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Corn fiber oil lowers plasma cholesterol levels and increases cholesterol excretion greater than corn oil and similar to diets containing soy sterols and soy stanols in hamsters

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The aims of this study were to compare the cholesterol-lowering properties of corn fiber oil (CFO) to corn oil (CO), whether the addition of soy stanols or soy sterols to CO at similar levels in CFO would increase CO's cholesterol-lowering properties, and the mechanism(s) of action of these dietary ingredients. Fifty male Golden Syrian hamsters were divided into 5 groups of 10 hamsters each, based on similar plasma total cholesterol (TC) levels. The first group of hamsters was fed a chow-based hypercholesterolemic diet containing either 5% coconut oil + 0.24% cholesterol (coconut oil), 5% CO, 5% CFO, 5% CO + 0.6% soy sterols (sterol), or 5% CO + 0.6% soy stanols (stanol) in place of the coconut oil for 4 weeks. The stanol diet significantly inhibited the elevation of plasma TC compared to all other dietary treatments. Also, the CFO and sterol diets significantly inhibited the elevation of plasma TC compared to the CO and coconut oil diets. The CFO, sterol, and stanol diets significantly inhibited the elevation of plasma non-high density lipoprotein cholesterol compared to the CO and coconut oil diets. The stanol diet significantly inhibited the elevation of plasma high density lipoprotein cholesterol (HDL-C) compared to all other dietary treatments. The sterol diet significantly inhibited the elevation of plasma HDL-C compared to the CO and coconut oil diets, whereas the CFO diet significantly inhibited the elevation of plasma HDL-C compared to the coconut oil diet only. No differences were observed between the CFO and CO for plasma HDL-C. There were no differences observed between groups for plasma triglycerides. The CO and CFO diets had significantly less hepatic TC compared to the coconut oil, sterol, and stanol diets. The CO and CFO diets had significantly less hepatic free cholesterol compared to the sterol and stanol diets but not compared to the coconut oil diet; whereas the coconut oil and sterol diets had significantly less hepatic free cholesterol compared to the stanol diet. The CFO, sterol, and stanol diets excreted significantly more fecal cholesterol compared to the coconut oil and CO diets. In summary, CFO reduces plasma and hepatic cholesterol concentrations and increases fecal cholesterol excretion greater than CO through some other mechanism(s) in addition to increase dietary sterols and stanols—possibly oryzanols. (J. Nutr. Biochem. 11:443–449, 2000)

Keywords: corn fiber oil; soy sterols; soy stanols; fecal cholesterol; LDL cholesterol

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Introduction

Corn fiber and corn bran (the analogous pericarp-rich processing fraction produced during the dry-milling of corn) have received little success as food products or as

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food ingredients. However, several investigators have shown that corn fiber and corn bran have valuable cholesterol-lowering properties.[1–4] The mechanisms by which dietary fibers affect metabolic lipid alterations are not well established. Soluble fibers are thought to function as gel or viscosity builders in the intestine that reduce translational diffusion and, hence, lower absorption of all food ingredients including cholesterol and reabsorption of bile acids in the lumen.[5,6] Some of the less soluble materials may physically entrap or ionically interact with sterols (bile acids), thereby interfering with the formation of micelles.[5,7] There is also considerable evidence that dietary fibers reduce plasma cholesterol indirectly via their short-chain fatty acid fermentation products (principally acetate, propionate, and butyrate).[8] Overall, high short-chain fatty acid concentrations also result in acid colonic pH, which could lower the solubility and reabsorption of bile acids.

