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Naringenin 7-*O*-cetyl Ether as Inhibitor of HMG-CoA Reductase and Modulator of Plasma and Hepatic Lipids in High Cholesterol-Fed Rats

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Abstract—Numerous studies in vitro have shown a close relationship between the chemical structure and biologic activity of flavonoids, whereby their basic structure is modified to increase or decrease their biologic activity. The effects of naringenin (**1**) and its synthetic derivative, naringenin 7-*O*-cetyl ether (**2**), on the lipid profile, the cholesterol-regulating enzyme activity and the excretion of sterol were compared in rats fed a high-cholesterol (1% wt/wt) diet. Either **1** or **2** was supplemented with a high-cholesterol diet for 6 weeks at a dose of 0.073 mmol/100 g diet. The supplementation of **1** or **2** significantly lowered the levels (mean ± SE) of the plasma total cholesterol (4.93 ± 0.19 and 4.75 ± 0.16 mmol/L vs 5.87 ± 0.36 mmol/L, $p < 0.05$) and hepatic triglyceride (0.12 ± 0.01 and 0.11 ± 0.01 mmol/g vs 0.18 ± 0.01 mmol/g, $p < 0.05$) and cholesterol (0.23 ± 0.01 and 0.21 ± 0.01 mmol/g vs 0.31 ± 0.01 mmol/g, $p < 0.05$) compared to those of the control. The compound **1** or **2** supplementation appeared to decrease the excretion of neutral sterols. The plasma HDL-cholesterol concentration and ratio of HDL to total cholesterol were significantly higher in **1** and **2** groups than in control group. Although the biological effect of **2** on inhibiting hepatic HMG-CoA reductase and ACAT activities was only significant compared to the control group, both compounds exhibited a significant hypocholesterolemic effect in rats fed a high-cholesterol diet. The results suggest that cholesterol biosynthesis and esterification were concomitantly reduced by **2**, as indicated by the decreased HMG-CoA reductase and ACAT activities.

Introduction

Flavonoids, a heterogeneous group of ubiquitous plant polyphenols, are a frequent component of the human diet.[1] Flavonoids have exhibited a variety of biological and pharmacological activities, including the inhibition of enzymes,[2] free radical scavenging,[3] anti-inflammation,[4] and inhibition of tumor promotion.[5]

A number of epidemiological studies have implied a role for flavonoids in reducing the risk of coronary heart disease.[6–8] Among naturally occurring flavonoids, hesperidin, hesperetin, naringin, and naringenin have all been evaluated as potential agents for improving the cholesterol metabolism in diet-induced hypercholesterolemic animals.[9,10] Wilcox et al.[11] and Borradaile et al.[12] provided evidence that citrus flavonoids, naringenin, and hesperetin, reduced the secretion of both apoB and newly synthesized cholesteryl ester, and inhibited acyl-CoA: cholesterol acyltransferase (ACAT) activity in HepG2 cells. In a previous report, the current authors demonstrated in vivo that two cholesterol-regulating enzymes, ACAT and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, would appear to account for the hypocholesterolemic effect of flavonoids.[10,13] However, it is still unclear whether all naturally derived flavonoids share these properties.

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Numerous studies in vitro have shown a close relationship between the chemical structure and biologic activity of flavonoids,[14,15] whereby their basic structure can be modified to increase or decrease their biologic activity.[6] Since we proved the efficacy of compound **1** in experimental animals,[13] we are interested in synthesizing a derivative based on chemical structure of **1**. Thus, compound **2** was synthesized in our laboratory to search a more potent compound than **1**. The structural difference between the two compounds is the presence of a C_{16} ether group instead of a hydroxyl group in compound **1**. Accordingly, the current study was performed to compare the effect of the low dose (0.02 g/100 g diet) **1** and an equivalent amount of its synthetic derivative (**2**) on the plasma and hepatic lipids levels, key enzymes in the liver involved in the regulation of cholesterol homeostasis, and the excretion of sterols in high cholesterol-fed rats.

Results

No significant differences were observed in the food intake, weight gain, or organ weights between the groups (data not shown). As such, the rats were not apparently adversely affected by the compound **1** or **2** supplement (Fig. 1).

