Mechanisms of Phytosterolemia in Stroke-Prone Spontaneously Hypertensive and WKY Rats

Ikuo Ikeda, Hideaki Nakagiri, Michihiro Sugano, Sumiko Ohara, Tadateru Hamada, Megumi Nonaka, and Katsumi Imaizumi

Analysis of sterol composition in serum, liver, adipose tissue, adrenals, and abdominal aorta demonstrated that the contents of plant sterols, campesterol and sitosterol, were evidently higher in WKY and stroke-prone spontaneously hypertensive (SHRSP) rats than in Wistar and WKA rats fed a diet containing a 0.5% plant sterol mixture. Lymphatic 24-hour recovery of ^{3}H-sitosterol was about 2-fold higher in the WKY and SHRSP rats than in the WKA rats. Lymphatic absorption of ^{14}C-cholesterol was also higher in WKY and SHRSP rats compared with WKA rats, but the difference was smaller than in the case of sitosterol. The remarkable increase of sitosterol absorption in WKY and SHRSP rats was observed between 9 and 24 hours after the administration. In SHRSP rats, lymphatic absorption of sitosterol between 0 and 3 hours was also higher than those in the other rat strains. Markedly less esterified ^{3}H-sitosterol was detected in lymph than ^{14}C-cholesterol in all strains, and in WKY and SHRSP rats, only a small increase in the esterified forms of sitosterol and cholesterol was observed. Although the incorporation of micellar ^{3}H-sitosterol and ^{14}C-cholesterol into intestinal brush border membranes was higher in SHRSP rats than in WKA rats, no difference was observed between WKY and WKA rats. These observations suggest that the incorporation into the brush border membranes and the esterification of sterols are not the major determinants for the hyperabsorption of sitosterol and cholesterol in SHRSP and WKY rats. Secretion of sitosterol and cholesterol in the bile of rats fed a plant sterol mixture was lower in SHRSP than in WKA rats. These results suggest that WKY and SHRSP strains deposit plant sterols in the body by enhancing the absorption and lowering the excretion of plant sterols. These strains of rats may be suitable models for studying mechanisms of differential absorption of various sterols.

IN ALMOST ALL animal models and humans, intestinal absorption of plant sterols is undoubtedly low compared with cholesterol.[1,2] The mechanism underlying the discrimination of absorption of these sterols is an enigma yet to be solved.[3] Our previous study suggested a possibility that the low absorbability of sitosterol may be due to the high affinity for bile salt micelles and hence, a release of sitosterol as a monomer from the micelles and the incorporation into intestinal brush border membranes are less efficient.[3] However, it is known that phytosterolemic patients who deposit plant sterols in their body absorb plant sterols more efficiently than normal subjects.[4,5] Because phytosterolemia is a rare inherited disease, it is assumed that carrier proteins are involved in the hyperabsorption of plant sterols. Although gene defects in 10 families with phytosterolemia were localized to chromosome 2p21,[6] no candidate genes were found in this region and hence, mechanisms underlying the high absorbability of plant sterols in these patients have not yet been elucidated. The reason for this uncertainty is attributed to a lack of an appropriate animal model for this issue. We report here that the 3 strains of WKY, spontaneously hypertensive (SHR) and stroke-prone spontaneously hypertensive (SHRSP) rats, can deposit plant sterols to a significant extent as compared with Wistar and WKA rats. The SHRSP strain was genetically separated from the SHR strain,[7] which was originated from the WKY strain.[8] To elucidate causes underlying the deposition of plant sterols in these rat strains, the lymphatic absorption and the incorporation into intestinal brush border membranes of plant sterols were examined. It has been reported that the secretion of plant sterols in bile in sitosterolemic patients is less efficient than that in normal subjects.[4] This may be another reason for the high deposition of plant sterols in their body. Therefore, biliary excretion of sterols was also measured in SHRSP and WKA rats.

MATERIALS AND METHODS

Materials

[22,23(n)-^{3}H] Sitosterol (specific activity 814 GBq/mmol, Amersham, Buckinghamshire, England) was kindly provided from Kao Co, Tokyo, Japan; [4-^{14}C]cholesterol (55 mCi/mmol) was purchased from Amersham.

Feeding Study of Plant Sterols

Groups of 9-week-old male WKY (WKY/NCrj, inbred, SPF, Charles River Japan, Kanagawa, Japan), SHRSP (SHRSP/Sea, inbred, SPF, Seac Yoshitomi, Fukuoka, Japan), Wistar (Sea: Wistar, outbred, SPF, Seac Yoshitomi) and WKA (WKA/Sea, inbred, SPF, Seac Yoshitomi) rats were fed a purified diet (AIN-93G)[9] containing 10% safflower oil and 0.5% plant sterol mixture (Merck, Darmstadt, Germany) for 2 weeks. The composition of the sterol preparation was in weight percent, sitosterol, 54.9; stigmasterol, 3.9; campesterol, 37.6; campestanol, 0.4; and sitostanol, 1.5. The chemical structure of some plant sterols is shown in Fig 1. At the termination of the feeding period, rats were fasted for 7 hours, and blood was then withdrawn from the abdominal

From the Laboratory of Nutrition Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, and the Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto, Japan.

Submitted January 19, 2001; accepted March 26, 2001.

Supported in part by a grant from the Vegetable Oil Association of Japan.

Address reprint requests to Ikuo Ikeda, PhD, Laboratory of Nutrition Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.

0026-0495/01/5011-0030$35.00/0

doi:10.1053/meta.2001.26739

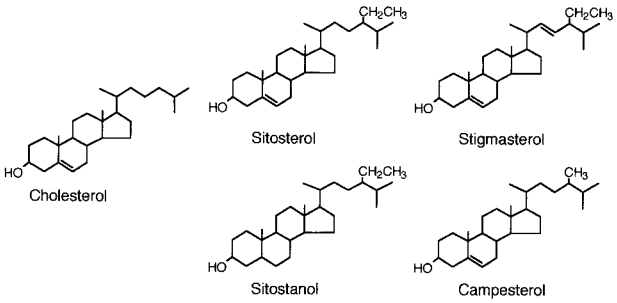


Fig 1. Chemical structure of various plant sterols and cholesterol.

aorta under diethyl ether anesthesia and it was centrifuged to obtain serum. Liver, adrenals, adipose tissue, aorta, and brain were also excised and frozen at −40°C until analysis. Kyushu University Animal Policy and Welfare Committee approved all aspects of our animal studies.

