

Relative Failure of Saturated Fat in the Diet to Produce Atherosclerosis in the Rabbit

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ABSTRACT

Three "saturated" fats of vegetable origin were fed to different groups of rabbits for periods up to 1 year. Cocoa butter and a hydrogenated vegetable oil shortening produced no hypercholesterolemia. Coconut oil feeding increased the serum cholesterol concentrations for 4 months, but a decline to baseline values occurred after 6 months. No gross atherosclerosis occurred in any animal fed coconut oil or the hydrogenated vegetable oil shortening. Slight atherosclerotic lesions were found in 50% of the rabbits fed cocoa butter. Aortic cholesterol content was slightly increased in animals fed coconut oil and cocoa butter. Dietary fats, even when highly saturated, had only a minimal capacity to produce atherosclerosis in the rabbit, a species usually highly susceptible to the induction of atherosclerosis. When a moderate amount of cholesterol was added to the diet, the serum cholesterol levels increased greatly and considerable atherosclerosis resulted.

ADDITIONAL KEY WORDS cocoa butter serum cholesterol
hydrogenated vegetable shortening dietary cholesterol and atherosclerosis
liver cholesterol aortic cholesterol coconut oil

■ The question asked in this investigation is: will saturated fat in the diet produce hypercholesterolemia and atherosclerosis in a susceptible animal species? This question is important because many proposals have been made to reduce the content of saturated fat in the American diet in order to prevent atherosclerosis and coronary heart disease (2-4). Ever since Anitschkow demonstrated in 1912 that atherosclerosis developed in animals fed cholesterol, the experimental animal model has been utilized to evaluate the role of dietary substances in the causation of atherosclerosis (5). However, the evidence from animal experiments to support the idea that

saturated fat produces atherosclerosis is not conclusive (6, 7). In some instances, the feeding of semisynthetic diets containing saturated fat has induced hypercholesterolemia and atherosclerosis, but the experimental animals generally failed to grow, became unhealthy, and may have suffered from some dietary deficiency (8-11).

In the experiments to be reported here, we fed diets rich in saturated fat to rabbits for periods up to 1 year. We selected rabbits for study because this species usually is highly susceptible to the development of experimental atherosclerosis. These animals were fed a diet of natural foods complete in all nutrients, and to this was added one of three "saturated" fats: cocoa butter, natural coconut oil, or a hydrogenated vegetable oil shortening. None of these fats contained cholesterol. All animals remained healthy for the duration of the study.

Materials and Methods

Four groups of white adult male New Zealand rabbits were fed different diets (Table 1). Six control animals were given only Purina rabbit

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TABLE 1
Groups of Animals According to the Fat Added to Diet

Diet	No. of Animals	Duration of feeding (months)	Fat content of diet	
			By weight (%)	% of total calories
Rabbit chow*	6	12	4.4	10.6
Cocoa butter	12	11	18.5	44.8
Coconut oil	10	6	18.5	44.8
Hydrogenated vegetable shortening†	4	8	18.5	44.8
Hydrogenated vegetable shortening plus 0.25% cholesterol	4	4½	18.5	44.8

*Ralston Purina Company.

†Crisco (Proctor and Gamble Co.).

chow for 12 months. This chow had a fat content of 4.4% by weight. In the three experimental groups of animals, different saturated fats were incorporated in the diet of chow, so that the added fat supplied 18.5% of the diet by weight or 44.8% of the total calories. The saturated fats fed were cocoa butter (the triglycerides of chocolate), coconut oil, and a partially hydrogenated vegetable oil shortening (Crisco). The fat-feeding periods ranged from 6 to 12 months. Except as specifically mentioned, all diets were free of cholesterol. The addition of saturated fat was the only variable. Finally, in a subgroup of animals, 0.25% crystalline cholesterol by weight was added to the diet containing the hydrogenated vegetable shortening.

The iodine numbers of the three saturated fats were quite low; for coconut oil, 9; for cocoa butter, 36; and for the hydrogenated vegetable shortening, 69 (Table 2). Sixty percent of the fatty acids of the coconut oil were saturated short-chain fatty acids. Each of the three fats contained some polyunsaturated fatty acid as linoleic acid, which, when added to the 1.8% total of linoleic acid already present in the chow, provided ample essential fatty acid for adequate nutrition.

The different diets were prepared by dissolving

the specified fat in ethyl ether. This solution was mixed thoroughly with the chow, and the ether was then completely evaporated from the chow. With this technique, the requisite amount of fat was impregnated uniformly into the pellets of chow. The rabbits readily ate chow so prepared. In the subgroup of animals given cholesterol as another addition, the cholesterol was dissolved in the ether-fat solution and the entire solution was mixed with the chow pellets.