Previous studies have indicated that hexane extraction of corn fiber (specifically corn hull fiber) or corn bran yields 1–2% of extractable lipids by weight.[9] In addition, alkaline hydrolysis of the defatted fiber yields an additional 1–2% of lipid material that was originally covalently bound. The extractable lipids are composed of phytosterol fatty acyl esters, triacylglycerols, tocopherols, free sterols, and ferulate phytosterol esters. The latter fraction includes compounds similar in structure to those in the "oryzanol" fraction, a generic term given to a group of ferulic acid esters shown to be the component of rice bran oil that imparts it with hypocholesterolemic activity.[10–12] The oryzanol-like lipids in corn fiber oil (CFO) differ from the oryzanol in rice bran oil, in that the former is comprised of phytosterols that are completely saturated, and there is evidence that this higher degree of saturation may result in greater hypocholesterolemic activity.[13] The non-triacylglycerol content of CFO is composed of ferulate esters of sitostanol, sterol fatty acyl esters, and free phytosterols.

The cholesterol-lowering effect of phytosterols has been studied since the 1950s and is well known.[14] Phytosterols interfere with the uptake of both dietary and biliary cholesterol from the intestinal tract in humans.[15] The reason for this is not well known; however, phytosterols appear to decrease the solubility of cholesterol in the oil and micellar phases, thus displacing cholesterol from bile salt micelles and interfering with its absorption.[16] Phytosterols differ from cholesterol by the presence of an extra methyl or ethyl group on the cholesterol side chain.[17] The major dietary phytosterols are sitosterol, campesterol, and stigmasterol. The most common dietary phytostanol—sitostanol—is a saturated derivative of sitosterol.[18]

The main objectives of the current study were to compare the cholesterol-lowering properties of CFO to corn oil (CO), whether the addition of soy stanols or soy sterols to CO at similar levels in CFO would increase CO's cholesterol-lowering properties, and the possible mechanism(s) of action of these dietary ingredients.

Methods and materials

Animals and diet

Fifty male 9-week-old Golden Syrian hamsters (Charles River Breeding Laboratories, Wilmington, MA USA) were fed a chow diet (Purina, St. Louis, MO USA) for 1 week prior to the treatment period. Following this acclimation period and after an overnight fast, hamsters were bled and plasma total cholesterol (TC) concentrations were evaluated. Hamsters were then divided into five groups based on similar mean plasma TC concentrations and body weight, and placed on the treatment diets for 4 weeks. The hamsters were fed a chow-based hypercholesterolemic diet (HCD) supplemented with either 5% coconut oil + 0.24% cholesterol (coconut oil), 5% CO, 5% CFO, 5% CO + 0.6% soy sterols (sterol), or 5% CO + 2% soy stanols (stanol) in place of the coconut oil. A chow-based, rather than a semipurified diet, was used because published data from our laboratory[19] and those from another[20] have demonstrated that animals on the chow-based diet are more responsive to various hypocholesterolemic interventions, and the resultant lipoprotein profile (predominantly non-high density lipoprotein cholesterol [non-HDL-C]) is more similar to that of humans. The composition of the soy sterols was sitosterol 48.8%, campesterol 26%, stigmasterol 16.5%, brassicasterol 2.7%, sitostanol 0.9%, campestanol 0.5%, and others 4.6%. The composition of the soy stanols was sitostanol 69%, campestanol 29%, sitosterol 0.7%, campesterol 0.3%, and others 1%. Whereas the soy sterols and stanols were esterified to fatty acids, the sterols and stanols found naturally in CFO are partially esterified to fatty acids and to ferulate. Blood samples were taken at Weeks 0, 2, and 4. All liver and fecal samples were taken at Week 4. Hamsters were housed in individual hanging cages at room temperature with a 12-hr light/dark cycle. Hamsters were given food and water ad libitum and were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Massachusetts Lowell, Lowell, MA USA.