Plasma and hepatic lipids

The supplementation of **1** or **2** significantly lowered the levels (mean ± SE) of the plasma total cholesterol (4.93 ± 0.19 and 4.75 ± 0.16 mmol/L vs 5.87 ± 0.36 mmol/L, $p < 0.05$) and hepatic triglyceride (0.12 ± 0.01 and 0.11 ± 0.01 mmol/g vs 0.18 ± 0.01 mmol/g, $p < 0.05$) and cholesterol (0.23 ± 0.01 and 0.21 ± 0.01 mmol/g vs 0.31 ± 0.01 mmol/g, $p < 0.05$) compared to those of the control. The plasma triglyceride concentration was only significantly lower in the compound **2** supplemented group when compared with the control group (1.34 ± 0.04 vs 1.73 ± 0.10 mmol/L, $p < 0.05$) (Table 1 and Fig. 2). The plasma HDL-cholesterol concentration and ratio of HDL to total cholesterol were significantly higher in the compound **1** and **2** groups than in the control group, whereas the opposite was true for the atherogenic index.

Hepatic HMG-CoA reductase and ACAT activities

The HMG-CoA reductase and ACAT activities were only significantly lower in **2** supplemented group when compared to the control group (Fig. 3). The compound **2** apparently inhibited hepatic cholesterol biosynthesis and esterification by 27.4 and 14.0%, respectively, when compared to the control group.

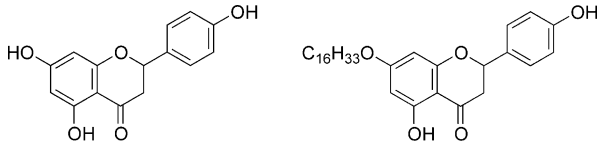

Figure 1. Structures of naringenin (**1**) and naringenin 7-*O*-cetyl ether (**2**).

Fecal sterols

The daily excretion of fecal sterols is shown in Figure 4. The effect of the compound **1** and **2** supplementation resulted in marked changes in the neutral sterols excretion under cholesterol-fed conditions. Among these fecal neutral sterols, excretion of each neutral sterol (cholesterol, coprostanol and coprostanone) was significantly lower in **1** and **2** group than in control group. However, the fecal acidic sterols were not significantly different between the groups. The total fecal sterol excretion was significantly lower in the compound **1** and **2** groups than in the control group. Accordingly, **1** or **2** supplementation would appear to decrease the excretion of neutral sterols.

Discussion

Bioflavonoids derived from citrus fruits have many biological functions, including preventive and therapeutic effects on several diseases.[16,17] Among naturally occurring citrus flavonoids, naringenin and naringin have already been pharmacologically evaluated as potential anticancer[18] and hypolipidemic agents.[19]

Table 1. Effects of **1** and **2** supplementation on plasma lipids in high cholesterol-fed rats[a]

	Control[b]	**1**[b]	**2**[b]
Total cholesterol (mmol/L)	5.87 ± 0.36[a]	4.93 ± 0.19[b]	4.75 ± 0.16[b]
Triglyceride (mmol/L)	1.73 ± 0.10[a]	1.62 ± 0.11[b]	1.34 ± 0.04[b]
HDL-cholesterol (mmol/L)	0.79 ± 0.05[a]	0.93 ± 0.04[b]	0.95 ± 0.03[b]
HDL-C/TC (%)	13.5 ± 1.20[a]	18.8 ± 0.90[b]	20.4 ± 0.70[b]
Athero. Index[c]	6.40 ± 0.50[a]	4.30 ± 0.30[b]	4.00 ± 0.20[b]

[a]Mean ± SE.
[b]Means in the same row not sharing a common letter are significantly different ($p < 0.05$) between groups.
[c]Athero. Index (atherogenic index): (total cholesterol-HDL-cholesterol)/HDL-cholesterol.

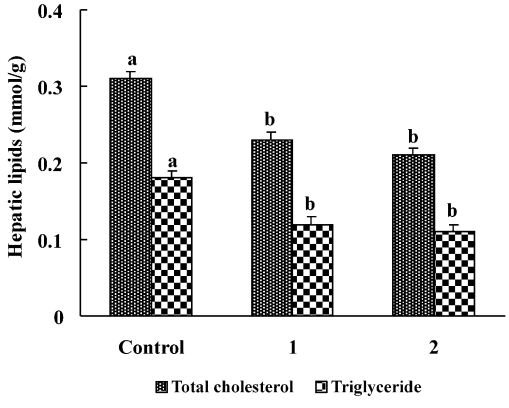

Figure 2. Effects of **1** and **2** supplementation on hepatic lipids level in high cholesterol-fed rats. Mean ± SE. The means not sharing a common letter are significantly different between groups ($p < 0.05$).

