pigs fed semipurified diets containing 0 (Control) 7.5%, or 10% lime-treated corn husks (LTCH)*

	Enzyme Activity (pmol/min.mg)		
	HMG-CoA reductase	ACAT	Cholesterol 7α-hydroxylase
Control	9.4 ± 3.9[a]	44.3 ± 16.8[a]	0.67 ± 0.18[a]
7.5% LTCH	17.6 ± 9.7[ab]	20.5 ± 16.2[b]	1.48 ± 0.76[b]
10% LTCH	20.7 ± 12.0[b]	16.6 ± 9.8[b]	1.87 ± 0.60[b]

*Values are presented as mean ± SD for Control (n = 10); 7.5% (n = 6), and 10% (n = 5) LTCH fed animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman Keuls post hoc test.

Table 8 LDL binding constants of guinea pigs fed semipurified diets containing 0% (control), 7.5%, or 10% (w/w) lime-treated corn husks (LTCH)*

Dietary treatment	LDL Binding Parameters	
	B_{max} (μg/mg)	K_D (μg/mL)
Control	2.36 ± 0.30[a]	17.1 ± 14.2
7.5% LTCH	3.31 ± 0.60[ab]	23.4 ± 20.1
10% LTCH	3.96 ± 0.41[b]	32.6 ± 2.8

*Values are presented as mean ± standard deviation for Control (n = 4); 7.5% (n = 3), and 10% (n = 3) LTCH animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA and the Newman Keuls post hoc test.

It is apparent from LDL composition and size that the smaller VLDL from LTCH-treated animals led to the formation of cholesteryl poor LDL particles that were smaller in size. It is possible that in addition to the increases in hepatic LDL receptor, the lower plasma LDL cholesterol concentrations observed in the animals fed LTCH could be associated to decreased conversion of VLDL to LDL. Studies conducted in guinea pigs have shown that sources of soluble fiber including pectin, guar gum and psyllium decrease the rates of VLDL conversion to LDL as well as direct VLDL removal from the circulation.[43] In the present studies, LTCH intake upregulated hepatic LDL receptors and significantly affected VLDL composition. These alterations suggest that VLDL could have been removed from the circulation without being converted to LDL, either by the apo B/E receptor that was upregulated by LTCH, or by the LDL-related protein receptor because of increased affinity of this receptor to the smaller, cholesteryl-ester-depleted VLDL.

Smaller LDL induced by dietary factors such as polyunsaturated fat are also correlated with faster LDL turnover in guinea pigs.[44] Another possible factor contributing to the lower plasma LDL cholesterol concentrations could be increased affinity of the apo B/E receptor to this small LDL, which could increase its turnover in plasma.

From these studies we conclude that LTCH, a by product of tortilla manufacture industry, significantly lowers plasma cholesterol levels by inducing specific alterations in the small intestine, which lower hepatic microsomal cholesterol and modify hepatic cholesterol metabolism. These modifications result in secretion of a modified VLDL particle containing less cholesterol which may be removed faster from circulation or have decreased rates of conversion to LDL. In addition, the upregulation of hepatic LDL receptors contributes to the accelerated removal of plasma LDL. The potential use of LTCH as a hypocholesterolemic agent for human consumption deserves further consideration.

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