The Effect of β -Sitosterol on the Metabolism of Cholesterol and Lipids in Rats on a Diet containing Coconut Oil

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1. Intraperitoneal injection of β -sitosterol (5mg./rat/day for 25 days) into 1-year-old male Wistar rats fed on a low-fat diet supplemented with 10% of coconut oil resulted in a lowering of cholesterol and lipid concentrations in the tissues.
2. β -Sitosterol increased the rate of biosynthesis of cholesterol and lipids in the tissues, but to an even greater extent enhanced their oxidative degradation.
3. The present results are similar to those previously obtained on a low-fat diet, indicating that the presence of fat had no marked effect on the action of β -sitosterol.

When β -sitosterol was injected intraperitoneally into rats fed on a low-fat diet (Gerson, Shorland & Dunckley, 1964), the weights and concentrations of cholesterol and lipids in the tissues were decreased. Moreover, the experimental rats, after the administration of sodium [1^{14}C]acetate, showed a greater incorporation of ^{14}C into the tissue cholesterol and fat, as well as into the expired carbon dioxide. These results, together with the fact that the amount of expired carbon dioxide was greater in the experimental animals than in the controls, were consistent with the hypothesis that injected β -sitosterol increased the biosynthesis of fatty acids and cholesterol, and accelerated their oxidative degradation to an even greater extent. The possibility that increased ^{14}C specific activities were due to higher concentrations of sodium [1^{14}C]acetate in depleted acetate pools was discussed.

In the work now reported, the effect of β -sitosterol in the presence of a cholesterogenic dietary fat was investigated. For this purpose coconut oil, which contains mainly saturated fatty acids and is among the most cholesterogenic of the common food fats as judged by its elevation of plasma cholesterol concentrations (Ahrens *et al.* 1957; Gunning, Imaichi, Splitter & Kinsell, 1964), was selected.

EXPERIMENTAL

Animals. The animals (male Wistar rats), when 9 months old, were fed *ad lib.* on a low-fat diet (cf. Gerson, Shorland & Adams, 1961) supplemented with 10% (w/w) of coconut oil, for a period of 3 months. At this stage they were divided into an experimental and a control group of 15 animals each. These groups were further subdivided into five subgroups

of three animals. The average weight per rat at the beginning of the experimental period was 395 g. (s.d. $\pm$ 38.2 g.) and 401 g. (s.d. $\pm$ 28.4 g.) for the control and experimental animals respectively.

Each animal was given a daily intraperitoneal injection of 0.5 ml. of 0.9% NaCl solution over a period of 25 days. In the experimental animals this contained 5 mg. of emulsified β -sitosterol. At 24 hr. after the last of these injections all animals were given an intraperitoneal injection of 40 μC of sodium [1^{14}C]acetate in 1 ml. of 0.9% NaCl. The expired CO_2 was collected during a further hour and the animals were then killed by heart puncture while under ether anaesthesia. The tissues and organs were removed for analysis. During the experimental period the faeces were collected separately for each subgroup.

The β -sitosterol in this experiment was the same as that used by Gerson *et al.* (1964). When subjected to gas-liquid chromatography on an SE30 column one peak only was observed.

Extraction of tissues and faeces. This was carried out as described by Gerson *et al.* (1964).

Cholesterol and faecal sterols. The methods of Zak, Luz & Fisher (1957) and Gerson (1960) respectively were used.

^{14}C specific activities. The determinations were carried out as described by Gerson *et al.* (1964), the only difference being a blank of 5 counts/min. at a counting efficiency of 46.5%. Except where otherwise stated all values represent the mean values of the combined lipids from any one tissue, plasma or faeces of each subgroup.

Statistical analysis. Values quoted for *P* are based on the standard *t* test.