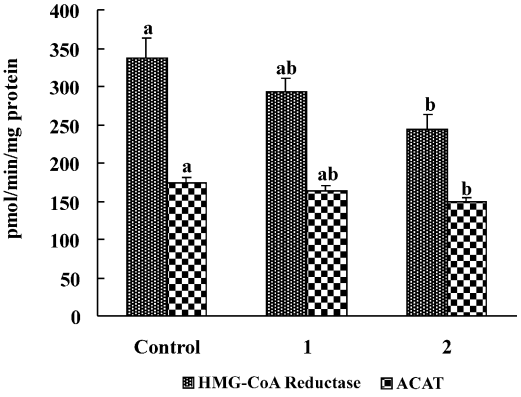

Figure 3. Effects of **1** and **2** supplementation on hepatic HMG-CoA reductase and ACAT activities in high cholesterol-fed rats. Mean ± SE. The means not sharing a common letter are significantly different between groups ($p < 0.05$).

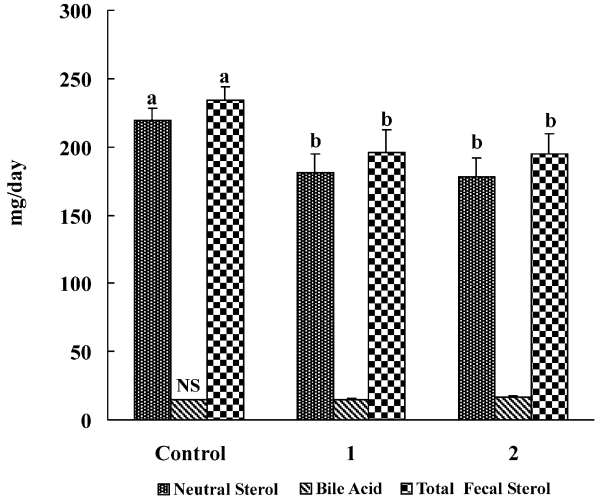

Figure 4. Effects of **1** and **2** supplementation on fecal sterol contents in high cholesterol-fed rats. The daily neutral sterol excretion was calculated from the sum of cholesterol, coprostanol, and coprostanone from the gas chromatographic peaks and bile acids were measured using an enzymatic method as total bile acids. Total fecal sterol refers to the sum of neutral sterols and bile acids. Mean ± SE. The means not sharing a common letter are significantly different between groups ($p < 0.05$).

Cellular cholesterol homeostasis is very important for the prevention of cardiovascular disease. In general, the plasma concentration in the body is regulated by cholesterol biosynthesis, the removal of circulating cholesterol, dietary cholesterol absorption, and the excretion of cholesterol via bile and feces. In the present study, although **1** did not significantly inhibit hepatic HMG-CoA reductase and ACAT activities the plasma cholesterol concentrations were lowered 16 and 19% by the compound **1** and **2** supplements, respectively. It is well known that two key enzymes, HMG-CoA reductase and ACAT, play a major role in the regulation of cholesterol metabolism. HMG-CoA reductase is a rate-limiting enzyme in the cholesterol biosynthesis pathway, while ACAT is involved in the formation of cholesteryl esters which is important for cholesterol absorption, hepatic very low density lipoprotein (VLDL) cholesterol secretion, and foam cell development in atherosclerosis.[20] Accordingly, numerous studies have reported on the beneficial effects of HMG-CoA reductase and ACAT inhibitors on hypercholesterolemia and atherosclerosis.[21,22]

In the cholesterol-fed rats, the compound **2** supplement significantly lowered the hepatic cholesterol biosynthesis and cholesterol esterification, which is beneficial for lowering the plasma cholesterol level. Meanwhile, although not statistically significant, the compound **1** supplement also slightly lowered the hepatic HMG-CoA reductase and ACAT activities, possibly contributing to the plasma cholesterol reduction observed. A previous report by the current authors already speculated on the cholesterol-lowering action of a 0.1% (wt/wt) naringenin diet via the inhibition of HMG-CoA reductase and ACAT in high cholesterol-fed rats.[13] The difference on inhibition of HMG-CoA reductase and ACAT activity could be due to the different dose of **1** used in present study and previous report that is 0.02 and 0.1% in diet, respectively. In addition, **1** also previously exhibited a dose-dependent inhibition of cholesterol esterification in HepG2 cells.[12]