Lymphatic Absorption of Sitosterol and Cholesterol

Nine-week-old WKA, WKY, and SHRSP rats were fed a commercial chow for 1 week until the operation. The left thoracic lymphatic duct cephalad to the cisterna chyli of these rats was cannulated as described previously.[10] Average body weights (mean ± SE) were 332 ± 4, 278 ± 2, and 234 ± 3 g in WKA, WKY, and SHRSP rats, respectively. A second indwelling catheter was placed in the stomach for administration of a test emulsion. After surgery, the animals were placed in restraining cages and intragastrically given a continuous infusion of a solution containing 139 mmol/L glucose and 85 mmol/L NaCl at a rate of 3.4 mL/h until the end of the experiment. The same solution was given as drinking water. On the next morning, animals with a constant lymph flow rate were administered 1 mL/100 g body weight of a test emulsion containing [^{14}C] cholesterol and [^{3}H] sitosterol. The test emulsion contained 67 mg sodium taurocholate (Nacalai tesque, Kyoto, Japan), 17 mg fatty acid–free bovine serum albumin fraction V (BSA, Bayer, Kankakee, IL), 67 mg triolein (Sigma, St Louis, MO), and 0.33 μCi (12.2 kBq) [^{14}C]cholesterol and 3.3 μCi (122 kBq) [^{3}H] sitosterol in 1 mL. Lymph was collected in ice-chilled tubes containing EDTA, and the radioactivity was measured.

Incorporation of Sitosterol and Cholesterol Into Intestinal Brush Border Membranes

Nine-week-old WKA, WKY, and SHRSP rats were fed a commercial chow for 1 week until use. Intestinal brush border membranes were prepared from the proximal half of rat small intestine as described by Kessler et al.[11] Trypsin-chymotrypsin inhibitor was added in a homogenizing buffer to prevent proteolysis. The membranes were suspended in a Hank's balanced salt solution containing 5 mmol/L EGTA and 4% BSA (fatty acid–free) in 15 mmol/L HEPES, pH 7.4, to give a final protein concentration of 2 mg/mL and kept at 0°C until use. They were consistently enriched regarding the activities of sucrase[12] and alkaline phosphatase[13] to 15-fold relative to the whole homogenate. Recovery of acyl CoA cholesterol acyltransferase,[14] succinic dehydrogenase,[15] and DNA[16] markers for microsomes, mitochondria, and nuclei, respectively, in the brush border membranes was less than 0.5% of the levels in the whole homogenate as observed in our previous study.[10]

Transfer of sitosterol and cholesterol from a micellar solution to brush border membranes was measured as reported previously.[3,10] A total of 4 mL of a micellar solution containing 6.6 mmol/L sodium taurocholate, 0.6 mmol/L phosphatidylcholine (Sigma), 50 μmol/L [^{3}H] sitosterol, and 50 μmol/L [^{14}C]cholesterol prepared in a Hank's balanced salt solution containing 5 mmol/L EGTA and 4% BSA (fatty acid–free) in 15 mmol/L HEPES buffer, pH 7.4, was incubated with 1 mL of brush border membrane suspension (2 mg protein) at 37°C. At 0 and 30 minutes, 1 mL of the incubation solution was withdrawn and released into 5 mL iced 0.9% NaCl containing 7 mmol/L sodium taurocholate. This was centrifuged at 27,000*g* for 30 minutes at 5°C. The resulting pellet was washed once with 5 mL of the same solution and then again collected by centrifugation. The brush border membrane pellet was suspended in 1.0 mL distilled water by sonication. An aliquot of the solution was subjected to sterol determination by radioactivity measurement and to protein determination.

Secretion of Sitosterol and Cholesterol in Bile

Male SHRSP and WKA rats (9-week-old) were fed a purified diet containing a 0.5% plant sterol mixture for 2 weeks as described. At the termination of the feeding period, rats were cannulated in the bile duct under diethyl ether anesthesia and were placed in restraining cages. Bile was collected for 3 hours.

Lipid Analyses

Lipids in blood serum, liver, adrenals, adipose tissue, abdominal aorta, brain, and bile were extracted and purified by the method of Folch et al.[17] After saponification of the lipids, unsaponifiable matters collected were derivatized to trimethylsilylethers[18] and were quantified by gas-liquid chromatography (GC) using a SUPELCO SPB-1 column (0.25 mm × 60 m, 0.25 μm film thickness, Sigma-Aldrich Japan, Tokyo) and 5α-cholestane (Sigma) as an internal calibration standard. Total bile acid concentration in bile was determined enzymatically.[19] The bile acid composition in bile was measured according to a method described previously.[20] Protein was measured according to the method of Lowry et al.[21]

Data were analyzed by a Duncan's new multiple-range test[22] or Student's *t* test. Differences were considered significant at $P < .05$.

RESULTS

Deposition of Plant Sterols in WKY, SHRSP, WKA, and Wistar Rats Fed a Plant Sterol Diet

Preliminary analysis of serum sterols in a range of experimental rats aged 8 to 13 weeks and fed commercial pellet showed that only WKY, SHR (SHR/NCrj, inbred, SPF, Charles River Japan) and SHRSP contained 12% to 15% of plant sterols in the sterol fraction, whereas in Sea: List H and Sea: SD (both are outbred, SPF, Seac Yoshitomi) and WKA/Sea, Dahl R/Jr Sea, Dahl S/Jr Sea, PVG/Sea, DRH/Sea, BN/Sea, and LEW/Sea (they are inbred, SPF, Seac Yoshitomi) rats, it was 2% to 6%. We chose WKY and SHRSP rats as strains highly depositing plant sterols and WKA and Wistar rats as controls.

As shown in Table 1, initial body weight, body weight gain, and food intake were lower in WKY and SHRSP rats and higher in WKA and Wistar rats. Relative liver weight (g/100 g body weight) of SHRSP and WKA rats tended to be higher than that of WKY and Wistar rats.