Plasma lipoprotein cholesterol and triglyceride measurements

Blood was collected via the retro-orbital sinus into heparinized tubes from hamsters fasted for 12 hr. Plasma was harvested after centrifugation at 1,500 × *g* at room temperature for 20 min and plasma TC[21] and triglyceride (TG)[22] concentrations were measured enzymatically (Sigma Chemicals, St. Louis, MO USA). Non-HDL-C (plasma very low-density cholesterol [VLDL] and low-density lipoprotein-cholesterol [LDL-C]) was precipitated with phosphotungstate reagent[23] and HDL-C was measured in the supernatant. The concentration of non-HDL-C was calculated as the difference between plasma TC and HDL-C.

Hepatic cholesterol measurements

Hepatic cholesterol concentrations were measured by a previously described method[24] in the following manner: A 100 mg portion of liver was homogenized with 50 mg of sodium sulfate. Five mL of methanol (Burdick and Jackson) was then added and the tissue was homogenized a second time followed by addition of 10 mL of chloroform (Allied Signal Inc., Muskegon, MI, USA). After mixing, 3 mL of a solution containing 1.25% KCl and 0.05% H_2SO_4 was added and centrifuged at 400 × *g* at room temperature for 10 min. The bottom layer was transferred and the supernatant re-extracted with 3 mL of chloroform/methanol (2:1) and centrifuged at 400 × *g* at room temperature for 10 min. The bottom layer was transferred and pooled with the previous step. The solution was placed in a 37°C water-bath and placed under N_2. When approximately half of the solution was evaporated, 1 mL of

Table 1 Plasma lipids and lipoprotein cholesterol concentrations (mmol/L, mean of Weeks 2 and 4) of hamsters after dietary treatment

Diet	TC	non-HDL-C	HDL-C	TC/HDL-C	TG
Coconut oil	7.42 ± 0.35*,†,a	3.33 ± 0.20[a]	4.09 ± 0.17[a]	1.81 ± 0.03	1.22 ± 0.08
CO	7.44 ± 0.22[a]	3.51 ± 0.13[a]	3.93 ± 0.15[a,b]	1.91 ± 0.05	1.04 ± 0.06
CFO	6.44 ± 0.19[b]	2.85 ± 0.10[b]	3.59 ± 0.16[b,c]	1.81 ± 0.04	1.13 ± 0.07
CO + 0.6% soy sterols	5.95 ± 0.12[b]	2.58 ± 0.08[b]	3.37 ± 0.06[c]	1.77 ± 0.02	0.98 ± 0.06
CO + 0.6% soy stanols	5.41 ± 0.27[c]	2.41 ± 0.24[b]	3.00 ± 0.14[d]	1.82 ± 0.10	0.97 ± 0.08

*Values represent mean ± SEM; $n = 10$.
†Values in a column not sharing a superscript are significantly different at $P < 0.05$.
TC–total cholesterol. non-HDL-C–non-high density lipoprotein cholesterol. HDL-C–high density lipoprotein. TG–triglyceride. CO–corn oil. CFO–corn fiber oil.

chloroform with 1% Triton-100 was added, mixed, and evaporated to dryness at 37°C under N_2. Five hundred μL of distilled water was added to the samples, mixed, and placed in a shaking water-bath at 37°C for 20 min to solubilize the lipid. After incubation, hepatic TC and free cholesterol concentrations were determined enzymatically (Waco Chemicals, Richmond, VA USA). Hepatic cholesteryl ester concentration was determined as the difference between the TC and free cholesterol concentrations.

Fecal neutral sterol measurements

Fecal samples were collected over the final 3 days of the study, freeze-dried (lyophilized), and ground-up prior to analysis.[24] Dry feces (200 mg) were extracted with 4 mL of methanol/water (80:20) for 1 hr at 100°C in a 5-mL Reacti-vial fitted with a mini-nert cap. Samples were then allowed to come to room temperature and centrifuged at 500 × g at room temperature for 10 min. The supernatant was removed from the fecal pellet and transferred to an 8-mL borosilicate vial. The fecal pellet was subsequently extracted with 4 mL of methanol/chloroform (50:50) and 4 mL of 1 M ammonium carbonate/methanol (20:80) using the same conditions as the first extraction. The three supernatants were pooled and evaporated to dryness at 100°C under N_2.

