

## Corn fiber oil and sitostanol decrease cholesterol absorption independently of intestinal sterol transporters in hamsters

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### Abstract

**Objective:** The aim of this study was to investigate the cholesterol-lowering mechanisms of corn fiber oil (CFO), ferulate phytostanyl esters (FPEs) and parent compounds of FPE, including sitostanol and ferulic acid, in hamsters.

**Method:** Seventy male Golden Syrian hamsters were randomly assigned to six experimental diets for 4 weeks: (1) cornstarch–casein–sucrose-based control diet (control); and (2) control diet plus 0.1% (wt/wt) cholesterol (cholesterol–control). The remaining four groups were given cholesterol–control diet with: (3) 10% (wt/wt) CFO; (4) 0.5% (wt/wt) sitostanol; (5) 0.23% (wt/wt) ferulic acid; and (6) 0.73% (wt/wt) FPE. At the end of dietary intervention, total plasma cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were determined. Parameters of cholesterol kinetics, including cholesterol absorption and synthesis, as well as mRNA expression of sterol transporters such as Niemann–Pick C1 like 1 (NPC1L1), ATP-binding cassette G5 (ABCG5) and ABCG8, were assessed.

**Results:** Supplementation with CFO decreased ( $P<0.001$ ) plasma total cholesterol levels by 29% as compared with the cholesterol–control group, while FPE and sitostanol reduced ( $P<0.02$ ) cholesterolemia by 15% and 14%, respectively. CFO and sitostanol decreased ( $P<0.05$ ) cholesterol absorption by 24% compared to the cholesterol–control group. Dietary intervention did not alter the intestinal gene expression of ABCG5, ABCG8 and NPC1L1.

**Conclusion:** The present results show that the CFO-induced and sitostanol-induced decrease in cholesterol absorption is independent of intestinal enterocyte sterol transporters such as ABCG5, ABCG8 and NPC1L1 in hamsters.

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**Keywords:** Corn fiber oil; Sitostanol; Ferulate phytostanyl esters; Total cholesterol; Non-HDL cholesterol; Gene expression; Hamster

### 1. Introduction

Alterations in cholesterol metabolism lead to clinical conditions that increase cardiovascular disease (CVD) risk. These clinical conditions could be manifest as hypercholesterolemia, hypertriglyceridemia, mixed hyperlipidemia, insulin resistance or lipoprotein disorders. Both genetic and environmental factors, including diet, influence cholesterol metabolism and predispose individuals to CVD. Lowering

serum cholesterol levels plays an important role in the prevention of CVD [1]. Recently, cholesterol-lowering dietary alternatives, nontraditional foods or nutraceuticals have become more appealing over other cholesterol-lowering approaches. Dietary ingredients such as plant sterols and stanols have been shown to lower serum cholesterol levels [2–4].

Nontraditional foods such as corn fiber oil (CFO) [5,6] and rice bran oil [7,8] contain high levels of plant sterols/stanols and their conjugates. These nontraditional foods have been shown to reduce circulatory cholesterol concentrations [9]. Dietary ingredients such as plant sterols/stanols have

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been well identified as cholesterol-lowering agents [2–4] by inhibiting intestinal cholesterol absorption [10–13]. However, the exact process through which plant sterols/stanols decrease cholesterol absorption is not fully understood. Recently, several diseases caused by disruption of normal subcellular cholesterol transport, such as Niemann–Pick C1 like 1 (NPC1L1) disease due to an NPC gene defect [14] and  $\beta$ -sitosterolemia due to mutations in ATP-binding cassette G5 (ABCG5) and ABCG8 [15], suggest the existence of specific sterol transport and sorting pathways. According to a recent study by Davis et al. [16], cholesterol and sitosterol absorption was markedly reduced in NPC1L1-null mice. Therefore, plant sterols and stanols may exert their cholesterol-lowering action by decreasing the influx of cholesterol through NPC1L1 transporter proteins. In addition to NPC1L1, plant sterols may also exert their cholesterol-lowering action through ATP-binding cassette transporter proteins. Recent studies have shown that ABCG5 and ABCG8 are involved in the intestinal absorption of sterols and in their excretion in bile [15,17].