The results shown in Table 2 clearly demonstrated that tissues from WKY and SHRSP rats contained markedly higher proportions of plant sterols compared with those from Wistar and WKA rats, although food intake of the former 2 strains was lower than that of the latter 2 strains. Except for the abdominal aorta, which contained half as much plant sterols than the other tissues analyzed, the proportion of plant sterols in total sterols ranged from 25% to 35% in WKY and SHRSP rats. The values were approximately 3 to 4 times higher than the corresponding

Table 1. Growth Parameters in Wistar, WKA, WKY, and SHRSP Rats

Strains	Body Weight		Food Intake (g/d)	Food Efficiency (g gain/g intake)	Liver Weight (g/100 g body weight)
	Initial (g)	Gain (g)			
Wistar	200 ± 4[a]	103 ± 7[a]	20.1 ± 0.7[a]	0.399 ± 0.014[a]	4.17 ± 0.11[a]
WKA	196 ± 2[a]	90.2 ± 3.3[c]	18.5 ± 0.2[b]	0.348 ± 0.012[ab]	4.63 ± 0.15[b]
WKY	140 ± 2[b]	77.5 ± 1.4[b]	14.9 ± 0.3[b]	0.368 ± 0.007[a]	4.18 ± 0.07[a]
SHRSP	143 ± 3[b]	72.2 ± 3.4[b]	15.7 ± 0.4[b]	0.328 ± 0.010[b]	4.45 ± 0.05[ab]

NOTE. These rats were fed a 0.5% plant sterol diet for 2 weeks. Values are mean ± SE for 6 rats/group.
Values with different superscript letters show significant difference at $P < .05$.

values (6% to 12%) in WKA and Wistar rats. Total plant sterols in the brain were extremely low (less than 1%) even in SHRSP and WKY rats (data not shown). Therefore, blood-brain barrier in these rat strains remain intact, as in the case of sitosterolemic patients.[4]

In all animal strains, the deposition of campesterol was higher than that of sitosterol in the tissues examined, although the content of campesterol in dietary plant sterols was lower than that of sitosterol.

Percentages of esterified cholesterol, sitosterol, and campesterol were measured in the liver. Percentage of esterified cholesterol was significantly higher in WKA rats (mean ± SE, 45.0% ± 3.0%) than the other 3 rat strains (Wistar, 28.9 ± 4.2; WKY, 19.3 ± 2.5; SHRSP, 23.1 ± 1.9%; $P < .05$). The same tendency was observed in campesterol (WKA, 25.1 ± 2.2 *v* Wistar, 15.4 ± 2.6; WKY, 13.1 ± 3.1; and SHRSP, 13.6% ± 0.9%; $P < .05$). No difference was observed in the percentage of esterified sitosterol among the 4 groups (WKA, 13.3 ± 1.0; Wistar, 9.89 ± 1.38; WKY, 11.3 ± 2.8; SHRSP, 10.4% ± 0.4%).

Lymphatic Recovery of Radioactive Cholesterol and Sitosterol in SHRSP, WKY, and WKA Rats

Lymphatic 24-hour recovery of sitosterol and cholesterol is shown in Figs 2 and 3, respectively. Lymphatic 24-hour recovery of sitosterol was 2.1 and 1.8 times higher in WKY and SHRSP rats than in WKA rats, respectively (Fig 2A). A major difference was observed between 9 and 24 hours after the administration (Fig 2B). The recovery between 0 and 3 hours was significantly higher in SHRSP rats than in the other strains. The absorption of sitosterol in WKY rats until 9 hours after the administration was lower than that in SHRSP rats, and it was similar to that of WKA rats.

Lymphatic 24-hour recovery of cholesterol was 1.4 and 1.2

Table 2. Sterol Compositions in Several Tissues of Wistar, WKA, WKY, and SHRSP Rats

Tissues	Total Sterol (mg/mL or mg/g)	Composition (wt %)				
		Cholesterol	Campesterol	Sitosterol	Sitostanol	Total Plant Sterol
Serum						
Wistar	88.4 ± 3.4[a]	88.5 ± 0.9[a]	6.63 ± 0.47[a]	4.69 ± 0.42[a]	0.16 ± 0.03[a]	11.5 ± 0.9[a]
WKA	69.1 ± 3.4[b]	90.4 ± 0.4[a]	5.35 ± 0.26[a]	4.11 ± 0.17[a]	0.11 ± 0.01[a]	9.57 ± 0.44[a]
WKY	114 ± 3[c]	69.1 ± 0.8[b]	15.8 ± 0.4[b]	14.5 ± 0.4[b]	0.61 ± 0.02[b]	30.9 ± 0.81[b]
SHRSP	93.8 ± 2.0[a]	65.7 ± 1.1[c]	18.1 ± 0.8[c]	15.5 ± 0.7[b]	0.71 ± 0.17[b]	34.3 ± 1.1[c]
Liver						
Wistar	2.84 ± 0.13[a]	90.8 ± 0.6[a]	6.15 ± 0.41[a]	2.99 ± 0.22[a]	0.08 ± 0.02[a]	9.23 ± 0.64[a]
WKA	3.48 ± 0.17[b]	93.8 ± 0.3[b]	4.34 ± 0.18[b]	1.81 ± 0.10[b]	0.08 ± 0.01[a]	6.23 ± 0.27[b]
WKY	2.41 ± 0.04[c]	70.8 ± 1.0[c]	16.6 ± 0.5[c]	12.1 ± 0.4[c]	0.55 ± 0.02[b]	29.2 ± 0.9[c]
SHRSP	2.43 ± 0.05[c]	70.2 ± 0.9[c]	18.0 ± 0.7[d]	11.4 ± 0.3[c]	0.47 ± 0.01[c]	29.8 ± 0.9[c]
Aorta						
Wistar	1.48 ± 0.04[a]	93.9 ± 0.6[a]	3.94 ± 0.36[a]	2.01 ± 0.26[a]	0.18 ± 0.02[a]	6.14 ± 0.63[a]
WKA	1.75 ± 0.07[a]	95.4 ± 0.2[a]	2.99 ± 0.12[a]	1.41 ± 0.06[a]	0.16 ± 0.04[a]	4.56 ± 0.18[a]
WKY	1.65 ± 0.10[a]	85.7 ± 1.1[b]	8.34 ± 0.56[b]	5.42 ± 0.48[b]	0.52 ± 0.07[b]	14.3 ± 1.1[b]
SHRSP	1.62 ± 0.15[a]	86.1 ± 0.9[b]	8.38 ± 0.52[b]	4.99 ± 0.35[b]	0.57 ± 0.06[b]	13.9 ± 0.9[b]
Adrenals						
Wistar	14.7 ± 1.3[ac]	89.9 ± 0.8[a]	6.77 ± 0.50[a]	3.29 ± 0.27[a]	0.10 ± 0.01[a]	10.2 ± 0.8[a]
WKA	18.1 ± 1.4[a]	91.7 ± 0.4[a]	5.63 ± 0.29[a]	2.64 ± 0.15[a]	0.07 ± 0.00[a]	8.34 ± 0.43[a]
WKY	27.0 ± 2.3[b]	71.9 ± 0.7[b]	16.6 ± 0.4[b]	10.9 ± 0.3[b]	0.57 ± 0.05[b]	28.1 ± 0.7[b]
SHRSP	11.4 ± 1.0[c]	68.2 ± 0.8[c]	19.2 ± 0.7[c]	12.1 ± 0.2[c]	0.50 ± 0.02[b]	31.8 ± 0.8[c]
Adipose tissue						
Wistar	0.27 ± 0.03[ac]	90.4 ± 0.7[a]	5.72 ± 0.38[a]	3.18 ± 0.25[a]	0.70 ± 0.05[a]	9.59 ± 0.65[a]
WKA	0.23 ± 0.03[a]	90.9 ± 0.3[a]	5.26 ± 0.23[a]	2.98 ± 0.13[a]	0.85 ± 0.07[a]	9.10 ± 0.34[a]
WKY	0.35 ± 0.03[bc]	75.2 ± 0.6[b]	13.4 ± 0.3[b]	10.2 ± 0.3[b]	1.25 ± 0.06[b]	24.8 ± 0.6[b]
SHRSP	0.36 ± 0.02[b]	72.5 ± 1.1[c]	15.7 ± 0.7[c]	10.6 ± 0.4[b]	1.20 ± 0.02[b]	27.5 ± 1.1[c]