RESULTS AND DISCUSSION

The average daily food consumption of the control animals on the coconut oil diet was 12.1 g./rat/day, as compared with 13.1 g./rat/day for similar animals

in a previous experiment with a low-fat diet (cf. Gerson *et al.* 1964). On a calorie basis, however, the intake on the coconut oil-supplemented diet was 7.6% higher. As found for the low-fat diet, β -sitosterol injections were associated with an initial decline in food consumption; the mean consumption did not fall below 8 g./rat/day and had returned to normal by the tenth day. Throughout the experiment the consumption of the control animals remained unchanged. The decrease in food intake was not reflected in a decrease in total faecal lipid excreted, as was found by Lee & Lucia (1963) with

rats on a calorically restricted diet. On the contrary, the effect of β -sitosterol was to increase the rate of excretion from 43.6 mg. (s.d. ± 7.2 mg.)/rat/day in the controls to 53.7 mg. (s.d. ± 6.6 mg.)/rat/day in the experimental animals ($P < 0.05$). This was equivalent to 0.8% of the dietary fat. Table 4 shows that this increase was not due to faecal sterols.

During the β -sitosterol injections the mean weight of the rats decreased, reaching a minimum on the fourth day, but thereafter did not change significantly. The mean loss of weight of 19.1 g. (s.d. ± 6.0 g.) in the experimental animals occurred

Table 1. *Effect of intraperitoneally injected β -sitosterol on the lipid concentrations and weights, as well as the weights of total cholesterol, in the tissue of rats on a coconut oil-supplemented diet*

Tissue	Concn. of lipid (g./100 g. fresh wt. of tissue)		Total lipids (g./rat)		Total cholesterol (mg./rat)	
	Control	Experimental	Control	Experimental	Control	Experimental
Pelt	22.0	22.0	16.80	17.30	125.4	114.3
Intestines	24.4	25.0 ($P < 0.7$)	4.40	6.33	30.7	43.0
Liver	5.8	4.7 ($P < 0.2$)	0.51	0.46	18.2	15.2
Adrenals	31.0	28.7 ($P < 0.5$)	0.02	0.02	1.2	0.8
Testes	5.8	5.1 ($P < 0.7$)	0.11	0.15	2.4	1.9
Heart	5.0	4.7 ($P < 0.7$)	0.06	0.06	1.4	1.3
Aorta	17.7	14.3 ($P < 0.1$)	0.03	0.03	0.5	0.4
Plasma	0.46	0.46				
Adipose tissue	86.3	79.3 ($P < 0.3$)	36.90	25.32	325.5	283.6
Muscle	11.0	6.3 ($P < 0.02$)				
Carcass	11.7	8.7 ($P < 0.001$)				
Total			58.83	49.67	505.3	460.5
Change				-9.2 (-15.6%)		-44.8 (-8.9%)

Table 2. *Effect of intraperitoneally injected β -sitosterol on the concentrations of cholesterol in the tissues of rats on a coconut oil-supplemented diet*

Cholesterol was determined by the method of Zak *et al.* (1957) and expressed as mg./100 g. fresh wt. of tissue. Other experimental details are given in the text.

Tissue	Concn. of total cholesterol			Concn. of free cholesterol			Concn. of esterified cholesterol (by difference)		
	Control	Experimental	Difference	Control	Experimental	Difference	Control	Experimental	Difference
Carcass	130.0	118.1	- 11.9 ($P < 0.01$)	115.3	106.7	- 8.6 ($P < 0.05$)	14.7	11.4	- 3.3
Adipose tissue	114.5	73.3	- 41.2 ($P < 0.001$)	69.7	61.0	- 8.7 ($P < 0.025$)	44.8	12.3	- 32.5
Muscle	66.2	50.8	- 15.4 ($P < 0.005$)	56.0	40.8	- 15.2 ($P < 0.005$)	6.2	10.0	+ 3.8
Pelt	158.2	148.8	- 9.4 ($P < 0.1$)	103.4	100.4	- 3.0 ($P < 0.5$)	54.8	48.4	- 6.4
Testes	132.5	110.0	- 21.5 ($P < 0.01$)	124.9	107.6	- 17.3 ($P < 0.02$)	7.6	3.4	- 4.2
Intestines	168.6	170.2	+ 1.6 ($P < 0.9$)	109.1	98.0	- 11.1 ($P < 0.5$)	59.5	72.2	+ 12.7
Liver	204.4	157.5	- 46.9 ($P < 0.05$)	178.1	146.7	- 31.4 ($P < 0.1$)	26.3	10.8	- 15.5
Adrenals	2435	1449	- 986 ($P < 0.1$)	452	420	- 32 ($P < 0.6$)	1983	1029	- 954
Heart	114.7	94.3	- 20.3 ($P < 0.005$)	89.7	75.4	- 14.3 ($P < 0.05$)	25.0	18.9	- 6.1
Aorta	308.2	218.9	- 89.3 ($P < 0.01$)	251.2	175.4	- 75.8 ($P < 0.05$)	57.0	43.5	- 13.5
Plasma	57.8	43.6	- 14.2 ($P < 0.02$)	19.0	11.8	- 7.2 ($P < 0.001$)	38.8	31.8	- 7.0