The present study was undertaken to investigate the cholesterol-lowering mechanisms of CFO, ferulate phytostanyl esters (FPEs) and parent compounds, including sitostanol and ferulic acid, in hamsters by determining the gene expression of intestinal sterol transporters including NPC1L1, ABCG5 and ABCG8. The hamster model was considered suitable for investigating the effect of our dietary interventions on parameters of cholesterol kinetics, including cholesterol absorption and synthesis, because of the close resemblance of cholesterol metabolism between humans and hamsters [18].

## 2. Materials and methods

### 2.1. Animals and study design

A 4-week hamster trial, approved by the McGill University Animal Care Committee, was conducted accord-

ing to Canadian Animal Care guidelines. Upon arrival, 70 male Golden Syrian hamsters weighing 80–110 g (Charles River Laboratories, Wilmington, MA) were acclimatized individually for 2 weeks in stainless steel cages. A light cycle of 12 h was set. During the acclimatization period, hamsters were given free access to water and were fed pelleted rodent chow ad libitum. Hamsters were then randomly assigned to and fed six experimental diets for 4 weeks. Semipurified diets (ICN Biomedicals, Inc., Aurora, OH) were prepared every 2 weeks and stored at 4°C. The dietary composition of the different experimental diets is shown in Table 1. Except for the noncholesterol–control group, all diets contained 0.1% (wt/wt) cholesterol. Other dietary groups contained: (a) 10% CFO (USDA, Wyndmoor, PA); (b) 0.5% sitostanol (Research Plus, Manasaquan, NJ); (c) 0.23% ferulic acid (Sigma Chemical, St. Louis, MO); or (d) 0.73% FPE [19]. All diets contained 10 wt.% added fat provided in the form of a mixture of safflower oil and beef tallow to yield a polyunsaturated fatty acid/saturated fatty acid ratio of 0.4, while the CFO group contained 10 wt.% of CFO, replacing the 10 wt.% added fat.

On Day 26 of feeding, 48 h prior to sacrifice, hamsters were given intragastrically 1.4 mg of  $^{13}\text{C}_2$  cholesterol suspended in 0.4 ml of equal amounts of olive oil and sunflower oil mix. Hamsters were returned to cages and provided with food and water as usual. At the end of the 28-day trial, 2 h prior to sacrifice, hamsters were injected intraperitoneally with 0.5 ml of deuterium oxide. Two hours after injection, hamsters were euthanized by carbon dioxide inhalation, and blood samples were collected by decapitation in EDTA tubes. Plasma and red blood cell (RBC) samples were separated by centrifugation at 136 rad/s for 10 min and stored at –20°C for further analysis. RBCs were used immediately for fragility measurement, and plasma was stored at –20°C. Immediately, proximal intestinal tissue samples were collected, frozen in liquid nitrogen and then stored at –80°C for later mRNA expression analysis.

Table 1  
Composition of experimental diets for hamsters

Diet (% wt/wt)	Noncholesterol	Cholesterol–control	10% CFO	0.73% FPE	0.50% Sitostanol	0.23% Ferulic acid
Casein	20	20	20	20	20	20
Cornstarch	26	25.96	25.96	25.64	25.74	25.86
Sucrose	33.29	33.24	33.24	32.83	32.96	33.11
Beef tallow/safflower oil mix	10	10	–	10	10	10
Cellulose	5	5	5	5	5	5
DL-methionine	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mixture	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2	0.2	0.2
Butylated hydroxytoluene	0.002	0.002	0.002	0.002	0.002	0.002
Cholesterol	–	0.1	0.1	0.1	0.1	0.1
CFO	–	–	10	–	–	–
FPEs	–	–	–	0.73	–	–
Sitostanol	–	–	–	–	0.5	–
Ferulic acid	–	–	–	–	–	0.23