NOTE. These rats were given a 0.5% plant sterol diet for 2 weeks. Mean ± SE of 6 rats.
Values with different superscript letter show significant difference at $P < .05$.

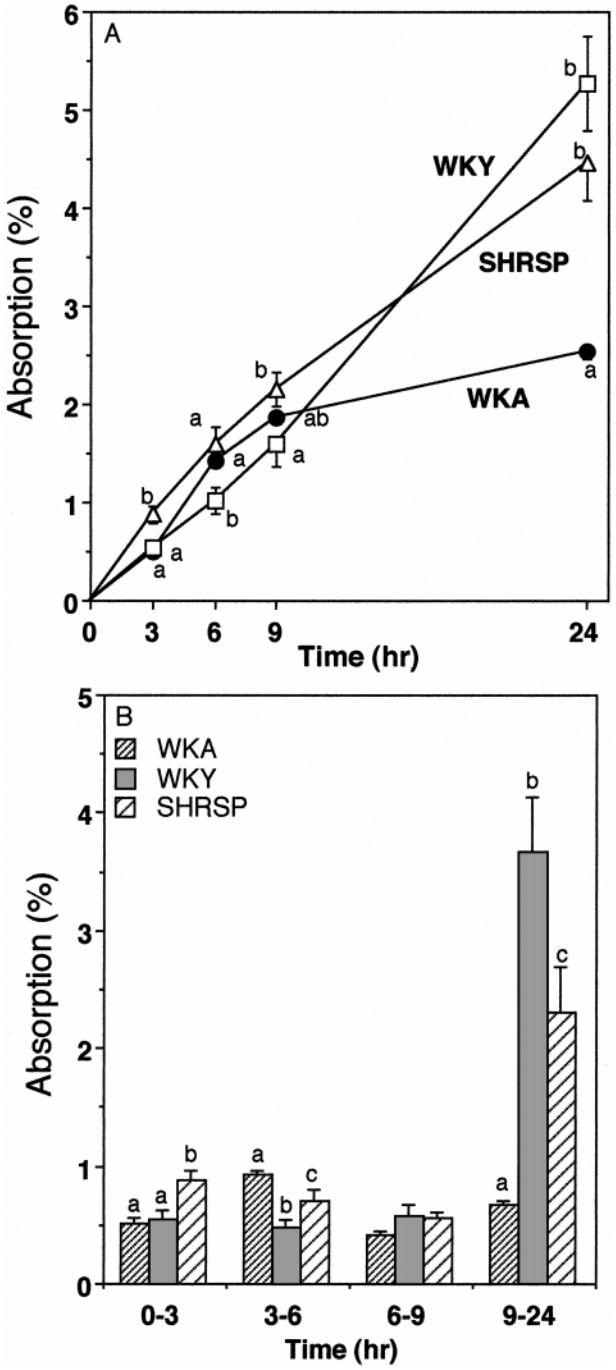


Fig 2. Lymphatic recovery of ^{3}H-sitosterol in WKA, WKY, and SHRSP rats cannulated thoracic duct. Rats were given a test emulsion containing 3.3 μCi ^{3}H-sitosterol and 0.33 μCi ^{14}C-cholesterol, 67 mg sodium taurocholate, 17 mg fatty acid–free albumin, 67 mg triolein/1 mL. The emulsion was given 1 mL/100 g body weight. Cumulative and periodic recoveries are shown in (A) and (B), respectively. Data are means ± SE of 6 rats; a, b, and c show significant difference at $P < .05$.

times higher in the WKY and SHRSP rats than in the WKA rats, respectively (Fig 3A). As in the case of sitosterol, cholesterol absorption in WKY rats was lower until 9 hours compared with SHRSP rats and then, increased between 9 and 24 hours (Fig 3B). The absorption of cholesterol until 3 hours was higher in SHRSP rats than in the other 2 rat strains. The periodic absorption pattern of cholesterol was similar to that of sitosterol (compare Figs 2 and 3).