Hepatic ACAT utilizes cholesterol as its substrate, which is either synthesized endogenously or acquired via the LDL and chylomicron remnant receptors. In the current study, the compound **2** supplement also inhibited hepatic ACAT activity. Two recently reported ACAT variants are ACAT1 and ACAT2. ACAT2 is predominant in the intestines and liver for the synthesis of cholesteryl esters or lipoprotein assembly, whereas ACAT1 accounts for most ACAT activity in the liver and macrophages.[23] Chang et al.[24] found that the activity of ACAT2 in HepG2 cells was approximately 15% of the total ACAT activity. In addition, Wilcox et al.[11] recently reported that **1** decreases the availability of lipids for the assembly of apoB-containing lipoproteins in a cell culture of HepG2 by reducing the activities of ACAT1 and ACAT2, and selectively decreases ACAT expression along with an enhanced expression of LDL-receptors and an inhibition of microsomal triglyceride transfer protein (MTP) activity. MTP is known to be essential for the assembly and hepatic secretion of apoB-containing lipoproteins such as VLDL and LDL.[25] Whether **1** or **2** produces similar effects in vivo remain to be determined. The treatment of different species with tissue-selective inhibitors of ACAT has led to variable decreases in the plasma cholesterol content.[26–28] Thus, inhibiting ACAT may also be a possible therapeutic approach for decreasing plasma cholesterol concentrations. However, it is still unclear as to how much of a role hepatic ACAT plays in cholesterol metabolism in human subjects.[29]

The current study identified decreased plasma cholesterol and hepatic cholesterol levels and decreased fecal neutral sterols in the animals supplemented with **1** or **2**. Furthermore, the results also suggest that cholesterol biosynthesis was concomitantly reduced by **2**, as indicated by the decreased HMG-CoA reductase activities. Since the cholesterol intake was about the same for all

groups, the supplementation of **1** or **2** seemed to promote an efficient utilization of dietary cholesterol. The combination of generally recognized concepts of hepatic cholesterol homeostasis and the present findings would seem to suggest that several mechanisms are responsible for the metabolic effects of these compounds. The current results imply that **2** reduced cholesterol esterification and cholesterol biosynthesis through the inhibition of hepatic HMG-CoA reductase and ACAT, resulting in a decreased hepatic cholesterol level. The compound **1** also exhibited a tendency to lower these enzyme activities as they were the same as those found in **2** group but lower than those in control group. These results are very similar to the hypocholesterolemic effect of a mixture of naringin and hesperidin in high cholesterol-fed rats.[10] HMG-CoA reductase activity is normally decreased with a high-cholesterol diet, whereas the activities of hepatic ACAT and cholesterol-7α-hydroxylase are increased.[30,31] However, the presence of **1** or **2** in a high-cholesterol diet tended to decrease the activities of both hepatic HMG-CoA reductase and ACAT. Reduced ACAT activity can lead to less cholesteryl ester being available for VLDL packing, thereby resulting in a reduction of VLDL secretion from the liver, as suggested by Carr et al.[32] In the current study, the plasma triglyceride levels were significantly lower in **2** group than in control group, which suggests that hepatic ACAT inhibition might affect VLDL secretion or VLDL removal. The data in **1** group is also supported by the observation of Choi et al.[33] where **1** exhibited a significant hypocholesterolemic effect, yet no hypotriglyceridemic effect in rats fed a high fat diet. Another possible hypothesis is that an increase in the hepatic cholesterol uptake by LDL receptors accelerated the cholesterol catabolism in **1** or **2** supplemented rats as evidenced by the decreased expression of the LDL-receptor by naringenin in cell culture.[11] Overall, it would appear that **2**, a synthetic **1** derivative, is clearly effective in altering lipid metabolism and lowering plasma cholesterol.