## 2.2. Plasma cholesterol analysis

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were measured in duplicate by enzymatic methods (Roche Diagnostics, Laval, QC, Canada) [20]. HDL-C in plasma was measured by the precipitation of apolipoprotein B containing lipoproteins with dextran sulfate and magnesium chloride [21]. Results were also expressed as non-HDL-C (very-low-density lipoprotein+intermediate-density lipoprotein+low-density-lipoprotein cholesterol) instead of low-density-lipoprotein cholesterol because the Friedewald equation [22] may not be applicable to hamsters. Non-HDL-C was calculated by subtracting HDL-C from total cholesterol levels.

## 2.3. RBC fragility

RBCs freshly collected at the end of plasma separation were used for RBC fragility measurement. RBCs were added to eight different concentrations of 2 ml of saline solution ranging from 0.3% to 0.65% in 5-ml disposable tubes and kept for 1 h. The tubes were then centrifuged at 100 rad/s for 5 min at room temperature. Supernatants were collected in cuvettes, and absorbance was measured at 520 nm [23]. Results were expressed as percent hemolysis for different saline concentrations ranging from 0.3% to 0.65%.

## 2.4. Determination of cholesterol absorption and biosynthesis

Cholesterol absorption was determined using a single-isotope-labeled cholesterol tracer approach [24]. The free cholesterol extracted from RBCs was used to determine  $^{13}\text{C}_2$  cholesterol enrichment. RBCs contain free cholesterol designated as part of a rapid changeover pool [25]. The area under the  $^{13}\text{C}_2$  cholesterol enrichment curve was used to calculate percent cholesterol absorption assuming that cholesterol tracers are entirely incorporated into the central cholesterol pool and no significant amount of cholesterol tracer is lost over the period of 48 h after oral administration. The rate of cholesterol absorption was used to compare the difference between treatment groups and their effect on cholesterol absorption relative to the cholesterol–control group.

Cholesterol biosynthesis rate was determined as the rate of deuterium incorporation into the RBC membrane of free cholesterol over the period of 2 h at the end of 4 weeks of dietary intervention. The increase in the ratio of deuterium/hydrogen in free cholesterol 2 h after deuterium injection, relative to baseline sample, represents the amount of newly synthesized cholesterol and was expressed as a percent rate of body cholesterol pool [26].

Lipids were extracted in duplicate from 0.1 g of RBC using a modified Folch, Lees and Sloane–Stanley procedure [27]. Extracted lipids were converted to trimethylsilyl (TMSi) ethers by adding 1.5 ml of TMSi reagent (pyridine–hexamethyldisilazan–trimethylchlorosilane, 9:3:1, vol/vol/

vol) and heating the samples at 70°C for 1 h [28]. Afterwards, silylating reagents were evaporated under a nitrogen flush evaporator. TMSi derivatives were dissolved in 1 ml of hexane and injected into a Clarus gas chromatograph with an autosampler injector and interfaced to a Clarus 500 mass spectrometer (Perkin Elmer, Inc., Connecticut). TMSi derivatives of extracted lipids were separated in a 30-m fused silica capillary column (HP SAC-5, inner diameter of 0.25 mm; Supelco Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Helium was used as a carrier gas with a flow rate of 1.0 ml/min. Oven temperature was set to an initial value of 250°C for 1 min followed by a temperature program, increasing by 1.3°C/min up to the final temperature of 280°C. The temperature of the transfer line was kept at 280°C. Electron impact ionization was applied with 70 eV. Ions of  $m/z$  458, 459 and 460 were monitored for quantification of cholesterol (M), deuterated cholesterol (M+1) and  $^{13}\text{C}_2$ -labeled cholesterol (M+2), respectively. Each ion peak area monitored consisted of detector response due to one type of cholesterol, but also included smaller interfering fragment responses due to other cholesterol. Ion peaks at masses 458, 459 and 460 were corrected by constants determined by running samples from pure natural cholesterol or labeled cholesterol, as described previously [29]. Cholesterol absorption was determined by measuring the increase in ratio between counts of ion representing  $^{13}\text{C}_2$ -labeled cholesterol ( $m/z=460$ ) and natural cholesterol ( $m/z=458$ ) relative to baseline sample. Cholesterol biosynthesis was determined by measuring the increase in ratio between counts of ion representing deuterated cholesterol ( $m/z=459$ ) and natural cholesterol ( $m/z=458$ ) relative to baseline sample.