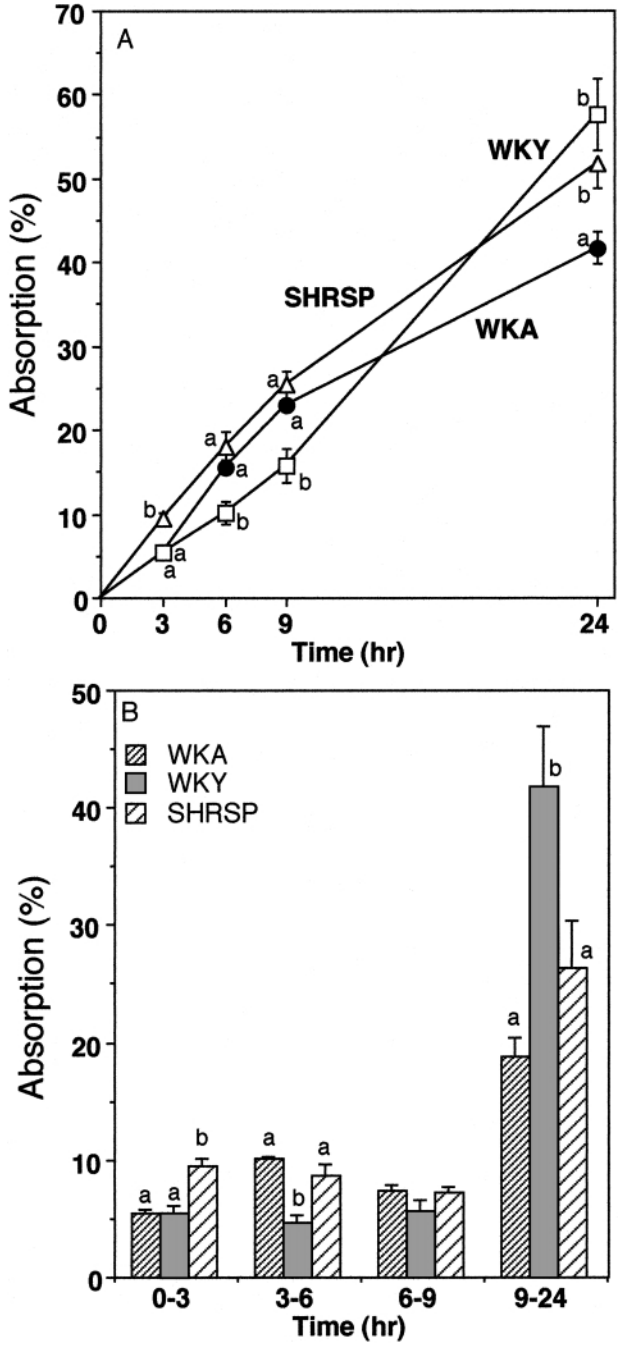


Fig 3. Lymphatic recovery of ^{14}C-cholesterol in WKA, WKY, and SHRSP rats cannulated thoracic duct. Rats were given a test emulsion containing 3.3 μCi ^{3}H-sitosterol and 0.33 μCi ^{14}C-cholesterol, 67 mg sodium taurocholate, 17 mg fatty acid–free albumin, 67 mg triolein/1 mL. The emulsion was given 1 mL/100 g body weight. Cumulative and periodic recoveries are shown in (A) and (B), respectively. Data are means ± SE of 6 rats; a and b show significant difference at $P < .05$.

Table 3. Percentages of Sterol Esters in Lymph

Rat Strains	^{14}C-Cholesterol		^{3}H-Sitosterol	
	0 to 9 Hours	9 to 24 Hours	0 to 9 Hours	9 to 24 Hours
WKA	70.5 ± 0.8[a]	74.0 ± 0.6[a]	8.62 ± 0.39[a]	7.15 ± 0.19[a]
WKY	74.0 ± 0.4[b]	76.4 ± 0.7[b]	8.38 ± 0.26[a]	8.44 ± 0.33[b]
SHRSP	76.2 ± 0.6[c]	77.4 ± 0.6[b]	9.84 ± 0.44[b]	8.55 ± 0.55[b]

NOTE. Rates were given a test emulsion containing 3.3 μCi ^{3}H-sitosterol and 0.33 μCi ^{14}C-cholesterol, 67 mg sodium taurocholate, 17 mg fatty acid–free albumin, 67 mg triolein/1 mL. The emulsion given was 1 mL/100 g body weight. Values are mean ± SE of 6 rats/group.

Values with different superscript letters show significant difference at $P < .05$.

The percentage of esterified radioactive sitosterol in lymph was markedly lower than that of cholesterol in all rat strains (Table 3). The percentage of esterified sitosterol and cholesterol was slightly higher in WKY and SHRSP rats compared with WKA rats.

Incorporation of Cholesterol and Sitosterol Into Intestinal Brush Border Membranes of SHRSP, WKY, and WKA Rats

The incorporation of [^{3}H]sitosterol and [^{14}C]cholesterol into intestinal brush border membranes was linear until 60 minutes of incubation (Fig 4A), and the uptake of sitosterol was 1.6 times and that of cholesterol was 1.4 times higher in the SHRSP than in the WKA rats. In contrast, there was no difference in their incorporation between WKA and WKY rats (Fig 4B).

Secretion of Cholesterol and Sitosterol in the Bile of Rats Fed a Plant Sterol Diet

Initial body weight and weight gain was lower in SHRSP than in WKA rats (171 ± 1 *v* 258 ± 3 and 91 ± 1 *v* 96 ± 1 g, respectively, $P < .05$). Food intake tended to be lower in SHRSP than in WKA rats (16.5 ± 0.3 *v* 17.9 ± 0.1 g/d). Secretion of campesterol and sitosterol as well as cholesterol via bile for 3 hours was significantly lower in SHRSP rats than in WKA rats (Fig 5). The total bile acid concentration in bile was 23.3 ± 2.1 μmol/mL and 17.1 ± 0.6 μmol/mL in SHRSP and WKA rats, respectively. Since bile flow was 1.44 ± 0.16 mL/3 h and 2.39 ± 0.07 mL/3 h, total bile acid secretion for 3 hours was 31.1 ± 0.6 and 40.6 ± 1.2 μmol in SHRSP and WKA rats, respectively. The percentage of the bile acid identified as cholic acid was significantly lower (17.5 ± 1.2 *v* 29.9 ± 3.3, $P < .05$), and those of the bile acids identified as chenodeoxycholic and hyodeoxycholic acids were significantly higher (7.8 ± 1.1 *v* 4.4 ± 0.5 and 6.2 ± 0.4 *v* 3.7 ± 0.5, respectively, $P < .05$) in SHRSP than in WKA rats. Although several minor unidentified peaks were detected in GC analyses in both SHRSP and WKA rats, no significant differences were observed between those peaks.