Four mL of 0.1 N NaOH/ethanol (10:90 by volume) were added to each sample, over-layered with N_2, and capped and heated at 100°C for 30 min. The samples were allowed to cool to room temperature followed by the removal of the solvent and transferred to 16 × 150 mm borosilicate test tubes. Five mL of water and 3 mL of hexane were added to the solvent followed by mixing and centrifugation at 500 × *g* for 2 min. The top hexane layer was removed and placed in vials. The hexane extraction was repeated two more times and pooled. The hexane extracts were stored at −80°C until analysis of neutral sterols.

To the hexane portion, 1 mL of 5-α-cholestane (240 μg/mL) was added and the solution was brought up to 10 mL with hexane in a volumetric flask. Exactly two mL were removed and evaporated to dryness at 100°C under N_2. One hundred μL of Tri-Sil reagent was added and the samples heated at 85°C for 20 min, followed by evaporation and reconstitution in 100 μL of methylene chloride. One μL was then injected and analyzed by capillary gas chromatography.

Gas chromatographic analyses

Bile acids and neutral sterols were analyzed using a Shimadzu GC-14A gas chromatograph with a flame ionization detector (Shimadzu, Kyoto, Japan) and a 50 m × 0.2 mm HP-1 capillary column (Hewlett Packard, Andover, MA USA). The injector and detector temperatures were set at 300°C. The initial column temperature was 220°C and was increased to 300°C at a rate of 2°C/min. The final temperature was held for 10 min. Column flow rate was 1.5 mL/min. Peak areas were quantitated using a Shimadzu CR501 integrator.

Statistical analyses

A one-way analysis of variance was used to examine the effect of treatment on the different variables using SigmaStat (Jandel Scientific, San Rafael, CA USA). Differences between group means were assessed by Student-Newman-Keuls test. All values are expressed as mean ± SEM and statistical significance was set at $P < 0.05$.[25]

Results

All 50 hamsters survived the 4-week diet regimen. No significant differences were observed between groups for body weight prior to and at the end of the treatment period and for food consumption during the 4 weeks (data not shown).

Plasma lipoprotein and triglyceride concentrations

There were no significant differences between groups for plasma TC concentrations prior to treatment; that is, at Week 0. The average plasma TC concentrations (mmol/L) prior to dietary treatment ranged from 2.45 to 2.47 for all groups.

Because lipid values analyzed at Weeks 2 and 4 were similar within each group, the mean lipid concentrations for each analysis point were averaged together and are presented in *Table 1*. Hamsters that were fed the stanol diet had significantly lower plasma TC concentrations compared to hamsters fed the coconut oil (−27%), CO (−27%), CFO (−16%), and sterol (−9%) diets ($P < 0.05$). Hamsters that were fed the CFO and sterol diets had significantly lower plasma TC compared to hamsters fed the coconut oil (−13% and −20%, respectively) and CO (−13% and −20%, respectively) diets ($P < 0.05$). The coconut oil- and CO-fed hamsters were not significantly different from each other for plasma TC concentrations (*Table 1*).

Hamsters that were fed the CFO, sterol, and stanol diets had significantly lower plasma non-HDL-C concentrations compared to the coconut oil- (−14%, −22%, and −28%, respectively) and CO- (−19%, −26%, −31%, respectively) fed hamsters ($P < 0.05$; *Table 1*). The coconut oil- and CO-fed hamsters were not significantly different from each other for plasma non-HDL-C concentrations (*Table 1*).

