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Cholesterol-Lowering Effects of Plant Steryl and Stanyl Laurate by Oral Administration in Mice

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ABSTRACT: The present study was conducted to investigate the efficacy of synthesized plant steryl and stanyl laurate in lowering the cholesterol level and to further examine the cholesterol-lowering potential of the free plant sterols and stanols dissolved in liquid emulsion on serum and liver lipids in mice by oral administration. Experimental results showed that both plant steryl and stanyl laurate could significantly decrease the serum levels of TC, LDL-C, LDL-C/HDL-C, and liver cholesterol contents and markedly increase fecal cholesterol concentrations but have no effect on serum TAG level, indicating that the produced plant steryl and stanyl laurate retained the cholesterol-lowering potential of natural plant sterols and stanols. However, no statistical difference in cholesterol-lowering efficacy was observed between plant steryl laurate and plant stanyl laurate, and free plant sterols and stanols dissolved in liquid emulsion could also significantly decrease serum cholesterol levels and markedly increase fecal cholesterol excretion. These results suggested that the esterified plant sterols/stanols had comparable effects to the free plant sterols/stanols in lowering serum TC levels but that they did gain a solubility advantage from the free plant sterols/stanols. Therefore, plant steryl/stanyl laurate could be considered as a potential nutraceutical or functional ingredient to reduce or prevent atherosclerosis and its related complications.

KEYWORDS: Plant sterols, stanols, steryl laurate, stanyl laurate, serum cholesterol, oral administration

INTRODUCTION

Atherosclerosis and its related complications, such as cardiovascular disease (CVD) and cerebrovascular disease, are the major causes of morbidity and mortality in many developed countries.[1,2] Abundant evidence in animals and humans has demonstrated that dyslipidemia, including the elevation of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and triacylglycerol (TAG) concentrations and a decrease in high density lipoprotein cholesterol (HDL-C) concentration in the blood, is the leading risk factor of atherosclerosis.[3−6] Therefore, the development of strategies that reduce both cholesterol and TAG levels has generated considerable interest in combatting hyperlipidemia-associated cardiovascular diseases.

As known to all, drug treatment is highly beneficial to the hyperlipidemic populations. Most drugs used for lowering serum cholesterol levels act as inhibitors of 3-hydroxy-3-methylglutary coenzyme A reductase (HMG-CoA reductase), the rate-controlling enzyme of the cholesterol synthetic pathway. These inhibitors reduce cholesterol levels by decreasing LDL cholesterol by 20% to 55%.[7] Even though statins remain the major hypolipidemic drugs at present, a growing number of patients treated with statins has risen along with the number of those suffering from side effects or not responding well to the therapy.[8] In recent years, nutraceutical and functional foods have attracted much interest as alternative therapies for lowering plasma TC, especially for hypercholesterolemia patients, whose blood cholesterol level is moderately high but not enough to reach values that require the prescription of cholesterol-lowering medications.[9]

Plant sterols and stanols are the most widely used natural products for lowering blood cholesterol.[4,10] However, their practical applications of free plant sterols and stanols in foods are greatly limited due to their poor solubility.[9] Esterification or transesterification of plant sterols or stanols with fatty acids or oils and fats could increase their lipid solubility and thus facilitate their incorporation into a variety of fat-based foods. The cholesterol-lowering effects of plant steryl esters or stanyl esters have also been demonstrated in both animals and humans.[1,11−14] These modified plant sterols/stanols mainly included sitosterol conjugated linoleic acid ester, steryl stearate, plant stanol rapeseed oil fatty acid esters, plant sterol/stanol erucic acid esters, plant sterol olive oil fatty acid esters, and so on.[15−18] Our previous work has shown that plant steryl laurate and stanyl laurate could be efficiently prepared from plant stanols and sterols via lipase-catalyzed esterification with lauric acid, which significantly improved the solubility of plant stanols and sterols in plant oil and contributed to their application in fatty foods.[19] In continuation of this work, the goals of the present research were to determine whether the produced plant steryl laurate and stanyl laurate retained the cholesterol-lowering properties of plant sterols and stanols and, if so, the possible differences between their respective activities.