## 2.5. mRNA expression analysis

Real-time quantitative polymerase chain reaction (PCR) was used for the analysis of the relative mRNA expression of NPC1L1, ABCG5 and ABCG8. RNA was extracted from proximal intestinal tissue using RNeasy mini kit (Qiagen, Valencia, CA). The ratio of absorbance at 260 and 280 nm ( $A_{260}/A_{280}$ ) was calculated to ensure the integrity and purity of extracted RNA. Two micrograms of RNA was reverse transcribed to cDNA using random nanomer primers and an Omniscript RT kit (Qiagen). Real-time quantitative PCR was performed using the MX 3000p thermal cycle system (Stratagene, California), with gene-specific primers (Alpha DNA, QC, Canada) and a Quantitect SYBR green Q-PCR master mix (Qiagen). PCR conditions for all genes consisted of one denaturing cycle at 95°C for 15 min, followed by 40 cycles consisting of denaturing for 15 s at 95°C and annealing and elongation for 1 min at 60°C. At the end of PCR cycles, samples were subjected to a melting curve analysis. To control for any variation due to the efficiencies of reverse transcription and PCR, all genes measured were normalized against 18S RNA. All PCR runs were performed in triplicate. Primer sequences for NPC1L1, ABCG5, ABCG8 and 18S RNA for hamsters have been published previously [30].

## 2.6. Statistical analysis

All data were tested for normality by the Kolmogorov–Smirnov test, and a log transformation for mRNA expression data was used to attain normality. To determine the effect of dietary intervention on the cholesterol absorption, cholesterol biosynthesis and mRNA expression of NPC1L1, ABCG5 and ABCG8, data were analyzed by one-way analysis of variance (ANOVA) using the general linear model procedure of SAS 8.0 software (SAS Institute, Inc., Cary, NC). Significant differences among dietary treatments were analyzed by the method of PDiff matrix of least squares means. Pearson's correlation was used to test correlations between parameters of cholesterol kinetics, including cholesterol absorption and cholesterol biosynthesis, and mRNA expression of intestinal sterol transporters such as NPC1L1, ABCG5 and ABCG8. Data were expressed as mean±S.D., and the difference between means was considered statistically significant at  $P<0.05$ .

## 3. Results

### 3.1. Feed intake and body weight

No significant differences were observed in average food intake (g/day) or body weight between different dietary intervention groups. Daily food intake across the groups ranged from  $6.90\pm0.34$  to  $8.44\pm0.27$  g. During the 4 weeks of trial, hamsters gained 15–22 g in body weight. The CFO group exhibited a higher weight gain of  $21.97\pm2.81$  g, while the ferulic acid group displayed a lower weight gain of  $15.79\pm1.70$  g; however, differences were not statistically significant.

### 3.2. Response of plasma lipid profile to treatment

Compared with the cholesterol–control group ( $6.16\pm0.78$  mmol/L), animals supplemented with 10% (wt/wt) CFO ( $4.34\pm0.87$  mmol/L) displayed plasma total cholesterol levels that were reduced by 29% ( $P<0.0001$ ) (Table 2). The mean plasma total cholesterol levels in the 0.5% (wt/wt) sitostanol group ( $5.28\pm1.00$  mmol/L) and in the 0.73% (wt/wt) FPE group ( $5.21\pm0.49$  mmol/L) were also reduced by 14% ( $P=.02$ ) and 15% ( $P=.02$ ), respectively, in