Table 2 Hepatic cholesterol concentrations (mg/g of tissue) after 4 weeks of dietary treatment

Diet	Free cholesterol	Cholesteryl ester	Total cholesterol
Coconut oil	1.66 ± 0.42[*,†,a,b]	2.69 ± 1.31	4.35 ± 1.40[a]
CO	1.00 ± 0.58[a]	0.27 ± 0.25	1.27 ± 0.67[b]
CFO	0.98 ± 0.23[a]	0.78 ± 0.60	1.76 ± 0.64[b]
CO + 0.6% soy sterols	2.37 ± 0.57[b]	1.86 ± 0.70	4.23 ± 0.96[a]
CO + 0.6% soy stanols	4.07 ± 0.65[c]	1.56 ± 0.57	4.97 ± 0.95[a]

*Values are mean ± SEM; $n = 10$.
†Values in a column not sharing a superscript are significantly different at $P < 0.05$.
CO–corn oil. CFO–corn fiber oil.

Hamsters that were fed the stanol diet had significantly lower plasma HDL-C concentrations than the coconut oil- (−27%), CO- (−24%), CFO- (−16%), and sterol- (−11%) fed hamsters ($P < 0.05$; *Table 1*). Hamsters fed the CFO and sterol diets had significantly lower plasma HDL-C concentrations compared to hamsters fed the coconut oil (−12% and −18%, respectively) diet ($P < 0.05$). Also, hamsters fed the sterol diet had significantly lower plasma HDL-C concentrations compared to hamsters fed the CO (−14%) diet ($P < 0.05$). The CFO- and CO-fed hamsters were not significantly different from each other for plasma HDL-C concentrations (*Table 1*).

No groups were significantly different for plasma triglyceride concentrations or for plasma TC/HDL-C ratio (*Table 1*).

Hepatic cholesterol concentrations

Hepatic free cholesterol, cholesteryl ester, and TC concentrations are presented in *Table 2*. Hamsters that were fed the CO and CFO diets had significantly less hepatic TC concentrations compared to the hamsters fed the coconut oil (−71% and −60%, respectively), sterol (−70% and −58%, respectively), and stanol (−74% and −65%, respectively) diets ($P < 0.05$). There were no significant differences between hamsters fed the coconut oil, sterol, and stanol diets for hepatic cholesterol concentrations (*Table 2*).

Hamsters that were fed the stanol diet had significantly higher hepatic free cholesterol concentrations compared to hamsters fed the coconut oil (146%), CO (307%), CFO (317%), and sterol (72%) diets ($P < 0.05$; *Table 2*). Also, hamsters fed the sterol diet had significantly higher hepatic free cholesterol concentration than hamsters fed the CO (137%) and CFO (143%) diets. The CO- and CFO-fed hamsters were not significantly different from each other for hepatic free cholesterol, nor were hamsters fed the coconut oil diet significantly different from hamsters fed the sterol diet (*Table 2*).

No groups were significantly different from each other for hepatic cholesteryl ester concentration (*Table 2*).

Fecal neutral sterol concentrations

Fecal neutral sterol concentrations are presented in *Table 3*. Hamsters that were fed the stanol diet had significantly higher concentrations of total fecal neutral sterols than hamsters fed the coconut oil (376%), CO (393%), and CFO (60%) diets ($P < 0.05$), but were not significantly different from hamsters fed the sterol diet. The sterol- and CFO-fed hamsters had significantly higher concentrations of total fecal neutral sterols than the coconut oil- (274% and 198%, respectively) and CO- (288% and 209%, respectively) fed hamsters ($P < 0.05$), but were not significantly different from each other. The coconut oil- and CO-fed hamsters were not significantly different from each other for total fecal neutral sterol concentrations (*Table 3*).

Hamsters that were fed the CFO, sterol, and stanol diets had significantly higher concentrations of fecal cholesterol compared to hamsters fed the coconut oil (187%, 261%, and 271%, respectively) and CO (227%, 311%, and 323%, respectively) diets ($P < 0.05$; *Table 3*). The CFO-, sterol-, and stanol-fed hamsters were not significantly different from each for fecal cholesterol concentrations, nor were the coconut oil- and CO-fed hamsters significantly different from each other (*Table 3*).