However, numerous studies in animals have explored the cholesterol-lowering effects of plant sterol or stanol esters by dietary administration,[3,18,20,21] whereas the dietary method has many disadvantages, such as the difference of feed consumption among different animals. The gavage method of dosing can deliver a more precise amount of the test compound. Thus, the

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objective of the present study was to evaluate whether the produced plant steryl and stanyl laurate restored the cholesterol-lowering potential of plant sterols and stanols and to compare the effects of plant steryl and stanyl laurate on the serum and liver lipids by gavage in mice. In addition, the current study also examined the cholesterol-lowering properties of free plant sterols and stanols dissolved in liquid emulsion. Although hamsters synthesized and excreted cholesterol and bile acids in a manner similar to that in humans,[22] the mice were chosen due to the ease of the daily operation of gavage.

MATERIALS AND METHODS

Materials. Mice, experimental diets, and sawdust bedding were purchased from Slac Animal Co., Ltd. (Shanghai, China). TC enzymatic kits, TAG enzymatic kits, HDL-C kits, and LDL-C kits were purchased from FengHui Medical Science & Tech. Co. Ltd. (Shanghai, China). Palladium−Carbon (10%, w/w) catalyst was purchased from Xukou Reagent Feaduct Factory (Suzhou, China). Novozym 435 lipase B from *Candida antarctica* was obtained from Novo Nordisk Co., Ltd. (Shanghai, China). Plant sterols were generous gifts from Jiangsu Spring Fruit Biological Products Co., Ltd. (Taixing, China), and the sucrose esters were generously provided by Hangzhou Jinhelai Food Additive Co., Ltd. (Hangzhou, China).

Preparation of Plant Stanols, Plant Steryl, and Stanyl Laurate. Plant stanols were prepared by catalytic hydrogenation of plant sterols under hydrogen pressure of 2 MPa, using Palladium−Carbon (10%, w/w) as catalyst. Plant stanyl and steryl laurate were prepared by esterification of plant stanols or sterols and lauric acid using Novozym 435 lipase B from *Candida antarctica* in non-aqueous media as in our previous work.[19] The reaction mixtures were purified by column chromatography on silica gel (100−200 mesh) and eluted with cyclohexane/ethyl acetate (4:1, v/v). The flow rate was 20 mL/h, and the eluent, 1 tube/15 min, was collected and then detected by thin-layer chromatography. The fractions containing the desired products were collected by rotary evaporator. The isolated plant stanyl and steryl laurate were used as test compounds. The compounds were analyzed by HPLC prior to use. The purity of plant sterols was >95% (42% β-sitosterol, 27% campesterol, 23% stigmasterol, and 3% brassicasterol). The purity of plant stanols was >93% (64% sitostanol and 29% campestanol). The purity of plant stanyl laurate was >99% (68% sitostanyl laurate and 31% campestanyl laurate). The purity of plant steryl laurate was >99% (44% sitosteryl laurate, 28% campesteryl laurate, 24% stigmasteryl laurate, and 3% brassicasteryl laurate).

Animals and Diets. Seventy male 6-week-old Kun Ming mice (Slac Animal Co., Ltd., Shanghai, China), weighing 22−24 g, were housed in polypropylene cages (5 per cage) in a room controlled at 25 ± 1 °C and 60% humidity with a 12-h light/dark cycle. The animal use and experimental procedures complied with the International Guiding Principles for Biomedical Research Involving Animals (1985), and the protocols were approved by the Animal Use and Care Committee of Jiangnan University.

After 3 days of adaptation, the mice were weighed and randomly divided into 7 groups (n = 10/group). In the normal group (NG), mice were given a regular rodent chow diet. In the control oil-based group (COG) and control emulsion-based group (CEG), mice were fed with a high-cholesterol−high-fat diet, which was prepared from regular rodent chow supplemented with 1% (w/w) cholesterol, 10% (w/w) pork oil, and 10% (w/w) egg yolk powder. The detailed ingredient and nutrient compositions of the experimental diets were shown in Tables 1 and 2, respectively. In the four treatment groups, the plant sterols group (PEG), plant stanols group (PAG), plant steryl laurate group (PELG), and plant stanyl laurate group (PALG) mice were fed with the control diet plus oral administration of plant sterols, plant stanols, plant steryl laurate, and plant stanyl laurate, respectively. Oral administration was performed at 13:00 o'clock by gavage feeding once a day with drinking water for NG, sunflower oil for COG, the sunflower oil solution of plant steryl laurate for PELG, the sunflower oil solution of plant stanyl laurate for PALG, liquid emulsion for CEG, the liquid emulsion solution of plant sterols for PEG, and the liquid emulsion solution of plant stanols for PAG.