Experimental

Synthesis of naringenin 7-*O*-cetyl ether (2)

Ten grams (36.73 mmol) of naringenin was dissolved in a mixture of 100 mL of acetone and 100 mL of dimethylformamide. Thirteen and a half millilitres of hexadecanyl bromide and 4.70 g of sodium carbonate were added to the mixture and stirred in a water bath at 80 °C for 12 h. The resulting solution was cooled, then 100 mL of water and 800 mL of ethyl acetate were added and the mixture extracted with ethyl acetate. The extract was then washed with water and concentrated under reduced pressure. The solid formed was filtered using a glass filter and dried under reduced pressure to give 10.1 g of compound **2**. In addition, the residue was concentrated and subjected to silica gel column chromatography (45 × 150 mm, 70–230 mesh, eluent: hexane/ethyl acetate (8:2)] to obtain an additional 3 g of naringenin-7-*O*-cetyl ether as a pale yellow solid that is very stable at room temperature.

Spectral data of naringenin 7-*O*-cetyl ether (2)

The structure of **2** was confirmed by NMR spectra. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 NMR spectrometer using the solvent peak as reference (^{1}H 7.25 ppm and ^{13}C 77.0 ppm of $CDCl_3$, respectively). Low- and HRFABMS were recorded on a high-resolution Tandem Mass (JMS-HX 110/110A, Jeol Ltd.) spectrometer. The structure was confirmed as shown in Figure 1. Compound **2** (naringenin 7-*O*-cetyl ether): white solid; mp 114–117 °C; ^{1}H NMR ($CDCl_3$) δ 12.0 (s, –OH), 7.32 (d, $J = 8.4$ Hz, 2H), 6.87 (d, $J = 8.4$ Hz, 2H), 6.04 (d, $J = 2.0$ Hz, 1H), 6.02 (d, $J = 2.0$ Hz, 1H), 5.33 (dd, $J = 13.2$, 2.8 Hz, 1H), 5.09 (s, –OH), 3.95 (t, $J = 6.8$ Hz, 2H), 3.07 (dd, $J = 17.2$, 13.2 Hz, 1H), 2.77 (dd, $J = 17.2$, 2.8 Hz, 1H), 1.75 (quin, $J = 6.8$ Hz, 2H), 1.44–1.36 (m, 2H), 1.34–1.22 (m, 24H), 0.87 (t, $J = 6.8$ Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 195.8, 167.6, 164.0, 162.8, 156.0, 130.6, 127.9 (2C), 115.6 (2C), 103.0, 95.6, 94.6, 78.9, 68.6, 43.2, 32.0, 29.75.69 (six carbons, –(CH_2)6–), 29.62, 29.57, 29.4, 29.3, 28.9, 25.9, 22.7, 14.2 ppm; 1D NOESY : NOE contacts were observed between H (6.04 and 6.02 ppms) and H (3.95 ppm); FABMS m/z 497 [M + H] + (100), 495 (44), 153 (85), 147 (39); HRFABMS m/z found for 497.3267 (calcd for $C_{31}H_{45}O_5$ 497.3267).

Animals and diets

Thirty male Sprague–Dawley rats weighing 75 ± 5 g were purchased from Bio Genomics. Inc. (Seoul, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperature (24 °C) and lighting (alternating 12-h periods of light and dark). All the rats were fed a pelletized commercial chow diet for 10 days after arrival. Next, the rats were randomly divided into three groups ($n = 10$) and fed a high-cholesterol diet (1%, wt/wt). Either compound **1** (Sigma Chemical Co.) or **2**, both at a concentration of 0.073 mmol/100 g diet, was supplemented in the experimental diet for 6 weeks. The structures of the test compounds are shown in Figure 1. The composition of the experimental diet, was based on the AIN-76 semisynthetic diet.[34,35] The dose of dietary **1** is based on the our finding in which the 0.02% naringin was as effective as 0.05% naringin[19] or 0.1% naringenin[13] for lowering plasma cholesterol concentration.

The animals were given food and distilled water ad libitum throughout the experimental period. The food consumption and weight gain were measured daily and weekly, respectively. The feces collected during the last 3 days using metabolic cages were used for determining the fecal sterol. At the end of the experimental period, the rats were anesthetized with ketamine following a 12 h fast. Blood samples were taken from the inferior vena cava to determine the plasma lipid profile. The livers were removed and rinsed with physiological saline. All samples were stored at −70 °C until analyzed.