Hamsters that were fed the CFO and sterol diets had significantly higher fecal concentrations of campesterol compared to hamsters fed the coconut oil (284% and 300%, respectively) and CO (134% and 144%, respectively) diets ($P < 0.05$), but were not significantly different from the hamsters fed the stanol diet (*Table 3*). Also, hamsters fed the stanol diet had significantly higher fecal concentrations of campesterol compared to the coconut oil (167%) diet ($P < 0.05$), but not significantly different from the CO-fed hamsters. The CO- and coconut oil-fed hamsters were not significantly different from each other for fecal campesterol concentrations (*Table 3*).

Table 3 Total fecal neutral sterol concentrations (mg/g of dry feces) after 4 weeks of dietary treatment

Diet	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Sitostanol	Total
Coconut oil	0.62 ± 0.20[*,†,a]	0.72 ± 0.08[a]	0.90 ± 0.79[a]	0.51 ± 0.26[a]	0.21 ± 0.04[a]	4.46 ± 1.08[a]
CO	0.54 ± 0.05[a]	1.18 ± 0.09[a,c]	0.14 ± 0.01[a]	0.31 ± 0.03[a]	0.73 ± 0.24[a]	4.30 ± 0.40[a]
CFO	1.78 ± 0.18[b]	2.76 ± 0.11[b]	0.35 ± 0.04[a]	2.84 ± 0.23[b]	4.04 ± 0.17[b]	13.29 ± 0.63[b]
CO + 0.6% soy sterols	2.23 ± 0.46[b]	2.87 ± 0.47[b]	2.45 ± 0.51[b]	7.37 ± 1.38[c]	0.60 ± 0.12[a]	16.68 ± 3.04[b,c]
CO + 0.6% soy stanols	2.30 ± 0.22[b]	1.92 ± 0.38[c]	0.28 ± 0.06[a]	1.12 ± 0.24[a,b]	13.67 ± 1.61[c]	21.21 ± 1.66[c]

*Values are mean ± SEM; $n = 10$.
†Values in a column not sharing a superscript are significantly different at $P < 0.05$.
CO–corn oil. CFO–corn fiber oil.

Hamsters that were fed the sterol diet had significantly higher fecal concentrations of stigmasterol compared to hamsters fed the coconut oil (173%), CO (1,704%), CFO (636%), and stanol (767%) diets ($P < 0.05$; *Table 3*). No other dietary treatments were significantly different from each other for fecal stigmasterol concentrations.

Hamsters that were fed the sterol diet had significantly higher fecal concentrations of sitosterol compared to hamsters fed the coconut oil (1,340%), CO (2,285%), CFO (159%), and stanol (559%) diets ($P < 0.05$; *Table 3*). Also, hamsters fed the CFO diet had significantly higher fecal concentrations of sitosterol compared to the coconut oil (455%) and CO (820%) diets ($P < 0.05$), but not compared to the stanol-fed hamsters. The coconut oil-, CO-, and stanol-fed hamsters were not significantly different from each other for fecal sitosterol concentrations (*Table 3*).

Hamsters that were fed the stanol diet had significantly higher fecal concentrations of sitostanol compared to the hamsters fed the coconut oil (6,318%), CO (1,778%), CFO (238%), and sterol (2,167%) diets ($P < 0.05$; *Table 3*). Also, hamsters fed the CFO diet had significantly higher fecal concentrations of sitostanol compared to the coconut oil- (1,799%), CO- (455%), and sterol- (571%) fed hamsters ($P < 0.05$). The coconut oil-, CO-, and sterol-fed hamsters were not significantly different from each other for fecal sitostanol concentrations (*Table 3*).