Table 1. Ingredient Composition of the Experimental Diets[a]

rodent chow diet (%)		high-cholesterol−high-fat diet (%)	
corn powder	26	rodent chow diet	79
flour	34	egg yolk powder	10
soybean meal	24.5	pork oil	10
fish meal	5	cholesterol	1
wheat bran	1.5		
clover fodder	3		
plant oil	2		
premix	4		

[a] Data were taken from product specification sheets provided by the manufacturer (Slac Animal Co., Ltd., Shanghai, China).

Table 2. Nutrient Composition of Experimental Diets[a]

nutrients	rodent chow diet (%)	high-cholesterol−high-fat diet (%)
protein	20.5	19.4
fat	4.6	18.0
nitrogen free extract	52.5	42.6
fiber	4.4	3.5
cholesterol	<0.1	1.0
plant sterols	<0.1	<0.1
calories (kcal/100 g)	345.0	418.0

[a] Data were taken from product specification sheets provided by the manufacturer (Slac Animal Co., Ltd., Shanghai, China).

Preparation of the Oral Sample and Dose Determination. The sunflower oil solution was prepared individually by adding the appropriate amount of test compound (plant steryl or stanyl laurate) and mixing in an ultrasonicator for at least 30 min and stored at 4 °C. The liquid emulsion of 1% (w/v) sugar ester was prepared by adding the appropriate amount of sucrose ester (HLB 13) into drinking water and mixed in an ultrasonicator for 30 min, and the liquid emulsion solution was prepared by adding plant sterols or plant stanols into the produced liquid emulsion and mixing with the help of an ultrasonicator.

In humans, 2−3 g/d of plant sterols, stanols, or their esters was recommended to lower serum cholesterol.[2,9,23] The animal dose should be extrapolated to a human equivalent dose by the body surface area normalization method.[24] The dose of 3 g/d of plant steryl or stanyl laurate was used in the present study, which was equivalent to free plant sterols or stanols of 2.1 g/d. Thus, the dose of 3 g/d and 2.1 g/d for humans with 70 kg of body weight corresponds to 42.9 mg/(kg·d) and 30 mg/(kg·d), which is in turn equivalent to 387 mg/(kg·d) and 271 mg/(kg·d) for mice, respectively. The produced plant steryl or stanyl laurate was added into sunflower oil, and the mice were dosed at 20 mL/(kg·d), giving a concentration of 19.4 mg/mL for the 387 mg/(kg·d). Plant sterols or stanols were added into liquid emulsion, and the mice were dosed at 20 mL/(kg·d), giving a concentration of 13.5 mg/mL for the 271 mg/(kg·d). The precise volume of gavage was calculated weekly on the basis of animal body weight. In a preliminary experiment, the dose used in this study was tried and confirmed to be suitable and effective in test mice.

Table 3. Body Weight and Liver Weight in Different Groups of Test Animals[a]

group	body weight (g)		liver weight (g)	liver index (liver weight/body weight, %)
	initial	final		
NG	23.1 ± 0.44	45.5 ± 0.60	2.29 ± 0.06	5.03 ± 0.07
COG	23.2 ± 0.48	47.3 ± 0.99	2.57 ± 0.11	5.44 ± 0.19
PELG	23.1 ± 0.67	47.1 ± 1.65	2.43 ± 0.20	5.12 ± 0.24
PALG	23.0 ± 0.37	46.7 ± 1.06	2.41 ± 0.13	5.15 ± 0.22
CEG	23.7 ± 0.56	45.8 ± 0.57	2.36 ± 0.08	5.13 ± 0.13
PEG	22.2 ± 0.28	46.5 ± 0.52	2.41 ± 0.08	5.20 ± 0.20
PAG	23.0 ± 0.33	46.3 ± 0.29	2.43 ± 0.06	5.27 ± 0.13

[a] Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student−Newman−Keuls test after a significant effect was detected. Results are the means ± SEM (n = 10).