DISCUSSION

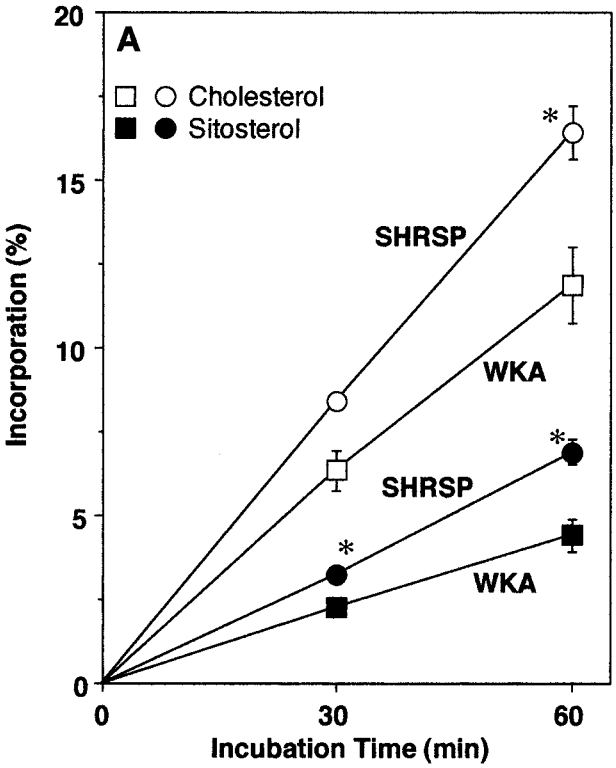
Our results clearly showed that SHRSP and WKY rats deposited considerable amounts of plant sterols in virtually all tissues except brain. A preliminary analysis showed that the content of plant sterols in serum of SHR rats fed a commercial chow was higher and comparable to those in WKY and SHRSP rats (data not shown). Therefore, it is apparent that these genetically related rat strains have an ability to deposit plant sterols efficiently. Because lymphatic 24-hour recovery of sitosterol was significantly higher in SHRSP and WKY rats than in WKA rats, the enhanced absorption of sitosterol can be a major cause in the deposition of plant sterols.

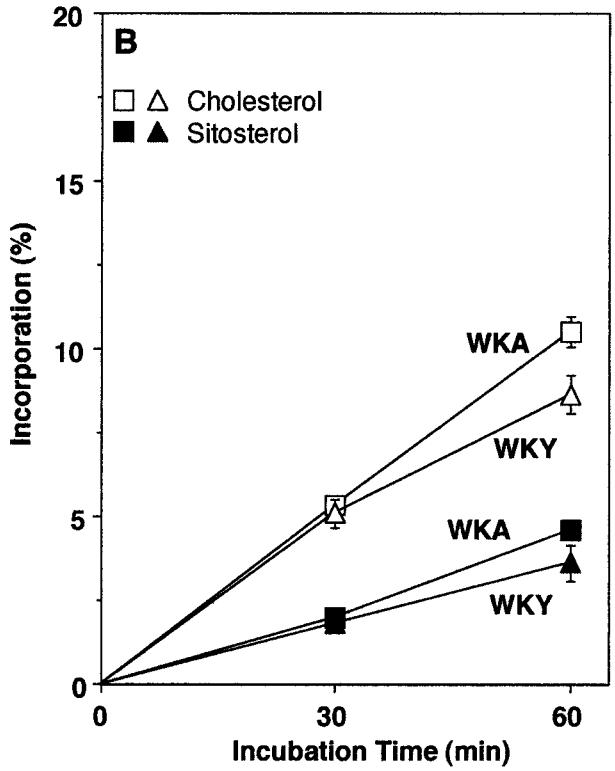
It is widely accepted that intestinal absorption of sitosterol is only less than one 10th that of cholesterol in humans and experimental animals.[2] We previously showed that sitosterol and cholesterol are mainly discriminated at the surface of intestinal brush border membranes, because the incorporation of dietary cholesterol into the brush border membranes was 5 times higher than that of sitosterol in rats.[23] Armstrong and Carey[24] showed a possibility that sitosterol, compared with cholesterol, has a higher affinity for bile salt micelles. We also demonstrated that the release of sitosterol from mixed micelles containing phosphatidylcholine was less efficient than that of cholesterol.[3] These observations suggest that a lower release of sitosterol as a monomer from bile salt micelles is responsible for a lower absorption of sitosterol than cholesterol. However, hyperabsorption of sitosterol in SHRSP and WKY rats cannot be explained only by this mechanism. Because the characteristics of SHRSP and WKY rats can be genetic, it is strongly suggested that carrier proteins in intestinal cells assist in the hyperabsorption of sitosterol in these rat strains.

In SHRSP rats, compared with WKA rats, 2-step acceleration in lymphatic absorption of sitosterol was observed between 0 and 3 hours and 9 and 24 hours (Fig 2). In contrast, the absorption in WKY rats was accelerated only between 9 and 24 hours. The major acceleration was observed between 9 and 24 hours in both WKY and SHRSP rats. The incorporation of sitosterol into intestinal brush border membranes separated from SHRSP rats was greater compared with that from WKA rats (Fig 4A). However, no differential incorporation of sitosterol was observed between WKA and WKY rats (Fig 4B). These observations suggest that the enhanced incorporation into brush border membranes is at least responsible for hyperabsorption of sitosterol in SHRSP rats. There is a possibility that a specific carrier protein that assists sitosterol absorption exists at the surface of brush border membranes more in SHRSP rats than in WKA rats. However, this is not the case for WKY rats. Therefore, it is thought that the incorporation of sitosterol into brush border membranes is not a major cause of the accelerated absorption in SHRSP and WKY rats. Recently, Hauser et al[25] suggested a possibility that the scavenger receptor class B type I (SR-BI) mediates intestinal cholesterol absorption. A more recent work identified several mutations in the adenosine triphosphate binding cassette (ABC) transporter family in patients with sitosterolemia.[26] One of the ABC transporter family has been suggested to participate in intestinal absorption of

cholesterol.[27] Whether these proteins are involved in the accelerated incorporation of sitosterol and cholesterol in SHRSP rats is now under investigation in our laboratory.