Plasma and hepatic lipids

The plasma cholesterol and HDL-cholesterol concentrations were determined using a commercial kit

(Sigma) based on a modification of the cholesterol oxidase method of Allain et al.[36] The HDL-fractions were separated using a Sigma kit based on the heparin-manganese precipitation procedure.[37] Atherogenic index was calculated as follow: (total cholesterol-HDL-cholesterol)/HDL-cholesterol. The plasma triglyceride concentrations were measured enzymatically using a kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method.[38] The hepatic lipids were extracted using the procedure developed by Folch et al.[39] The dried lipid residues were dissolved in 1 mL of ethanol for the cholesterol and triglyceride assays. Triton X-100 and a sodium cholate solution (in distilled H_2O) were added to 200 μL of the dissolved lipid solution to produce final concentrations of 5 g/L and 3 mmol/L, respectively. The hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit as used in the plasma analysis.

HMG-CoA reductase and ACAT activities

The microsomes were prepared according to the method developed by Hulcher and Oleson[40] with a slight modification. Two grams of liver tissue were homogenized in 8 mL of an ice-cold buffer (pH 7.0) containing 0.1 M of triethanolamine, 0.02 M of EDTA, and 2 mM of dithiothreitol, pH 7.0. The homogenates were centrifuged for 10 min at 10,000 *g* and then at 12,000 *g* at 4 °C. Next, the supernatants were ultracentrifuged twice at 100,000 *g* for 60 min at 4 °C. The resulting microsomal pellets were then redissolved in 1 mL of a homogenation buffer for protein determination[41] and finally analyzed for HMG-CoA reductase and ACAT activities.

The HMG-CoA reductase activities were determined as described by Shapiro et al.[42] with a slight modification using freshly prepared hepatic microsomes. An incubation mixture (120 μL) containing microsomes (150 ~ 200 μg) and 500 nmol of NADPH (dissolved in a reaction buffer containing 0.1 M of triethanolamine and 10 mM of EDTA) was preincubated at 37 °C for 5 min. Next, 50 nmol of [^{14}C]-HMG-CoA (specific activity; 2.1420 GBq/mmol; NEM™ Life Science Products, Inc., Boston, MA) was added and the incubation continued for 15 min at 37 °C. The reaction was terminated by the addition 30 μL of 6 M of HCl, then the resultant reaction mixture was incubated at 37 °C for a further 15 min to convert the mevalonate into mevalonolactone. The incubation mixture was centrifuged at 10,000 *g* for 5 min, then the supernatant was spotted on a Silica Gel 60 F_{254} TLC plate using a mevalonolactone standard. The plate was developed in benzene/acetone (1:1, v/v) and air-dried. Finally, the R_f 0.3.6 region was removed by scraping with a clean razor blade and the ^{14}C radioactivity determined using a liquid scintillation counter (Packard Tricarb 1600TR; Packard Instrument Company, Meriden, CT). The results were expressed as pmol mevalonate synthesized per min per mg protein.

The ACAT activities were determined according to the method developed by Erickson et al.[43] and modified by Gillies et al.[44] using freshly prepared hepatic microsomes. To prepare the cholesterol substrate, 6 mg of cholesterol and 600 mg of Tyloxapol (Triton WR-1339, Sigma) were each dissolved in 6 mL of acetone, mixed well, and completely dried in N_2 gas. The dried substrate was then redissolved in 20 mL of distilled water to a final concentration of 300 μg of cholesterol/mL. Next, reaction mixtures containing 20 μL of a cholesterol solution (6 μg of cholesterol), 20 μL of a 1 M of potassium-phosphate buffer (pH 7.4), 5 μL of 0.6 mM bovine serum albumin, 150–200 μg of the microsomal fraction, and distilled water (up to 180 μL) were preincubated at 37 °C for 30 min. The reaction was then initiated by adding 5 nmol of [^{14}C]-Oleoyl CoA (specific activity; 2.0202 GBq/mmol; NEM™ Life Science Products, Inc.) to a final volume of 200 μL; the reaction time was 30 min at 37 °C. The reaction was stopped by the addition of 500 μL of isopropanol/heptane (4:1, v/v), 300 μL of heptane, and 200 μL of 0.1 M potassium phosphate (pH 7.4), then the reaction mixture was allowed to stand at room temperature for 2 min. Finally, an aliquot (200 μL) of the supernatant was subjected to scintillation counting. The ACAT activity was expressed as pmol cholesteryl oleate synthesized per min per mg protein.