Animal Experiment and Sample Collection. During the 4-week experimental period, mice were given free access to drinking water. The food was available ad libitum, unless mice were fasted for four hours prior to dosing, and experimental diets and drinking water were replaced every day. Diets were prepared weekly and stored at 4 °C. Body weights were recorded weekly, and sawdust bedding was changed twice a week. After 4 weeks, mice were kept fasted overnight (16 h), then weighed and sacrificed. Blood was collected from the retro-orbital sinus. Serum was separated by centrifugation (8000 rpm) for 15 min at 4 °C and stored at −20 °C for further measurement of serum TC, TAG, HDL-C, and LDL-C. The liver was carefully dissected, perfused and cleaned with saline, blotted on filter paper, weighed and frozen in liquid nitrogen, and stored at −80 °C for the measurement of liver cholesterol and TAG contents.

Analysis of Serum Lipids. Serum TC, HDL-C, and LDL-C were measured by an enzymatic CHOD-PAP (cholesterol oxidase-peroxidase aminophenazone) method with the corresponding test kit (FengHui Co. Ltd., Shanghai, China) using an automatic FH-400 biochemical analyzer (FengHui Co. Ltd., Shanghai, China). Serum TAG was analyzed according to the fully enzymatic GPO (glycerol phosphate oxidase)-PAP method using an automatic FH-400 biochemical analyzer (FengHui Co. Ltd., Shanghai, China). The presence of the sterols or stanols even at the same concentration as that of cholesterol did not influence the accuracy of cholesterol determination.[25]

Analysis of Liver Lipids. Liver lipids were extracted using the previous method with minor modification.[3] Briefly, 0.3 g of liver tissue was homogenized in 6 mL of chloroform/ethanol (2/1, v/v) mixture in an ice bath. The homogenate was centrifuged at 8000 rpm for 15 min, and the supernatant was collected. After drying up under nitrogen gas, the lipids were redissolved in isopropanol. Liver cholesterol and TAG contents were measured according to the enzymatic CHOD-PAP and GPO-PAP methods, respectively.

Fecal Analysis. Dried fecal samples were ground into fine powder and then extracted with chloroform/ethanol (2/1, v/v) to a final volume of 20 times (0.5 g in 10 mL of solvent mixture) in a 25-mL screw-capped vial. The vial was placed in a water-bath at room temperature and shaken at 150 rpm for 24 h. The extract was centrifuged at 8000 rpm for 15 min, and the supernatant was collected. An aliquot of 10 μL of supernatant was evaporated under a nitrogen stream in an Eppendroff tube and then dissolved in isopropanol. Fecal total cholesterol concentrations were measured according to the enzymatic CHOD-PAP.

Fecal sterols/stanols were obtained by saponification with 0.5 mol/L ethanolic KOH at 100 °C for 2 h as described previously.[26] After having been dried under nitrogen flux, the residues were dissolved in n-hexane and analyzed for free sterol/stanol concentration using GC-MS (Varian 1200 L GC/MS-MS, Varian China Inc., Shanghai, China). α-Cholestane (Sigma-Aldrich, Shanghai, China) was used as the internal standard to calculate the free sterol/stanol contents. The column was Agilent DB-5, 30 m × 0.25 mm i.d. with a film thickness of 0.25 μm. The injector temperature was 300 °C. Chromatographic conditions were 150 °C for 2 min, then increased to 280 °C at a rate of 10 °C min^{-1}, and kept at 280 °C for 30 min. Ultrapure helium was used as carrier gas at a flow rate of 1.0 mL/min.

Statistical Analysis. The statistical analyses were performed using SPSS 16.0. One-way ANOVA was used to analyze the overall treatment effects on serum, liver, and fecal lipids. When a statistically significant effect was obtained, the Student−Newman−Keuls test was performed to determine the differences between treatment groups. Significance level was set at $p < 0.05$. All data are presented as means ± SEM.

RESULTS

Body Weight, Liver Weight, and Food Consumption. The six groups (COG, PELG, PALG, CEG, PEG, and PAG) fed with the high-cholesterol−high-fat diet had slightly more body weight gains than the normal group (NG) (Table 3). However, there was no significant difference among the six experimental groups, suggesting that plant sterols, plant stanols, plant stanyl, and steryl laurate administration did not significantly influence the weight gain in mice. This result was similar to a previous report, which showed that polysaccharide administration did not significantly suppress the weight gain of the high-fat diet-induced obesity in mice.[27] At the same time, there was only a slight increase in liver weight and liver index of these groups compared with those in NG, and there were no significant differences among the groups in average or total food consumption during the 4-week experimental period.