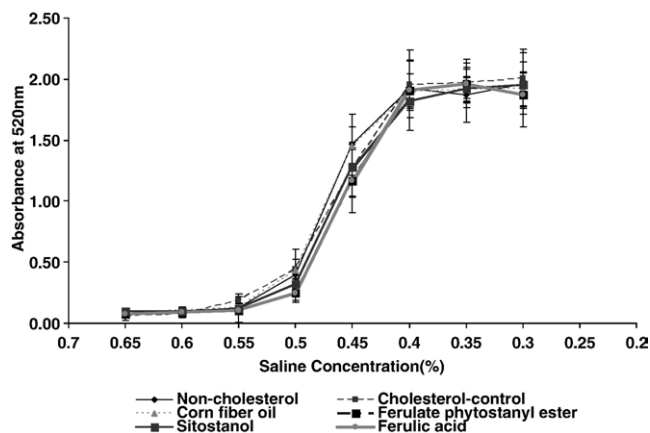


Fig. 1. Effect of dietary intervention on RBC fragility in hamsters.

comparison to the cholesterol–control group. However, plasma total cholesterol levels of 0.23% (wt/wt) the ferulic acid group ( $5.56\pm0.68$  mmol/L) were not statistically different from the cholesterol–control group. Moreover, HDL-C levels in hamsters supplemented with the CFO, sitostanol and FPE diets decreased by 19% ( $P=.029$ ), 39% ( $P=.001$ ) and 22% ( $P=.01$ ), respectively, in comparison to hamsters fed the cholesterol–control diet. Plasma TG levels were also reduced in the CFO ( $P=.039$ ), sitostanol ( $P=.041$ ) and FPE ( $P=.049$ ) groups, in comparison with the cholesterol–control group. Mean plasma non-HDL-C levels did not differ across the six treatment groups.

### 3.3. RBC fragility in response to treatment

There was no significant difference in RBC fragility between the different dietary intervention groups at saline concentrations ranging from 0.3% to 0.65% (Fig. 1).

### 3.4. Cholesterol absorption in response to treatment

Enrichment values of  $^{13}\text{C}_2$ -labeled cholesterol in RBC-free cholesterol relative to baseline sample were taken to represent the extent of cholesterol absorption [24]. Cholesterol absorption data, provided as percent cholesterol absorption relative to the cholesterol–control group, are presented in Fig. 2. Forty-eight hours after isotope administration, mean percent cholesterol absorption was lower

Table 2  
Effect of dietary intervention on the plasma lipid profile of hamsters<sup>1</sup>

Treatment group	n <sup>2</sup>	Total cholesterol	HDL-C	Non-HDL-C	TG
Noncholesterol	12	$4.64\pm1.03^{\text{c,d}}$	$2.60\pm0.51^{\text{a,b}}$	$2.04\pm1.16^{\text{b}}$	$2.38\pm0.39^{\text{b}}$
Cholesterol–control	12	$6.16\pm0.78^{\text{a}}$	$3.09\pm0.88^{\text{a}}$	$3.07\pm1.15^{\text{a,b}}$	$3.19\pm0.83^{\text{a}}$
Sitostanol	12	$5.28\pm1.00^{\text{b,c}}$	$2.49\pm0.48^{\text{b}}$	$2.80\pm1.08^{\text{a,b}}$	$2.46\pm0.52^{\text{b}}$
CFO	12	$4.34\pm0.87^{\text{d}}$	$1.88\pm0.63^{\text{c}}$	$2.46\pm0.96^{\text{a,b}}$	$2.45\pm0.36^{\text{b}}$
FPEs	10	$5.21\pm0.49^{\text{b,c}}$	$2.40\pm0.65^{\text{b,c}}$	$2.81\pm0.77^{\text{a,b}}$	$2.43\pm0.72^{\text{b}}$
Ferulic acid	12	$5.56\pm0.68^{\text{a,b}}$	$2.28\pm0.71^{\text{b,c}}$	$3.28\pm0.84^{\text{a}}$	$2.88\pm0.70^{\text{a,b}}$

Values carrying different superscript letters indicate significant differences between treatment groups ( $P<0.05$ ).