Fecal sterols

The fecal neutral sterols were determined using a simplified micro-method, as developed by Czubayko et al.[45] Gas–liquid chromatography was carried out with a Hewlett-Packard gas chromatograph (Model 5809; Palo Alto, CA) equipped with a hydrogen flame-ionization detector and Sac^{TM}-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film; Supelco Inc., Bellefonate, PA, USA). Helium was used as the carrier gas. The temperatures were set at 230 °C for the column and 280 °C for the injector/detector. 5-α-cholestane (Supelco Inc.) was used as the internal standard. The daily neutral sterol excretion was calculated based on the amount of cholesterol, coprostanol, and coprostanone in each sample. The fecal bile acid was extracted with methanol and quantified enzymatically with 3-α-hydroxysteroid dehydrogenase.[46]

Statistical analysis

All data is presented as the mean ± SE. The data was evaluated by one-way ANOVA using the SPSS program, and the differences between the means assessed using Duncan's multiple-range test. Statistical significance was considered at $p < 0.05$.

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References and Notes

1. Di Carlo, G.; Madcolo, N.; Izzo, A. A.; Capasso, F. *Life Sci.* **1999**, *65*, 337.

2. Havesteen, B. *Biochem. Pharmacol.* **1983**, *32*, 1141.
3. Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1990**, *186*, 343.
4. Di Perri, T.; Auteri, A. *Int. Angiol.* **1988**, *7*, 11.
5. Nishino, H.; Nagao, M.; Fujiki, H.; Sugimura, T. *Cancer Lett.* **1983**, *21*, 1.
6. Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. *Lancet* **1993**, *342*, 1007.
7. Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackbum, H.; Buzina, R.; Fidanza, F.; Giampaoli, S. *Arch Intern. Med.* **1995**, *155*, 381.
8. Knekt, P.; Jarvine, R.; Reunanen, A.; Maatela, J. *Brit. Med. L.* **1996**, *312*, 478.
9. Monforte, M. T.; Trovato, A.; Kirjavainen, S.; Forestieri, A. M.; Galati, E. M.; LoCurto, R. B. *Farmaco* **1995**, *50*, 595.
10. Bok, S. H.; Lee, S. H.; Park, Y. B.; Bae, K. H.; Son, K. H.; Jeong, T. S.; Choi, M. S. *J. Nutr.* **1999**, *129*, 1182.
11. Wilcox, L. J.; Borradaile, N. M.; de Dreu, L. F.; Huff, M. W. *J. Lipid Res.* **2001**, *42*, 725.
12. Borradaile, N. M.; Carroll, K. K.; Kurowska, E. M. *Lipids* **1999**, *34*, 591.
13. Lee, S. H.; Park, Y. B.; Bea, K. H.; Bok, S. H.; Kwon, Y. K.; Lee, E. S.; Choi, M. S. *Ann. Nutr. Metab.* **1999**, *43*, 173.
14. Krol, W.; Czuba, Z.; Scheller, S.; Paradowski, Z.; Shani, J. *J. Ethnopamarcol.* **1994**, *41*, 121.
15. Ferriola, P. C.; Cody, V.; Middleton, E. *Biochem. Pharmacol.* **1989**, *38*, 1617.
16. Aboobaker, V. S.; Balgi, A. D.; Bhattacharya, R. K. *In vivo*. **1994**, *8*, 1095.
17. Buening, M. K.; Chang, R. L.; Huang, M. T.; Fortner, J. G.; Wood, A. W.; Conney, A. H. *Cancer Res.* **1981**, *41*, 67.
18. So, F. V.; Guthrie, N.; Chambers, A. F.; Moussa, M.; Carroll, K. K. *Nutr. Cancer* **1996**, *26*, 167.