Serum cholesterol determinations were made monthly by the method of Abell et al. (12). At autopsy, the entire aorta was graded 0 to 4 for visible atheromatous changes of the intima. The grading was done without knowledge of the experimental regimen of the animals. The sterol content of the aortas was measured in terms of Liebermann-Burchard positive material. A uniform length of aorta from aortic valve to the bifurcation of the abdominal aorta was excised and extraneous tissue removed. The specimen was dried at 105°C until it reached a constant weight. It was then ground to a fine powder and extracted in 20 parts 2:1 chloroform-methanol overnight. At the end of this extraction, the mixture was brought to a boil. The residual material was extracted two more times to make certain that all of the sterol was obtained. The

TABLE 2
Fatty Acid Composition and Iodine Value of the Dietary Fats Fed to Different Groups of Animals

Diet	Saturated fatty acids (% of total fat)							Unsaturated fatty acids (% of total fat)				Iodine value
	< C10:0	C10:0	C12:0	C14:0	C16:0	C18:0	Total	C18:1	C18:2	C18:3	Total	
Rabbit chow ¹			0.7	0.4	18.0	4.0	23.1	26.3	42.8	7.5	76.3	122
Cocoa butter ²					24	36	61*	33.7	3		37	32
Coconut oil ²	6.2	8.4	45.4	18.0	10.5	2.3	91	8.4	0.4		9	9
Hydrogenated vegetable shortening ³			3.5†	1.2	14.3	13.0	32	59.0	8.7		68	69

*Includes 1% of C20:0.

†< C14.

¹Fatty acid analysis by the Woodson-Tenent Laboratories, Memphis, Tenn.

²Fatty acid composition derived from reference 21.

³Fatty acid analysis from the Proctor and Gamble Co.

chloroform-methanol extracts of the aortas were evaporated to dryness and sterol content measured by a modification of the method of Abell et al. (12). The cholesterol content of the liver was determined by the same procedure. Standard methods were used for statistical analyses (14).

Results

In rabbits fed cocoa butter, serum cholesterol concentrations remained low during the entire 12-month feeding period (Fig. 1). Al-

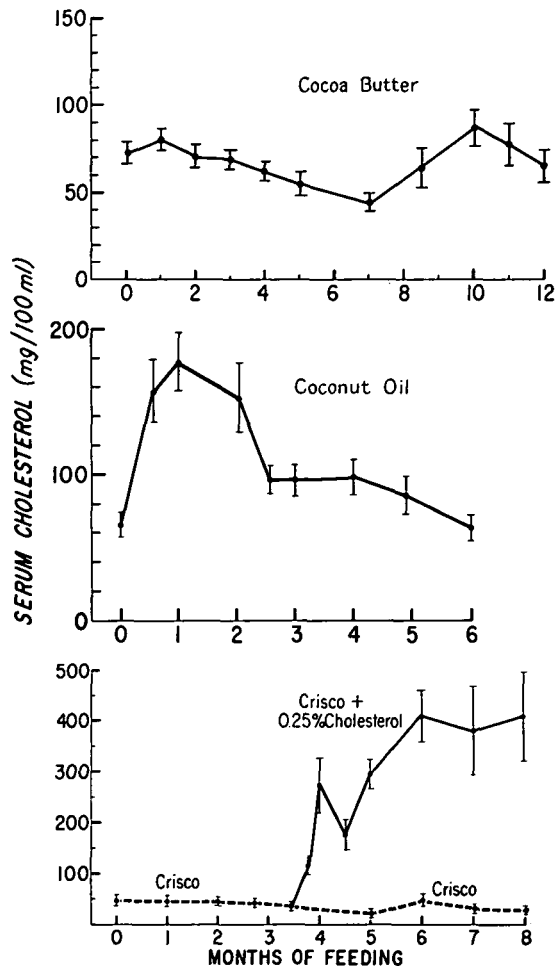


FIGURE 1

Mean serum cholesterol values of 12 rabbits fed cocoa butter for 12 months, 10 rabbits fed natural coconut oil for 6 months, and 8 rabbits fed a partially hydrogenated vegetable oil shortening for 8 months. Four of these 8 rabbits were also fed 0.25% cholesterol beginning at 3½ months. Standard errors of the mean values are indicated by the vertical lines.

though there was some fluctuation from month to month, the final mean value of 73 mg/100 ml was nearly identical to the initial level.

The consumption of coconut oil led to a rise in the serum cholesterol level to a maximum of 177 mg/100 ml after 1 month (Fig. 1). With continued feeding of coconut oil, however, an adaptation occurred. By 6 months, the serum cholesterol levels had declined to the pretesting values.

None of the 8 rabbits fed hydrogenated vegetable shortening had hypercholesteremia during the first 3½ months (Fig. 1). When cholesterol was added to the diet of 4 of these rabbits, serum cholesterol rose promptly, and its mean value at the end of the 8-month study period was 391 mg/100 ml. As shown in Figure 1, the cholesterol levels in the other 4 rabbits fed only vegetable shortening remained low.

At autopsy, the animals fed these different saturated fats had either little or no atherosclerosis (Table 3). No gross aortic lesions were found in the control animals or in those fed coconut oil or hydrogenated vegetable shortening. Several small fatty streaks and small discrete plaques were seen in 6 of the 12 rabbits fed cocoa butter; the average grade was 0.4. These lesions were in the ascending aorta and in the arch of the aorta. However, the very potent effect of a small amount of dietary cholesterol was observed in the rabbits to whose diet 0.25% cholesterol was added. In these animals atherosclerosis with a mean grade of 1.5 involved the entire length of the aorta.