almost entirely in the first 5 days. The mean loss of weight of 10.2 g. (s.d. ± 2.7 g.) in the controls, on the other hand, was distributed uniformly through the experiment. The difference due to β -sitosterol was 8.9 g. ($P < 0.02$).

β -Sitosterol significantly decreased the concentration of lipids in the aorta, muscle and carcass, as well as the total lipids, the greater part of which occurred in the carcass (Table 1).

The concentration of total cholesterol was lowered in most of the tissues investigated (Table 2). The decreases in carcass, muscle, testes, liver, heart and aorta were due primarily to decreases in the concentration of free cholesterol. In pelt, adipose tissue and adrenal glands, on the other hand, esterified cholesterol concentrations were diminished. In plasma both free and esterified cholesterol concentrations declined. These patterns were similar to those obtained on a low-fat diet (Gerson *et al.* 1964).

The administration of β -sitosterol increased the incorporation of ^{14}C from injected acetate in the tissue fatty acids with the exception of those of the adipose tissue and in the cholesterol of the tissues apart from the heart (Table 3).

From these results the increases in ^{14}C incorporation into fatty acids due to β -sitosterol injection were: liver (7%), heart (179%), aorta (24%) and plasma (142%); into cholesterol the increases were: carcass (23%), intestines (157%), liver (63%), adrenals (97%) and plasma (26%), and decreases in

heart (-27%) and aorta (-12%). The overall recovery of ^{14}C from cholesterol was increased by 22% after the injection of β -sitosterol.

The evidence thus indicates that the injection of β -sitosterol increased the biosynthesis of fatty acids and cholesterol, and also their oxidative degradation, as shown by the increased ^{14}C activity and rate of expiration of carbon dioxide, whereas the excretion of lipids and cholesterol in the faeces was relatively unaffected (Table 4).

The fatty acid compositions of the tissue and plasma lipids (Table 5) confirmed previous observations (Shorland, 1955) that lauric acid (12:0), which is the main constituent of coconut oil, is rapidly metabolized and not stored in the depot fat. The muscle, adipose tissue and aorta lipids contained considerable amounts (10–24%) of this acid. With the exception of the aorta lipids, the amounts of lauric acid appeared to be inversely related to the specific activities of the fatty acids (Table 3), i.e. where there was little fatty acid biosynthesis, the proportion of fatty acids deposited from dietary fat was high. The disappearance of lauric acid from muscle and aortas after β -sitosterol treatment appears to reflect an increase in oxidative degradation. However, in adipose tissue where little oxidative degradation takes place the proportion of lauric acid remained unchanged. In the aorta the administration of β -sitosterol appears to have brought about a far-reaching change in fatty acid

Table 3. Changes in ^{14}C specific activities of cholesterol and fatty acids due to β -sitosterol injections in the presence of a coconut oil-supplemented diet

Results for cholesterol are given in roman print, those for fatty acids in *italics*. The determinations were carried out as by Gerson *et al.* (1964). All specific activities are expressed as disintegrations/min./mg. Other experimental details are given in the text.