Serum Lipid Profiles. Serum lipid profiles of all test mice are shown in Figure 1. Obviously, mice in COG and CEG displayed a significant increase in levels of serum TC, HDL-C, and LDL-C after 4 weeks of feeding a high-cholesterol−high-fat diet in comparison with those in NG, indicating that the high-cholesterol−high-fat diet had induced hypercholesterolemia in test animals. Meanwhile, plant steryl and stanyl laurate significantly decreased serum TC levels as compared with that in COG. Plant sterols and stanols also remarkably lowered serum TC contents when compared with that in CEG (Figure1A). These results in serum TC level indicated that plant sterols, plant stanols, plant steryl, and stanyl laurate could markedly reduce serum TC level, although no remarkable differences were found among PELG, PALG, PEG, and PAG. However, no significant differences in the levels of serum TAG were observed in PELG and PALG compared with that in COG or in PEG and PAG compared with that in CEG (Figure1B), which suggested that the level of serum TAG was not noticeably affected by plant sterols, stanols, and their laurate.

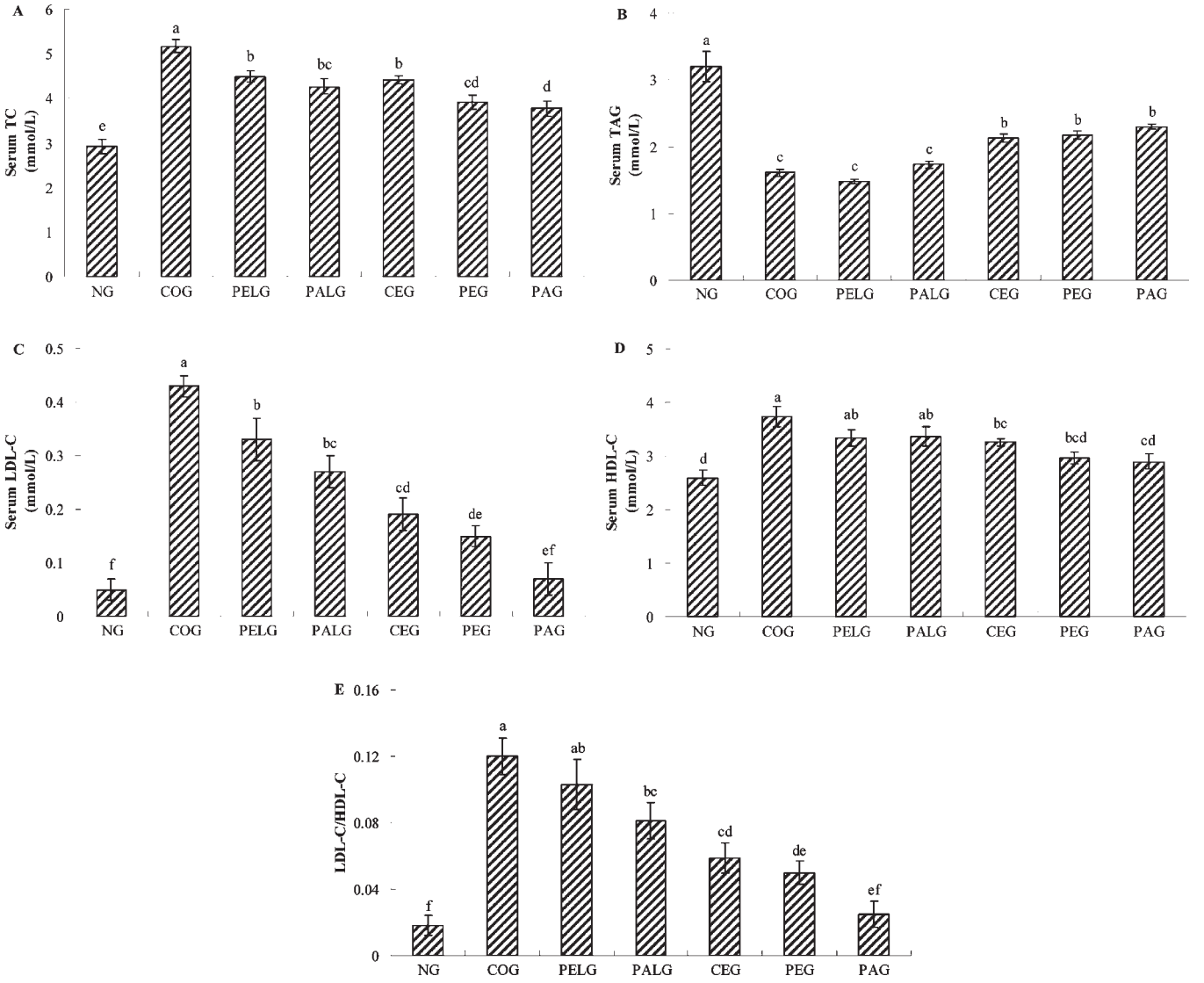

Figure 1. Serum TC (A), serum TAG (B), serum LDL-C (C), serum HDL-C (D), and LDL-C/HDL-C (E) in mice fed the regular rodent chow diet, the high-cholesterol−high-fat diet, or the high-cholesterol−high-fat diet plus oral administration of plant sterols, plant stanols, plant steryl laurate, or plant stanyl laurate. Results are the means ± SEM (n = 10). Values bearing different lowercase letters (a, b, c, d, e, and f) are significantly different ($p < 0.05$).

In the lipoprotein profile, plant stanols, plant steryl, and stanyl laurate evidently reduced the serum LDL-C level. In comparison with COG, the serum LDL-C level in PELG and PALG were markedly decreased, and compared with CEG, serum LDL-C of PAG was significantly reduced, but PEG only showed a not statistical significant decrease (Figure1C). Regarding the serum HDL-C level, no significant differences were observed between experimental groups (PELG, PALG; PEG, PAG) and positive control groups (COG; CEG) (Figure1D). As for atherogenicity index (LDL-C/HDL-C), there had been a remarkable increase in COG, PELG, PALG, CEG, PEG, and PAG compared with that in NG (Figure1E). In contrast to COG, there was a significant decrease in PALG. However, only a slight decrease was observed for PELG in the level of LDL-C/HDL-C when compared with that in COG. Similarly, a dramatic decrease in PAG and a slight decrease in PEG were observed when compared with the LDL-C/HDL-C level of CEG.