<sup>1</sup> Values are expressed as mean±S.D. (mmol/L).

<sup>2</sup> Number of hamsters.



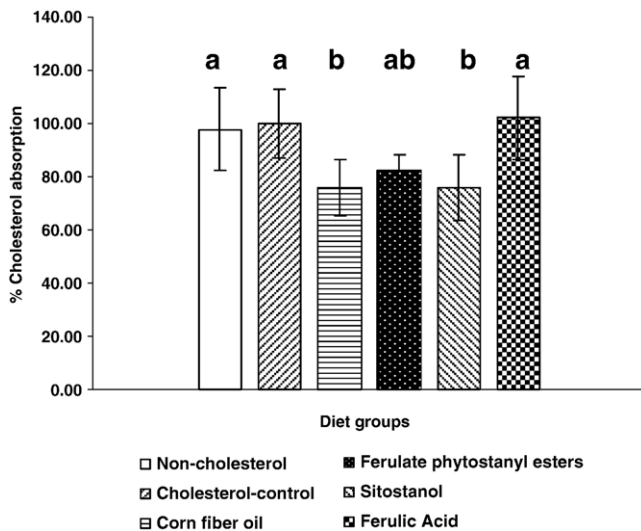


Fig. 2. Effect of dietary intervention on percent cholesterol absorption in hamsters. Groups carrying different letters are significantly different (ANOVA).

( $P < 0.05$ ) for the CFO and sitostanol groups by 24%, compared to the cholesterol–control group. No significant correlations were detected between percent cholesterol absorption and plasma lipid profile measured within each treatment group.

### 3.5. Cholesterol biosynthesis in response to treatment

The estimated amount of newly synthesized cholesterol was not significantly different among treatment groups (Table 3). However, CFO, sitostanol and FPE showed a trend of a 6% to an 8% increase in newly synthesized cholesterol compared to the cholesterol–control group. No significant correlations were detected between cholesterol biosynthesis and plasma lipid profile measured within each treatment group.

### 3.6. mRNA expression of intestinal sterol transporters

mRNA expression levels for intestinal sterol transporters, including NPC1L1, ABCG5 and ABCG8, in the proximal

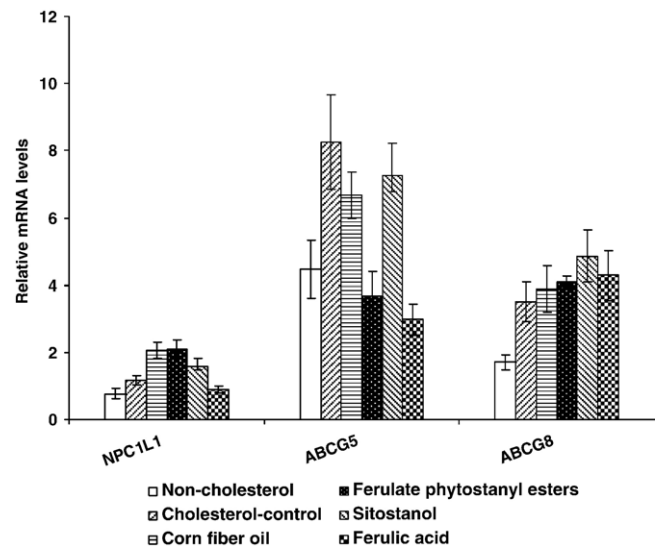


Fig. 3. Effect of dietary intervention on intestinal NPC1L1 and ABCG5/ABCG8 gene expression.

intestine, provided as mRNA expression relative to the cholesterol–control group, are presented in Fig. 3. The CFO, sitostanol and FPE groups showed onefold to twofold up-regulation and down-regulation of NPC1L1 and ABCG5/ABCG8 mRNA expression levels, respectively, compared to the cholesterol–control group; however, these changes were not statistically significant.

