Effect of Soybean Sterols on Liver Deposition of Cholesterol-C^{14}. (21294)

KENNETH A. BURKE,* R. F. J. MCCANDLESS, AND DAVID KRITCHEVSKY.
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From the Divisions of Nutrition and Physiology and Viral and Rickettsial Research, Lederle Laboratories Division, Amer. Cyanamid Co., Pearl River, N. Y.

The prevention of hypercholesterolemia in cholesterol-fed chickens(1,2) and rabbits(3) has been successfully accomplished by the concomitant feeding of soybean sterols. This effect has been explained as an interference phenomenon, the plant sterols somehow altering absorption of cholesterol(3). Using cholesterol-4-C^{14} in rats, Hernandez *et al.*(4) demonstrated that either mixed soybean sterols or β-sitosterol resulted in a marked decrease in the amount of cholesterol recovered from thoracic duct lymph. This finding has recently been challenged by Rosenman and coworkers(5) who found no reduction of cholesterol absorption into the thoracic duct lymph in a similar experiment. The latter authors, who fed 100 mg of cholesterol, suggest that the difference is due to the amount (4 mg) of labeled cholesterol administered by Hernandez. This amount is supposedly less than the average amount of cholesterol ingested daily by rats on a stock diet.

We have completed experiments involving feeding of labeled cholesterol to rats on various sitosterol regimens with a view to gauging the effect of the soy sterols on liver storage of the marked sterol as well as on fecal excretion of labeled material. The results would indicate that a large excess of soybean sterol may, indeed, reduce liver deposition of ingested cholesterol. These results tend to substantiate the findings of Hernandez *et al.*

Experimental. Three groups of 4 male Wistar strain rats (500 g) were used. Groups 1 and 2 were maintained on normal rat chow (Wayne Rat Pellets, Allied Mills, Chicago, Ill.) augmented with 3% cholesterol in 4% corn oil for 120 days. Group 3 was maintained on rat chow augmented with 3% cholesterol plus 5% mixed soy sterols (Distillation Products, Rochester, N. Y.) in 4% corn oil for the same period. Each rat was then given about 10 mg random-labeled cholesterol-C^{14}(6) in one cc of corn oil by stomach tube. In the case of Group 2, the cholesterol was followed immediately by one g of mixed soy sterols in corn oil. All animals were maintained in individual metabolism cages for 4 days; the feces and urine of each animal being pooled and assayed for carbon-14 activity. After 4 days the rats were sacrificed and the livers removed, dissolved in warm alcoholic KOH and the solution separated into non-saponifiable and acidic fractions, each of which was assayed for radioactivity. Radioactivity measurements were made in a windowless gas-flow counter using infinitely thick plates of aliquots of the solutions obtained. *Results.* Data are given in Table I.

Discussion. Inspection of the average values reveals that while the total activity recovered for the 3 groups was approximately the same, its distribution differed widely. The dose of administered cholesterol-C^{14} was small compared with the dose of cholesterol which the animals had been receiving, so that one cannot argue that the labeled cholesterol represented a unique dietary addition. The soy sterols evidently interfere with the adsorption of cholesterol but whether the mechanism is a competition for sites of esterification or a mechanical mixing which decreases cholesterol availability is not clear from these data. It is interesting to note that it is the amount of non-saponifiable material in the liver that differs, the activity recovered in the acidic fraction being about equal for the 3 groups.

The dose of soy sterols required to reduce the amount of cholesterol deposited in the liver after a single feeding of a small quantity of cholesterol appears to be rather large, since in this experiment even a hundredfold excess of soy sterol (Group 2) did not fully inhibit liver storage of cholesterol-C^{14}. In the animals fed 5% soy sterol with their diet for 3 months (Group 3) liver deposition of labeled choles-

* Deceased.

TABLE I. Per Cent Administered C^{14} Recovered after Feeding Cholesterol-C^{14}. Three groups.

Regimen	Rat No.	Dose chol.-C^{14} (mg)	Liver NS*	Liver Acid	Feces NS	Feces Acid	Urine	Total
3% cholesterol (120 days) then cholesterol-C^{14}	1	9.4	5.14	.54	16.74	.71	ND†	23.13
	2	9.4	7.82	.78	24.19	1.58	.04	34.41
	3	9.0	2.74	.49	16.16	.43	ND	19.82
	4	10.3	10.75	.28	23.47	1.08	"	35.58
Avg			6.61	.52	20.14	.95	.01	28.24
3% cholesterol (120 days) then cholesterol-C^{14} and 1 g soy sterol	5	11.1	1.60	.44	26.45	1.69	.03	30.21
	6	9.6	.87	.13	23.62	1.38	.05	26.05
	7	9.1	4.55	1.10	32.69	2.03	ND	40.37
	8	13.1	.18	.57	17.77	.84	"	19.36
Avg			1.80	.56	25.13	1.49	.02	29.00
3% cholesterol + 5% soy sterol (120 days) then cholesterol-C^{14}	9	11.5	lost	.72	32.90	1.10	.08	—
	10	10.0	.02	.79	25.55	2.74	ND	29.10
	11	10.2	.89	1.01	34.10	1.91	"	37.91
	12	13.1	.50	ND	18.49	1.98	"	20.97
Avg			.47	.63	27.76	1.93	.02	30.81

* NS = non-saponifiable. † ND = no detectable radioactivity.

terol was still less than that in Group 2, but no estimate of the amount of soy sterol which may have been present in the gut of these animals has been made.

In work with humans, Pollak(7) reported that 10 g of sitosterol daily lowered serum cholesterol in his subjects; on the other hand, Wilkinson *et al.*(8) have found that doses of sitosterol as high as 60 g/day do not reduce serum cholesterol levels. The timing of the sitosterol feedings may have some bearing on these discrepancies.

Summary. 1. The effect of soybean sterols on liver deposition and fecal excretion of cholesterol-C^{14} following a single feeding of the labeled material has been studied using cholesterol-fed rats. 2. Average recoveries of labeled cholesterol in liver (measured as radioactivity recovered from liver non-saponifiable fraction) were 6.61% in a cholesterol-fed group, 1.80% in a cholesterol-fed group receiving one g of soy sterol immediately after cholesterol-C^{14} administration and 0.47% in a group fed both cholesterol and soybean sterol for 120 days. 3. Average recoveries of radioactivity in the non-saponifiable fraction of feces were 20.14%, 25.13%, and 27.76%, respectively, for the 3 groups described above.

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