19. Bok, S. H.; Shin, Y. W.; Bae, K. H.; Jeong, T. S.; Kwon, Y. K.; Park, Y. B.; Choi, M. S. *Nutr. Res.* **2000**, *20*, 1007.
20. Chang, T. Y.; Chang, C. C. Y.; Cheng, D. *Annu. Rev. Biochem.* **1997**, *66*, 613.
21. Bocan, T. M.; Mueller, S. B.; Brown, E. Q.; Lee, P.; Bocan, M. J.; Rea, T.; Pape, M. E. *Atherosclerosis* **1998**, *139*, 21.
22. Hay, J. M.; Yu, W. M.; Ashraf, T. *Pharmacoeconomics* **1999**, *15*, 47.
23. Anderson, R. A.; Joyce, C.; Davis, M.; Regan, J. W.; Clark, M.; Shelness, G. S.; Rudel, L. L. *J. Biol. Chem.* **1998**, *273*, 26747.
24. Chang, C. C.; Sakashita, N.; Ornvold, K.; Lee, O.; Chang, E. T.; Dong, R.; Lin, S.; Lee, C. Y.; Strom, S.; Kashyap, R.; Fung, J.; Farese, J.; Patoiseau, J. F.; Delhon, A.; Chang, T. Y. *J. Biol. Chem.* **2000**, *275*, 28083.
25. Jamil, H.; Dickson, J. K.; Chu, C.; Lago, M. W.; Rinehart, J. K.; Biller, S. A.; Gregg, R. E.; Wetterau, J. R. *J. Biol. Chem.* **1995**, *270*, 6549.
26. Largis, E. F.; Wnag, C. H.; DeVries, V. G.; Schaffer, S. A. *J. Lipid Res.* **1989**, *30*, 681.
27. Williams, R. J.; McCarthy, A. D.; Sutherland, C. D. *Biochim. Biophys. Acta* **1989**, *1003*, 213.
28. Marezetta, C. A.; Savoy, Y. E.; Ferrman, A. M.; Long, C. A.; Pettini, J. L.; Hagar, R. E.; Inskeep, P. B.; Davis, K.; Stucchi, A. F.; Nicolosi, R. J.; Hamanaka, E. S. *J. Lipid Res.* **1994**, *35*, 1829.
29. Harris, W. S.; Dujovne, C. A.; von Bergmann, K.; Neal, J.; Akester, J.; Windsor, S. L.; Greene, D.; Look, Z. *Clin. Pharmacol. Ther.* **1990**, *48*, 189.
30. Bjorkhem, I. Mechanism of Bile Acid Biosynthesis in Mammalian Liver. In *New Comprehensive Biochemistry*, Elsevier, Amsterdam, 1995 pp 231–278.
31. Field, F. J.; Cooper, A. D.; Erickson, S. K. *Gasteroenterology* **1982**, *83*, 873.
32. Carr, T. P.; Parks, J. S.; Rudel, L. L. *Ateriacler. Thromb.* **1992**, *12*, 1274.
33. Choi, J. S.; Yokozawa, T.; Oura, H. *J. Nat. Prod.* **1991**, *54*, 218.
34. American Institute of Nutrition *J. Nutr.* **1977**, *107*, 1340.
35. American Institute of Nutrition *J. Nutr.* **1980**, *110*, 1717.
36. Allain, C. C.; Poon, L. S.; Chan, C. S. G. *Clin. Chem.* **1974**, *20*, 470.
37. Waenic, R. G.; Albers, J. J. *J. Lipid Res.* **1978**, *19*, 65.
38. McGowan, M. W.; Artiss, J. D.; Strandbergh, D. R.; Zak, B. *Clin. Chem.* **1983**, *29*, 538.
39. Folch, J.; Lees, M.; Sloan-Stanley, G. H. *J. Biol. Chem.* **1957**, *1957*, 226 497.
40. Hulcher, F. H.; Oleson, W. H. *J. Lipid Res.* **1973**, *14*, 625.
41. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
42. Shapiro, D. J.; Nordstrom, J. L.; Mitschelen, J. J.; Rodwell, V. W.; Schimke, R. T. *Biochim. Biophys. Acta* **1974**, *370*, 369.
43. Erickson, S. K.; Schrewsbery, M. A.; Brooks, C.; Meyer, D. J. *J. Lipid Res.* **1980**, *21*, 930.
44. Gillies, P. J.; Rathgeb, K. A.; Robinson, C. S. *Exp. Mol. Path.* **1986**, *44*, 320.
45. Czubayko, F.; Beumers, B.; Lutjohann, D.; von Bergmann, K. *J. Lipid Res.* **1992**, *32*, 1861.
46. Michael, J. C.; Ian, A. M. *Clin. Chem.* **1980**, *26*, 1298.