Discussion

Plant material contains phytosterols (analogues to cholesterol in mammalian organs) as structural and functional components of different membranes.[26] Vegetable oils are usually the richest sources of phytosterols. Structurally, phytosterols are close to cholesterol. Among the major phytosterols, sitosterol contains an additional ethyl group and campesterol an additional methyl group in the side chain of the cholesterol structure. Intestinal bacteria convert cholesterol or phytosterols into their corresponding 5β-saturated sterol (i.e., coprostanol) or 3-keto derivative (coprostanone).[27] Previous work[10] has shown that CFO has greater levels of these phytosterols compared to other vegetable oils including CO. The present study examined not only the cholesterol-lowering mechanism(s) of action of CFO, but also examined the mechanism(s) of action of phytosterols added to the diet.

In this controlled study, 50 hamsters were divided into five parallel treatment groups and fed a HCD for a period of 4 weeks. As expected, the HCD alone produced hypercholesterolemia in these hamsters, evidenced by the greater than 3-fold increase from Week 0 in plasma cholesterol concentrations. Hamsters fed the CFO, sterol, or stanol diets demonstrated inhibited elevations of plasma TC and non-HDL-C concentrations relative to both the coconut and the CO diets. In the present study, the stanol diet exhibited a slightly greater efficacy in preventing the development of hypercholesterolemia than hamsters fed the sterol or the CFO diets (observed only as a significant reduction in plasma TC but not non-HDL-C concentrations compared to the CFO and sterol diets). These findings are consistent with previous work in animals[28–32] and humans[15,33,34] that showed a reduction in plasma cholesterol concentrations with the addition of phytosterols or phytostanols to the diet, and that showed that the phytostanols were more effective than the phytosterols at lowering plasma cholesterol concentrations. The present study showed similar differences in plasma non-HDL-C concentrations between the sterol diet and stanol diet; this is consistent with another study[35] in which soybean sterol ester margarine lowered plasma LDL-C as much as the soybean stanol ester margarine. The current study also added soybean sterol and stanol esters to the diets of the hamsters.

Whereas the hamsters that were fed the coconut and CO diets had higher plasma HDL-C concentrations, hamsters fed the CFO diet had significantly higher concentrations of plasma HDL-C compared to hamsters fed the stanol diet, but not the sterol diet. In contrast, previous work had shown that phytosterols have no effect on other plasma lipoproteins other than LDL-C.[36–38] Although the addition of CFO, sterols, and stanols to a HCD reduced plasma TC and non-HDL-C concentrations, it did not significantly reduce plasma triglyceride concentrations. This is consistent with previous studies in humans fed vegetable oil–stanol ester margarine[38,39] and in animals fed soluble or insoluble fibers.[40–42]

The present study showed no effect of CO on plasma cholesterol and triglyceride concentrations; this is probably due to the low amount of CO (5%) in the diet. Previous work in animals[43–45] has shown reductions in plasma cholesterol concentrations with greater amounts of CO in the diet (>30% of energy). Also, the hamsters were only on the treatment diets for 4 weeks, which may not be sufficient time for a low-CO diet to produce reductions in plasma cholesterol concentrations. Although the CFO diet and the sterol- and stanol-containing diets, which have similar fatty acid profiles to CO, did produce significant reductions in plasma cholesterol concentrations, this reduction was probably the result of the phytosterols added to the CO diets (sterol and stanol diets) and the naturally occurring phytosterols and oryzanol found in CFO.

Higher concentrations of hepatic cholesterol is associated with increased secretion of VLDL.[46] In addition, hepatic lipids also influence VLDL triglyceride secretion by the liver.[47] Studies have shown that intakes of high cholesterol result in increased hepatic free and esterified cholesterol due to the increased delivery of exogenous cholesterol.[48] The consumption of CFO and CO decreased hepatic TC concentrations compared to the coconut oil diet, although CO feeding at 5% of the diet had no effect on plasma cholesterol concentrations. This result suggests that reductions in hepatic cholesterol concentrations can be observed without reductions in plasma cholesterol concentrations with CO. It also suggests that the hepatic cholesterol-lowering for CO and CFO is primarily due to the fatty acid profile of the vegetable oils and not due to other naturally occurring ingredients found in CFO and not in CO (i.e., phytosterols or oryzanols). Also, plasma LDL-C catabolism in vivo is highly correlated with hepatic apo B/E receptor modification.[49] Our results indicate that CFO could partially mediate its hypocholesterolemic effect by up-regulation of LDL receptor in response to a depleted hepatic cholesterol pool.