There were no significant differences between PELG and PALG in terms of the level of TC, TAG, HDL-C, LDL-C, and LDL-C/HDL-C, suggesting that no obvious differences were observed in antihyperlipidemic potential between plant steryl and stanyl laurate although plant stanyl laurate showed no statistical significance in serum TC, LDL-C, and LDL-C/HDL-C. Meanwhile, no evident discrepancies were also observed between PEG and PAG with regard to the levels of TC, TAG, HDL-C, LDL-C, and LDL-C/HDL-C.

Liver Lipid Profiles. Table 4 showed the liver lipid profiles of all test mice after 4 weeks of experimental feeding. Evidently, plant steryl and stanyl laurate significantly lowered liver cholesterol contents, but no statistical effects were observed between plant sterols and stanols. Compared with COG, plant steryl and stanyl laurate decreased liver cholesterol contents by 46.0% and 61.5%, respectively. As to liver TAG, only slight reductions were found in PELG and PALG compared to those in COG. Similarly, slight changes were also observed in PAG although PEG showed a dramatic reduction when compared with CEG.

Fecal Total Cholesterol, Plant Sterols, and Stanols Analysis. In NG, COG and CEG, fecal plant sterols and plant stanols

were not detected (Table 5). Compared with NG, the groups fed with the high-cholesterol−high-fat diet showed a significant increase in fecal total cholesterol contents, which could be attributed to the addition of 1% cholesterol in the diet. As compared with COG, PELG and PALG showed a significant increase in the fecal total cholesterol concentrations. At the same time, large amounts of fecal plant sterols and plant stanols were found in PELG and PALG, respectively. When compared with PELG, PALG significantly increased fecal total cholesterol, which indicated that plant stanyl laurate was preferable to plant sterols in fecal excretion.

Similarly, plant sterols and stanols contributed markedly to fecal excretion of cholesterol. The fecal total cholesterol content in PEG and PAG increased by 80.7% and 111.1%, respectively compared with that in CEG. Meanwhile, PAG increased the fecal total cholesterol content when compared with PEG, which suggested that plant stanols were better than plant sterols in fecal excretion of cholesterol.