Tissue	Sp. activity of total cholesterol			Sp. activity of free cholesterol			Sp. activity of esterified cholesterol		
	Control	Experi- mental	Difference	Control	Experi- mental	Difference	Control	Experi- mental	Difference
Carcass	282	386	+ 37% ($P < 0.01$)	317	421	+ 33% ($P < 0.05$)	7	58	+ 729%
Adipose	585	1534	+ 162% ($P < 0.001$)	791	1747	+ 133% ($P < 0.001$)	265	478	+ 80%
tissue	<i>1.05</i>	<i>0.87</i>	- 17%						
Muscle	298	397	+ 33% ($P < 0.05$)	331	454	+ 37% ($P < 0.005$)	192	365	+ 90%
	<i>1.57</i>	<i>1.72</i>	+ 10%						
Pelt	121	139	+ 15% ($P < 0.1$)	190	216	+ 14% ($P < 0.2$)	4	0	- 100%
Testes	175	237	+ 35% ($P < 0.02$)	191	247	+ 29% ($P < 0.05$)	0	0	0%
Intestines	710	1300	+ 69% ($P < 0.005$)	1044	1935	+ 85% ($P < 0.001$)	98	438	+ 347%
Liver	337	657	+ 95% ($P < 0.001$)	374	697	+ 86% ($P < 0.001$)	86	114	+ 33%
	<i>37.8</i>	<i>45.4</i>	+ 20%						
Adrenals	323	955	+ 196% ($P < 0.001$)	1041	2548	+ 145% ($P < 0.001$)	159	305	+ 92%
Heart	760	600	- 21% ($P < 0.005$)	899	710	- 19% ($P < 0.01$)	155	161	+ 4%
	<i>16.6</i>	<i>45.3</i>	+ 173%						
Aorta	980	1079	+ 10% ($P < 0.1$)	1063	1255	+ 18% ($P < 0.1$)	6.4	369	- 40%
	<i>39.0</i>	<i>49.1</i>	+ 26%						
Plasma	369	708	+ 92% ($P < 0.005$)	688	2071	+ 201% ($P < 0.001$)	213	202	- 5%
	<i>21.4</i>	<i>60.4</i>	+ 182%						

Table 4. *Changes in faecal sterol excretion and relative expiration of carbon dioxide and ^{14}C due to β -sitosterol injections in the presence of dietary coconut oil*

The determinations were carried out as described by Gerson *et al.* (1964). Faecal excretion is expressed as mg./rat/day. Rates of expiration are relative to the controls. Other experimental details are given in the text.

	Control	Experimental	Difference
Total cholesterol	1.0	1.1	
Total coprosterol	10.0	10.7	
Total sterols	11.0	11.8	+ 7% ($P < 0.8$)
Free cholesterol	0.8	1.1	
Free coprosterol	9.4	10.0	
Total free sterols	10.2	11.1	+ 9% ($P < 0.7$)
Esterified cholesterol (by difference)	0.2	0.0	
Esterified coprosterol (by difference)	0.6	0.7	
Total esterified sterols	0.8	0.7	- 13%
Total CO_2 expired	100.0	106.3	+ 6.3%
^{14}C sp. activity of CO_2	100.0	154.2	+ 54.2%
Total ^{14}C expired	100.0	163.9	+ 63.9%

Table 5. *Effect of β -sitosterol injections on the fatty acid composition of the tissues of rats receiving dietary coconut oil*

The analyses were carried out as described by Gerson *et al.* (1964). The designation of the fatty acids is also as used by Gerson *et al.* (1964). Other experimental details are given in the text.

Fatty acid	Fatty acid composition (% w/w)											
	Muscle		Adipose tissue		Liver		Plasma		Heart		Aorta	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
10:0	0.1	0.6	Trace	0.6	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
12:0	17.2	10.6	20.5	19.4	3.3	3.3	4.1	3.8	5.1	7.7	24.2	5.2
14:0	8.7	10.3	9.2	12.4	2.3	1.6	5.3	4.0	3.9	4.7	4.8	4.8
15:0	Trace	1.0	Trace	Trace	—	—	1.8	Trace	0.4	0.7	—	—
15:br	Trace	0.6	Trace	Trace	—	—	—	—	0.4	Trace	—	—
16:0	25.0	24.0	24.3	23.7	25.9	27.2	34.7	27.0	23.7	25.3	37.0	20.7
16:1	14.2	9.3	8.6	7.7	4.1	4.2	3.2	8.4	5.8	4.5	2.4	6.2
17:0	Trace	1.0	—	—	—	—	Trace	Trace	0.4	0.9	—	—
18:0	3.7	2.6	3.5	2.0	14.7	17.2	10.7	10.1	14.1	12.8	2.3	6.4
18:1	26.1	33.4	29.5	29.4	24.4	21.6	27.8	32.4	27.3	28.2	25.9	41.8
18:2	5.0	6.7	4.3	4.9	8.6	11.6	6.6	5.7	11.7	9.7	2.4	6.4
20:4	Trace	Trace	Trace	Trace	11.7	14.5	5.9	8.6	7.6	5.7	1.0	8.6