## 4. Discussion

Our results demonstrate that CFO was more effective in reducing plasma total cholesterol levels than FPE or sitostanol. The cholesterol-lowering effects of CFO of approximately the same extent have been previously reported in other animal studies [5,6]. The cholesterol-lowering efficacy of CFO is mostly attributed to its composition of free plant sterols and plant stanol conjugates such as fatty acyl phytosterol esters and ferulate phytostanol esters. Many studies have reported the cholesterol-lowering effect of free plant sterols and fatty acyl phytosterol esters [11,12]; however, this study is the first to report a hypocholesterolemic effect of FPE found in CFO. Although both sitostanols and ferulic acid [31] have been shown to possess cholesterol-lowering effects individually, the results of the present study demonstrate that there is no synergistic effect of the esterification of these compounds. This absence of synergism might be due to the decreased solubility of the newly synthesized larger compound.

In addition to affecting plasma total cholesterol levels, CFO, sitostanol and FPE lowered plasma HDL-C and TG levels in hamsters consuming a 0.1% cholesterol-containing diet. The effects of CFO on HDL-C are consistent with the results of a previous study by Wilson et al. [6] in hamsters. The lack of a significant lowering effect on non-HDL-C

Table 3  
Effects of dietary intervention on cholesterol biosynthesis<sup>1</sup>

Treatment group	n <sup>2</sup>	Newly synthesized cholesterol (%) <sup>3</sup>
Noncholesterol–control	12	1.81±0.14 <sup>a</sup>
Cholesterol–control	12	1.62±0.16 <sup>a</sup>
CFO	12	1.72±0.17 <sup>a</sup>
FPEs	10	1.75±0.13 <sup>b</sup>
Sitostanol	12	1.73±0.16 <sup>a</sup>
Ferulic acid	12	1.66±0.37 <sup>a</sup>

Values carrying different superscript letters indicate significant differences between treatment groups ( $P < 0.05$ ).

<sup>1</sup> Values are expressed as mean±S.D.

<sup>2</sup> Number of hamsters.

<sup>3</sup> Estimated amount of newly synthesized cholesterol expressed as a percentage of the body cholesterol pool 2 h after deuterium injection and relative to baseline.

levels could be due to the fact that hamsters carry a larger proportion of plasma cholesterol in HDL versus non-HDL particles. Since HDL is a major antiatherosclerotic factor, the dietary intervention might actually have acted as proatherogenic. Hence, the question of proatherogenic or antiatherogenic effects needs to be studied further in other rodents, in nonrodents or in humans. The effects of sitostanol on TGs have been variable due to the different amounts of dietary cholesterol and fats used in different animal models. Similar to what was observed in the present results, Trautwein et al. [32] showed a 25% decrease in TG levels by 0.48% dietary stanols in hamsters ingesting 0.08% cholesterol-containing diets.

RBC fragility and deformability tests are measures of RBC hemolysis due to changes in temperature, cell membrane intactness and osmotic pressure. Ratnayake et al. [33] showed that 0.2% wt/wt plant sterol supplementation lowered RBC deformability in the SHRSP rat model. Contrary to this finding, Hendriks et al. [34] showed that long-term supplementation of plant-sterol-enriched spreads for 1 year in humans had no effect on RBC deformability. Likewise, the results of the present study indicate that plant stanol (0.5% wt/wt) supplementation has no effect on RBC fragility in hamsters. Similar results were observed in different clinical [35,36] and animal [37] trials using RBC fragility as a measure of RBC lifespan. Hence, the present study suggests that CFO, sitostanol, ferulate sitostanyl esters and ferulic acid have no detrimental effects on RBC fragility in hamsters.