DISCUSSION

The cholesterol-lowering property of plant sterols in experimental animals and humans has been demonstrated since the 1950s.[6,11,12,23,25,28] Since then, there have been many studies on the effects of plant sterols or stanols on cholesterol metabolism. The current study revealed the significant cholesterol-lowering properties of plant sterols, stanols, plant stanyl, and steryl laurate on serum TC, especially LDL-C, without affecting HDL-C or serum TAG concentrations in mice by oral administration for 4 weeks, which was in agreement with previous studies.[4,13−16,23,26]

Table 4. Liver Lipid Profiles in Different Groups of Test Animals[a]

group	cholesterol (mg/g liver)	TAG (mg/g liver)
NG	10.04 ± 2.49 b	22.14 ± 1.47 bc
COG	21.98 ± 3.95 a	27.31 ± 1.84 ab
PELG	11.88 ± 2.14 b	27.16 ± 1.70 ab
PALG	8.47 ± 1.56 b	26.23 ± 0.58 abc
CEG	9.50 ± 0.83 b	29.63 ± 3.77 a
PEG	8.89 ± 2.17 b	20.67 ± 2.33 c
PAG	10.26 ± 2.69 b	25.88 ± 1.60 abc

[a] Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student−Newman−Keuls test after a significant effect was detected. Results are the means ± SEM (n = 10). Values bearing different lowercase letters (a, b, and c) are significantly different ($p < 0.05$).

Previous studies by our laboratory have shown that feeding conjugated linoleyl β-sitosterol-containing hyperlipidemic feed (at dose of 100 mg/kg·d) reduced serum TC by 38.1% and LDL-C by 56.8% of mice on 2% cholesterol-containing diet after 4-weeks of feeding.[18] Y. Lin et al. demonstrated that plant sterol rapeseed oil fatty acid esters significantly lowered the plasma total cholesterol concentration by 13% in cholesterol-fed hamsters.[20] D. Chung et al. reported that β-sitosterol oleate reduced cholesterol uptake by 29.8% (±14.63%) in rats.[28] In our experiments, the prepared plant stanyl and steryl laurate could significantly lower serum cholesterol by 13.2% and 17.6%, suggesting that the esterified plant sterols/stanols retained the cholesterol-lowering properties of free plant sterols and stanols. Earlier studies reported that plant sterol and stanol esters were rapidly hydrolyzed by intestinal enzymes, producing the physiologically active free plant sterols and stanols.[9] The chemical structures of plant sterols and stanols were similar to that of cholesterol, which could displace cholesterol from bile salt micelles and could compete the site for absorption, suppressing the absorption of exogenous cholesterol.[3]

As to fecal excretion, our results showed that these groups fed with the high-cholesterol−high-fat diet had markedly higher fecal total cholesterol contents, which could be due to the fact that total cholesterol excretion was directly correlated with cholesterol intake. Furthermore, it has also been found that treatment groups (PELG, PALG, PEG, and PAG) dramatically enhanced fecal cholesterol excretion, suggesting that plant sterols, stanols, plant steryl, and stanyl laurate greatly contributed to reducing cholesterol intake. Chien et al. reported that diets supplemented with plant sterols markedly increased fecal total cholesterol by 582% in hamsters.[3] Lin and co-workers also revealed that plant sterol esters increased fecal excretion of neutral sterols by 190% in cholesterol-fed hamsters.[20] In this study, we adopted enzymatic assays using chloroform/ethanol as the extraction solvent to measure total cholesterol in fecal samples. It would be interesting to know whether the total amount of cholesterol and free-form cholesterol correlate with their metabolites. Hopefully, this will be addressed in our future study by GC-MS. These studies certainly would help us to understand the biological process of cholesterol in depth.

Similarly, liver cholesterol contents were found to be significantly decreased by oral administration of plant steryl and stanyl esters. This result may be explained by Jia's view that the cholesterol content of the liver was significantly correlated with plasma TC and non-HDL-C levels.[4] Lin and co-workers also reported that feeding of 0.24% plant sterol esters for 5 weeks could decrease liver cholesterol concentration by 44.3% in hamsters.[20] This may also