After the incorporation of sterols into absorptive cells, it has been believed that sterols are transported from brush border membranes to endoplasmic reticulum by the concentration gradient. The esterification of cholesterol in intestinal cells can be a crucial factor to maintain the concentration gradient. In fact, the inhibition of intestinal acyl CoA cholesterol acyltransferase (ACAT), an enzyme esterifying cholesterol, decreased cholesterol absorption.[28] We previously showed less effective esterification and secretion to lymph of sitosterol than those of cholesterol.[3] Thus, it seems likely that the low rate of esterification is at least responsible for the lower absorbability of sitosterol. Therefore, the accelerated esterification of sitosterol





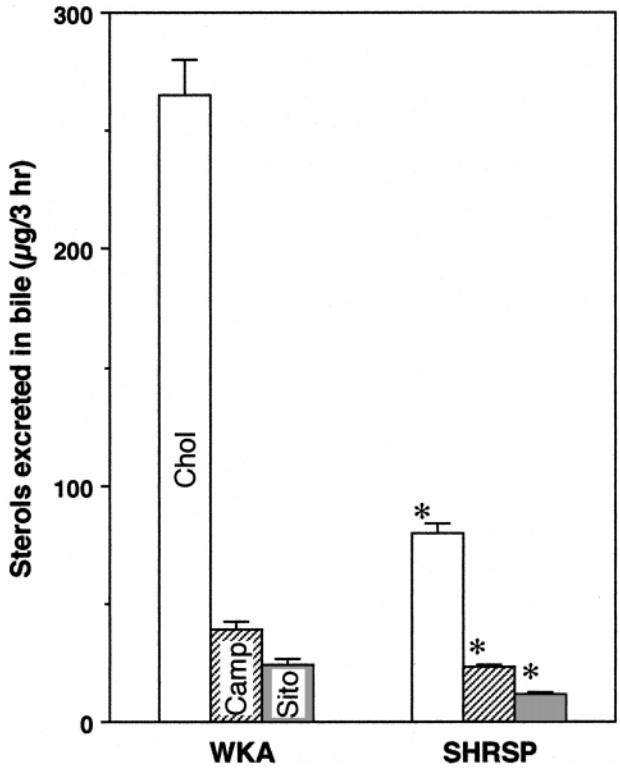


Fig 5. Excretion of biliary sterols in WKA and SHRSP rats cannulated bile duct. These rats were fed a 0.5% plant sterol diet for 2 weeks. At the termination of the feeding period, rats were cannulated bile duct and bile was collected for 3 hours. Data are means ± SE of 6 rats. *Significantly different from WKA rats at $P < .05$.

Fig 4. The incorporation of micellar ^{3}H-sitosterol and ^{14}C-cholesterol into intestinal brush border membranes separated from WKA, WKY, and SHRSP rats. A total of 4 mL of a micellar solution containing 6.6 mmol/L sodium taurocholate, 0.6 mmol/L phosphatidylcholine, 50 μmol/L ^{14}C-cholesterol, and 50 μmol/L ^{3}H-sitosterol prepared in a Hank's balanced salt solution containing 5 mmol/L EGTA and 4% BSA (fatty acid–free) in 15 mmol/L HEPES buffer, pH 7.4 was incubated with 1 mL of brush border membrane suspension (2 mg protein) at 37°C. Open and closed symbols show the incorporation of cholesterol and sitosterol, respectively. ○●, □■, △▲: brush border membranes from SHRSP, WKA, and WKY rats, respectively. The incorporation of cholesterol and sitosterol into intestinal brush border membranes is compared between SHRSP and WKA (A) and WKY and WKA (B). Data are means ± SE of 3 rats. *Significantly different from WKA rats at $P < .05$.

and cholesterol may increase their absorption. However, percentage of esterified sitosterol in lymph was only marginally higher in SHRSP and WKY rats. A slight increase in cholesterol esterification was also observed in SHRSP and WKY rats. Therefore, it is plausible that the contribution of the slightly accelerated esterification of cholesterol and plant sterols to their enhanced absorption is marginal, if any.

The precise mechanisms by which absorption of sitosterol and cholesterol is accelerated in SHRSP and WKY rats were not revealed in the present study. Although mechanisms of the desorption from intestinal brush border membranes and the diffusion to endoplasmic reticulum of sterols is not well understood,[29] our study suggests a possibility that some carrier proteins may promote a transfer of the sterols from brush border membranes to endoplasmic reticulum in SHRSP and WKY rats.

The present study showed that lymphatic 24-hour recovery of cholesterol was also accelerated in WKY and SHRSP rats, although the extent of the acceleration was smaller than the case of sitosterol. The periodic pattern of the lymphatic absorption of cholesterol was similar to that of sitosterol (compare Figs 2 and 3). The incorporation of cholesterol into intestinal brush border membranes obtained from SHRSP rats was higher than that from WKA rats as in the case of sitosterol (Fig 4). In this regard, Tilvis and Miettinen[30] indicated that the absorption of cholesterol is positively correlated to that of plant sterols in normal subjects. It was also reported that the absorption of both sitosterol and cholesterol is increased in sitosterolemic patients.[4] Thus, a common genetic factor may be involved in the absorption of cholesterol and plant sterols. Further studies on genetic factors involved in sitosterol absorption in WKY and SHRSP rats will provide definite information on transporter proteins related to cholesterol absorption.

Although plant sterols were deposited in the liver of SHRSP rats, the excretion of the sterols in bile was lower in this strain of rats (Fig 5). One of the causes of the accumulation of plant sterols in SHRSP rats can, therefore, be attributed to the restrained catabolism. The excretion of cholesterol was also lower in this rat strain. In normal humans, plant sterols incorporated into the body are preferentially secreted into bile.[31] In contrast, phytosterolemic patients secreted less plant sterols and cholesterol into bile.[4,32] Therefore, SHRSP rats may have similar characteristics as phytosterolemic patients. These similarities suggest a possibility that phytosterolemia could be explained by mutation in a common transporter protein expressed in intestinal and hepatic cells.

It has been reported that C_{21}-bile acids were synthesized from sitosterol in a rat strain.[33,34] There is a possibility that bile acids synthesized from plant sterols would be higher in SHRSP rats. Although we analyzed bile acid composition in bile in SHRSP and WKA rats, no candidate bile acids were detected in bile from SHRSP rats. More detailed studies will be necessary on metabolism of sitosterol in this rat strain.