composition. Despite the relatively high specific activity of the fatty acids, a high proportion of lauric acid was found in the controls. This was restored by the administration of β -sitosterol to a concentration similar to that found in the other tissues, except muscle and adipose tissues. Similarly, there was a decrease in the proportion of palmitic acid (16:0). These decreases were compensated for by stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and arachidonic acid (20:4), which, with the exception of oleic acid, were restored to proportions comparable with those found in liver, plasma and heart.

The present results show that the effects of β -sitosterol injections into rats on a coconut oil-supplemented diet are similar to those described by Gerson *et al.* (1964) with rats on a low-fat diet. Further, the evidence supports the hypothesis that there is an increase in biosynthesis of fatty acids and cholesterol and that the decreases in total weights and in the concentrations of these substances in the tissues result from an even greater increase in oxidative degradation.

A comparison of the present results with those for the low-fat diet (Gerson *et al.* 1964) reveals that there is no difference in the patterns of food intake

and weight change. However, on the fat-supplemented diet the weight loss (8.9 g./rat) was much smaller and roughly equal to the mean loss of body lipids (9.2 g.; Table 1), whereas in animals on a low-fat diet only about 50% of the weight loss was accounted for by loss of body lipids. This observation may be explained by the protein-sparing action of dietary fat, a subject that has been discussed by Deuel (1954). In addition in the presence of dietary fat the loss of lipid was confined primarily to the carcass, i.e. muscle and adipose tissue, during β -sitosterol treatment.

The present experiment was carried out under conditions as nearly identical as possible with those used by Gerson *et al.* (1964) for their studies on the effects of β -sitosterol on a low-fat diet. Comparison of these experiments showed that the coconut oil-supplemented controls had 505.3 mg. of total cholesterol/rat whereas the low-fat controls had only 445.7 mg./rat. Thus the dietary fat resulted in an increase of 59.6 mg. of cholesterol/rat. However, the administration of β -sitosterol diminished this increase to 14.8 mg./rat, the residual difference being accounted for mainly by the fact that the intestinal cholesterol in the experimental animals increased by 12.3 mg./rat.

A comparison of the effect of β -sitosterol on total cholesterol concentrations in the low-fat and fat-supplemented rats reveals that in the aorta coconut oil increased the concentration by 135 mg./100 g. fresh wt. of tissue (i.e. 78%) and that this increase can be confined by β -sitosterol to 46 mg./100 g. fresh wt. of tissue (i.e. 27%). This residual difference is due solely to free cholesterol, the concentration of esterified cholesterol having been diminished by β -sitosterol to that found in the controls on a low-fat diet. Trends similar to those described for the aorta can be found to apply to carcass, adrenals and adipose tissue (Table 3). In liver and plasma the effect of β -sitosterol on cholesterol concentrations appears to be greater in the presence than in the absence of fat in the diet.

Specific activities of fatty acids in rats on a fat-supplemented diet were found to be much smaller

than on a low-fat diet, in agreement with the findings of Hill, Webster, Linazasoro & Chaikoff (1960) as well as Reiser, Williams, Sorrels & Murtz (1963). On the other hand, in the presence of dietary fat the increase in $^{14}\text{CO}_2$ expiration was very much greater than in its absence.

The evidence described above shows that in the presence of dietary fat β -sitosterol helps to maintain lipid and cholesterol concentrations and weights at levels similar to those found in animals on a low-fat diet. This is particularly important within the aorta, which appears to be more sensitive to the presence of dietary fat than any other tissue investigated in this work. The evidence further supports the mechanism suggested by Gerson *et al.* (1964) for the effect of injected β -sitosterol on lipid and cholesterol metabolism.

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