Table 5. Fecal Cholesterol, Plant Sterol, and Plant Stanol Contents in Different Groups of Test Animals[a]

group	cholesterol (mg/g feces)	plant sterols (mg/g feces)	plant stanols (mg/g feces)
NG	1.27 ± 0.05 f	$<4 \times 10^{-3}$	$<4 \times 10^{-3}$
COG	3.65 ± 0.12 e	$<4 \times 10^{-3}$	$<4 \times 10^{-3}$
PELG	9.35 ± 0.03 b	36.94 ± 0.89	$<4 \times 10^{-3}$
PALG	15.20 ± 0.32 a	$<4 \times 10^{-3}$	38.38 ± 0.74
CEG	3.78 ± 0.23 e	$<4 \times 10^{-3}$	$<4 \times 10^{-3}$
PEG	6.83 ± 0.34 d	29.68 ± 1.03	$<4 \times 10^{-3}$
PAG	7.98 ± 0.46 c	$<4 \times 10^{-3}$	31.94 ± 0.53

[a] Data were analyzed using one-way ANOVA. Differences between treatment groups were further analyzed using the Student−Newman−Keuls test after a significant effect was detected. Results are the means ± SEM (n = 10). Values bearing different lowercase letters (a, b, c, d, e, and f) are significantly different ($p < 0.05$).

be ascribed to reduced cholesterol input resulting from the inhibition of intestinal cholesterol absorption, increased output from enhanced biosynthesis of bile acids, and secretion of cholesterol and bile salts.[4,29] However, the current study also showed lower liver lipid and fecal lipid contents when compared with those reported in the literature.[3,4] This discrepancy may have resulted from the differences in the animal species, dose and intake frequency of test compounds, and diet composition, especially in the dietary cholesterol and fat levels.[3,4,17,25]

There remains a controversy over the relative cholesterol-lowering efficacy of plant sterol esters versus stanol esters or plant sterols versus stanols in previous reports. The cholesterol-lowering efficacy of sterol and stanol esters was similar in several studies, apart from the study by Jones and co-workers, which favored sterol esters.[13] In this study, plant stanyl laurate significantly increased fecal cholesterol contents when compared with that by plant steryl laurate. Meanwhile, plant stanyl laurate also showed a slight strength in lowering the serum TC level, LDL-C level, LDL-C/HDL-C, and liver cholesterol contents than plant steryl laurate. The results may be explained by Plat's report, which held that plant stanols are more hydrophobic than plant sterols and have a higher affinity for micelles than cholesterol, resulting in reduced intestinal cholesterol and a higher fecal excretion of cholesterol.[1]

Both free plant sterols and stanols dissolved in liquid emulsion could significantly lower the levels of serum cholesterol, LDL-C and greatly contribute to fecal cholesterol excretion in our finding. Our study also suggested that the esterified plant sterols/stanols have comparable effects to the free plant sterols/stanols in lowering serum TC levels but that plant sterol/stanol esters have a solubility advantage over plant sterols/stanols. The insignificant effects of plant sterols, stanols, plant steryl, and stanyl laurate on serum TAG in our findings were consistent with that reported in previous studies.[3,4,20,21,26] However, an interesting finding in this study was that the mice fed with high-cholesterol−high-fat diets had dramatically lowered serum TAG levels than those fed with normal diets. It was found that one ingredient of the high-cholesterol−high-fat diet, yolk powder, may play an important role. Previous studies had reported that egg yolk could significantly reduce plasma TAG, without changes in TC,[30] which may account for this trend. Different discrepancies in serum TC, TAG, HDL-C, and LDL-C between the two control groups (COG and CEG) were also observed. This may be attributed to the difference of the two different vehicles used for administering the treatments.

In conclusion, the present study has demonstrated that the produced plant steryl and stanyl laurate retained the cholesterol-lowering properties of free plant sterols and stanols, while showing no statistical difference in the cholesterol-lowering property between the two. Our results suggested that the esterified plant sterols/stanols had comparable effects to the free plant sterols/stanols in lowering serum TC levels but that they had better solubility in oils with respect to plant sterols/stanols. Therefore, plant steryl/stanyl laurate could be considered as a potential nutraceutical or functional ingredient to reduce or prevent atherosclerosis and its related complications.

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Author Contributions

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ABBREVIATIONS USED

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TAG, triacylglycerol; CVD, cardiovascular disease; HPLC, high performance liquid chromatography; NG, normal group; COG, control oil-based group; CEG, control emulsion-based group; PEG, plant sterols group; PAG, plant stanols group; PELG, plant steryl laurate group; PALG, plant stanyl laurate group; HLB, value of hydrophile lipophile balance; CHOD-PAP, cholesterol oxidase-peroxidase aminophenazone; ANOVA, analysis of variance; GPO-PAP, glycerol phosphate oxidase-peroxidase aminophenazone.

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