In the present study, cholesterol absorption was analyzed by measuring the enrichment of single-isotope-labeled cholesterol tracer ( $^{13}\text{C}_2$  cholesterol) by gas chromatography/mass spectrometry (GC/MS). In the present study, the reduction in the rate of cholesterol absorption by sitostanol was comparable to that reported previously and was measured using the plasma dual stable isotope ratio method by differential isotope ratio mass spectrometry (IRMS) [3]. Cholesterol biosynthesis was analyzed using deuterium uptake and GC/MS methodology [26,28]. The results of the present study were not consistent with those reported previously, which reported an up-regulation of cholesterol biosynthesis rate in hamsters fed 1% (wt/wt) sitostanol, analyzed as deuterium enrichment of plasma water by differential IRMS [3]. However, our results demonstrate a trend of elevated newly synthesized cholesterol in hamsters fed 10% CFO, 0.5% (wt/wt) sitostanol and 0.73% FPE (Table 3). Cholesterol homeostasis in vivo is maintained by an interplay of exogenous cholesterol absorption and endogenous cholesterol synthesis [38]. Intracellular cholesterol from both sources combine to form the free cholesterol pool. Cholesterol biosynthesis is feedback inhibited, with low levels of circulating cholesterol up-regulating the rate of de novo cholesterol biosynthesis to maintain cholesterol homeostasis [38]. However, our data suggest that hamsters fed 10% CFO or 0.5% (wt/wt) sitostanol exhibit lower rates of cholesterol absorption without up-regulating the compen-

satory effect of cholesterol biosynthesis. These results do not support previous research, which demonstrated a reciprocal increase in cholesterol synthesis with attenuation of cholesterol absorption with plant sterol administration in animal and human studies [3,39]. This difference could be due to the fact that both GC/MS and gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) have varying levels of detection limits and sensitivity. According to a study by Gua et al. [40], both GC/C/IRMS and GC/MS have excellent sensitivity; however, GC/C/IRMS showed about a 15-fold better detection limit for cholesterol tracer compared to GC/MS. Although the dosages of the stable isotope cholesterol tracer were increased by 10- fold to 15-fold in the present study, it is possible that the imprecision of the method could have accounted for the results observed presently.

The exact mechanism of the reduction in the rate of cholesterol absorption by plant sterols remains to be clearly understood. Several mechanisms, such as preventing the incorporation of free cholesterol into micelles, preventing the esterification of free cholesterol into cholesterol esters [10] and increasing bile salt excretion [40], have been proposed. There also exists an increasing interest in understanding the genetic control of the cholesterol-absorption-lowering effect of plant sterols at the subcellular level and the role of such modulators in the regulation of cholesterol flux. This interest has been driven by recent studies showing the critical role played by sterol transporters such as NPC1L1 [41], ABCG5 and ABCG8 [42] in regulating cholesterol flux at the intestinal enterocyte level. In the present study, the effects of dietary intervention on cholesterol kinetics and the gene expression of NPC1L1, ABCG5 and ABCG8 were studied in non-gene-treated and diet-induced hypercholesterolemic hamsters to try to gain insight into the genetic control of the cholesterol-lowering effect of plant sterols at the intestinal enterocyte level. The present results indicate that the CFO and sitostanol interventions change the intestinal expression of ABCG5, ABCG8 and NPC1L1 in hamsters. However, these onefold to twofold changes in mRNA expression were neither statistically significant nor large enough to modulate intestinal cholesterol absorption [30]. Our observations are in line with recent findings in hamsters [29] and C57Bl/6J mice [17,43,44]. In these studies, hamsters and C57Bl/6J mice were fed a diet with and without phytosterols, and the intestinal expression of sterol transporter genes was not altered.

In summary, CFO was shown to possess a greater cholesterol-lowering effect than pure sitostanol and its ferulate ester. In addition, esterification of sitostanol and ferulic acid did not yield any additive synergistic effects over free sitostanol. The present data also suggest that reductions in the rate of cholesterol absorption induced by CFO and sitostanol are independent of intestinal enterocyte sterol transporters such as ABCG5/ABCG8 and NPC1L1. Therefore, CFO and its constituents may act as effective dietary

adjuncts that lower the levels of circulating cholesterol (a major risk factor in coronary vascular disease) by reducing the absorption of dietary cholesterol.

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