An unexpected finding was that hamsters fed the stanol

and sterol diets had significantly higher hepatic TC and free cholesterol concentrations compared to the hamsters fed the CFO and CO diets. It was previously thought that a decrease in cholesterol absorption efficiency with phytosterols reduces the flow of intestinal cholesterol to the liver, resulting in enhanced cholesterol synthesis (probably through reduced liver cholesterol),[27] which is inconsistent with the current study. Studies on compounds that reduce cholesterol absorption such as Benecol (a mixture of sitostanol esters[50] that prevent bile acid reabsorption) are oftentimes associated with increased 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity. However, despite this increase in cholesterol synthesis, reductions in plasma LDL-C concentrations of up to 20% are still obtainable. Although the present study did not measure HMG-CoA reductase activity, it is possible that in the CFO and the sterol and stanol diets, by inhibiting cholesterol absorption and decreasing plasma cholesterol concentrations, an increase in HMG-CoA reductase would have been observed, which would be consistent with previous studies[51–53] and/or greater amounts of plasma cholesterol being sequestered by the liver out of the plasma. In addition, the CFO diet may have increased the cholesterol metabolism into biliary acids via increased 7 α-hydroxylase activity in hepatic tissues, and thereby decreased hepatic cholesterol concentrations; previous studies, however, have shown that phytosterols do not increase bile acid synthesis,[54,55] which may have led to the increases in hepatic cholesterol concentrations that were observed in the present study.

Phytosterol/cholesterol absorption is influenced by the total sterol concentration in the oil, micellar as well as the solid phase.[54] As a metabolic consequence of lipolysis and CFO, sterol, and stanol treatment, free sterol could accumulate in the oil phase in the lumen of the intestine and selectively increase the precipitation rates of sterol.[32] Similar to previous observations,[34,56] CFO and phytosterols could have its hypocholesterolemic effect by replacing cholesterol from the micellar mix, and thereby rendering it less absorbable. The current study is consistent with these previous findings,[32,56–58] in that the CFO, sterol, or stanol diets increased the fecal excretion of total neutral sterols as well as cholesterol compared to hamsters fed the coconut oil and corn diets.

The present study also showed that sterol feeding to hamsters increased fecal excretion of other neutral sterols including campesterol, stigmasterol, and sitosterol compared to the coconut oil, CO, and stanol diets, but was not significantly greater for campesterol compared to the CFO diet. Stanol feeding, however, increased the fecal excretion of the corresponding saturated neutral sterols (i.e., sitostanol) compared to the other dietary treatments. Hamsters fed the CFO diet excreted significantly more campesterol, sitosterol, and sitostanol compared to the coconut oil and CO diets. The differences in fecal excretion of campesterol, stigmasterol, sitosterol, and sitostanol are due to the increased consumption and poor bioavailability of these neutral sterols by hamsters.

In conclusion, CFO reduces plasma cholesterol concentrations similarly to the addition of soy sterols and stanols to CO as a result of modulating hepatic cholesterol pools and inhibiting the absorption of cholesterol in the intestines. These mechanisms may possibly up-regulate the LDL receptor and remove cholesterol from plasma. However, because the addition of soy sterols and stanols to CO do not produce a reduction in hepatic cholesterol concentrations, the hepatic cholesterol-lowering effect of CFO may be due to its fatty acid profile or some other natural cholesterol-lowering component found in CFO such as oryzanol.

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