The SHRSP rat strain is an animal model for stroke with hypertension. A series of studies by this rat strain examined the effect of dietary vegetable oils on death due to stroke.[35-37] We report here that plant sterols were deposited in the aorta of SHRSP rats. Higher content of plant sterols in vessels may severely influence the pathogenesis of stroke and the longevity of SHRSP rats, because there is a possibility that plant sterols incorporated into cell membranes influence the membrane integrity. Therefore, care should be taken when the observations in this animal model are extrapolated to humans, because no accumulation of plant sterols was observed in humans suffering from stroke.

In conclusion, by using SHRSP and the related rat strains, it will become possible to know the molecular mechanism that the intestines can discriminate plant sterols from cholesterol and that sitosterolemic patients can deposit plant sterols.

REFERENCES

1. Ikeda I, Sugano M: Inhibition of cholesterol absorption by plant sterols for mass intervention. Curr Opin Lipidol 9:527-531, 1998
2. Ling WH, Jones PJH: Dietary phytosterols: A review of metabolism, benefits and side effects. Life Sci 57:195-206, 1995
3. Ikeda I, Tanaka K, Sugano M, et al: Discrimination between cholesterol and sitosterol for absorption in rats. J Lipid Res 29:1583-1591, 1988
4. Salen G, Shefer S, Nguyen L, et al: Sitosterolemia, in Bittman R (ed): Subcellular Biochemistry, vol 28, Cholesterol: Its Functions and Metabolism in Biology and Medicine. New York, NY, Plenum, 1997, pp 453-476
5. Salen G, Shore V, Tint GS, et al: Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. J Lipid Res 30:1319-1330, 1989
6. Patel SB, Salen G, Hidaka H, et al: Mapping a gene involved regulating dietary cholesterol absorption. The sitosterolemia locus is found at chromosome 2p21. J Clin Invest 102:1041-1044, 1998
7. Okamoto K, Aoki K: Development of a strain of spontaneously hypertensive rats. Jpn Circ J 27:282-293, 1963
8. Yamori Y, Nagaoka A, Okamoto K: Importance of genetic factors in stroke: An evidence obtained by selective breeding of stroke-prone and -resistant SHR. Jpn Circ J 38:1095-1100, 1974
9. Reeves PG, Nielsen FH, Fahey GC: AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939-1951, 1993
10. Ikeda I, Tanaka K, Sugano M, et al: Inhibition of cholesterol absorption in rats by plant sterols. J Lipid Res 29:1573-1582, 1988
11. Kessler M, Acuto O, Storelli C, et al: A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport system. Biochim Biophys Acta 506:136-154, 1978
12. Dalqvist A: Method for assay of intestinal disaccharidases. Anal Biochem 7:18-25, 1964
13. Forstner GG, Sabesin SM, Isselbacher KJ: Rat intestinal microvillus membranes. Biochem J 106:381-390, 1968
14. Gallo LL, Myers S, Vahouny GV: Rat intestinal acyl coenzyme A: cholesterol acyltransferase properties and localization. Proc Soc Exp Biol Med 177:188-196, 1984
15. Pennington RJ: Biochemistry of dystrophic muscle: Mitochondrial succinate-tetrazolium reductase and adenosine triphosphatase. Biochem J 80:649-654, 1961
16. Burton K: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315-323, 1945
17. Folch J, Lees M, Sloane-Stanley GH: A simple method for the

isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957

18. Tanaka K, Ikeda I, Sugano M: Effect of glyco-and tauro-cholic and chenodeoxycholic acids on lymphatic absorption of micellar cholesterol and sitosterol in rats. Biosci Biotechnol Biochem 57:2059-2062, 1993

19. Eaton DL, Klaassen CD: Effects of acute administration of taurocholic and taurochenodeoxycholic acid on biliary lipid excretion in the rat. Proc Soc Exp Biol Med 151:198-202, 1976

20. Ikeda I, Wakamatsu K, Inayoshi A, et al: α-Linolenic, eicosapentaenoic and docosahexaenoic acids affect lipid metabolism differently in rats. J Nutr 124:1898-1906, 1994

21. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

22. Duncan DB: Multiple range and multiple F tests. Biometrics 11:1-42, 1955

23. Ikeda I, Sugano M: Some aspects of mechanism of inhibition of cholesterol absorption by β-sitosterol. Biochim Biophys Acta 732:651-658, 1983

24. Armstrong MJ, Carey MC: Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. J Lipid Res 28:1144-1155, 1987

25. Hauser H, Dyer JH, Nandy A, et al: Identification of a receptor mediating absorption of dietary cholesterol in the intestine. Biochemistry 37:17843-17850, 1998

26. Berge KE, Tian H, Graf GA, et al: Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290:1771-1775, 2000

27. Repa JJ, Turley SD, Lobaccaro JMA, et al: Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289:1524-1529, 2000

28. Cases S, Novak S, Zheng Y-W, et al: ACAT-2, a second mammalian acyl CoA:cholesterol acyltransferase: Its cloning, expression, and characterization. J Biol Chem 273:26755-26764, 1998

29. Dawson PA, Rudel LL: Intestinal cholesterol absorption. Curr Opin Lipidol 10:315-320, 1999

30. Tilvis RS, Miettinen TA: Serum plant sterols and their relation to cholesterol absorption. Am J Clin Nutr 43:92-97, 1986

31. Salen G, Grundy SM, Ahrens EH Jr: The metabolism of β-sitosterol in man. J Clin Invest 49:952-967, 1970

32. Bhattacharyya AK, Connor WE, Lin DS, et al: Sluggish sitosterol turnover and hepatic failure to excrete sitosterol into bile cause expansion of body pool of sitosterol in patients with sitosterolemia and xanthomatosis. Atheroscler Thromb 11:1287-1294, 1991

33. Boberg KM, Lund E, Ölund J, et al: Formation of C21 bile acids from plant sterols in the rat. J Biol Chem 265:7967-7975, 1990

34. Björkhem I: Mechanism of degradation of the steroid side chain in the formation of bile acids. J Lipid Res 33:455-471, 1992

35. Huang MZ, Naito Y, Watanabe S, et al: Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats. Biol Pharm Bull 19:554-557, 1996

36. Huang MZ, Watanabe S, Kobayashi T, et al: Unusual effects of some vegetable oils on the survival time of stroke-prone spontaneously hypertensive rats. Lipids 32:745-751, 1997

37. Miyazaki M, Huang MZ, Takemura N, et al: Free fatty acid fractions from some vegetable oils exhibit reduced survival time-shortening activity in stroke-prone spontaneously hypertensive rats. Lipids 33:655-661, 1998