The average cholesterol content of the aorta generally paralleled the gross grade of atherosclerosis (Table 3). There were slight but significant elevations in the cholesterol content of the aortas of rabbits fed coconut oil or cocoa butter. The rise in aortic cholesterol content was far greater when a moderate amount of cholesterol was added to the diet. For comparison, the data of another study (15) in rabbits fed 0.25% cholesterol and 44.8% of the total calories as corn oil are also included in Table 3. These animals had final

TABLE 3

The Effects of Saturated Fat Feeding on Aortic Atherosclerosis and Aortic and Liver Cholesterol Content

Diet	Gross aortic atherosclerosis (0-4)	Aortic cholesterol* (mg/g)	Liver cholesterol* (mg/g)
Control chow	0	3.55 ± 0.17	9.54 ± 0.21
Cocoa butter	0.4	4.52 ± 0.33†	15.00 ± 0.74†
Coconut oil	0	5.18 ± 0.16†	
Hydrogenated vegetable oil shortening	0	2.43 ± 0.30†	
Hydrogenated vegetable oil shortening and 0.25% cholesterol	1.5	18.26 ± 10.75†	
Corn oil and 0.25% cholesterol§	1.9	21.30 ± 1.92†	86.90 ± 37.9†

*Mean ± SE.

†Significant difference from diet I, control rabbits ($P < 0.01$).

‡All individual values were greater than the control aortic cholesterol content.

§Results derived from reference 15.

serum cholesterol levels of 596 mg/100 ml. They developed gross atherosclerosis of grade 1.9 and had comparably elevated aortic cholesterol content. Liver cholesterol was somewhat increased in the rabbits fed cocoa butter and greatly elevated in the rabbits fed cholesterol plus corn oil.

Animals fed these different saturated-fat diets were healthy and active for the duration of the experiment. Their fur remained in good condition. Weight gain occurred steadily in all animals; adiposity was found at autopsy. For example, animals fed cocoa butter gained a mean weight of 1,017 g over the 12-month period of feeding. The control rabbits fed only chow without added fat had a similar mean weight gain of 997 g.

Discussion

In this study, the feeding of large quantities of saturated fat to rabbits for long periods failed to produce either a sustained hypercholesterolemia or more than slight atherosclerosis. The saturated fats tested in these experiments were completely free of cholesterol, and thus the effects of dietary triglycerides could be evaluated independently. We avoided the "saturated" type of fats derived from animal sources (i.e. butter or lard) in the study because such fats always contain cholesterol, which is itself a known

atherogenic substance. The amount of fat fed was 44.8% of the total calories, a greater amount than has usually been used in animal experiments of the same character (6).

Coconut oil did cause an initial mild hypercholesterolemia, which completely subsided with continued feeding. Perhaps this response occurred from the effects of short-chain fatty acids. Fatty acids of the chain length C12 and under are transported from the intestine via the portal blood to the liver instead of reaching the systemic venous circulation via the lymph as chylomicrons, the transport form of the absorbed long-chain fatty acids. Coconut oil does induce hypercholesterolemia in man (16). The periods of feeding have usually been 60 days or less, so that there is the possibility that even man might eventually adapt to coconut oil feeding.

It is instructive to consider those rabbit experiments in which the feeding of fat alone has been emphasized as a cause of atherosclerosis. Typically, investigators have used a semisynthetic diet in which varying amounts and types of fat have been incorporated (8-11, 17). Malmros and Wigand found that their basal fat-free semisynthetic diet caused an average hypercholesterolemia of 200 mg/100 ml (9). The incorporation of hydrogenated coconut oil caused an average serum cholesterol rise to more than 500 mg/100 ml

(10). Diets in which corn oil or rapeseed oil was incorporated resulted in only slight elevation of serum cholesterol above baseline values. Atherosclerosis has occurred with these semisynthetic diets whenever fat has been absent or essential fatty acids have been deficient, as with the feeding of hydrogenated coconut oil (8-11). As has already been stressed, rabbits fed such diets do not thrive, lose weight, and appear unhealthy. In the study of Funch, Krogh, and Dam, the vegetable oil margarine (iodine number of 69-75) added to a semisynthetic diet did contain sufficient essential fatty acids (17). Only slight atherosclerosis occurred in some animals.

In other studies the dietary fat has been added to the usual rabbit chow, as in our experiments. Steiner and Dayton found minimal atherosclerotic lesions in the aortas of rabbits fed a diet with 40% peanut meal containing a large quantity of peanut oil for 1 year (18). Kritchevsky and co-workers also found only minimal atherosclerosis in a few animals from the feeding of either corn oil or a hydrogenated vegetable oil (19). Other investigators have also failed to note atherosclerosis from the feeding of various fats in the rabbit over periods up to 4 months (20). In our study low grade atherosclerosis occurred in 50% of the animals fed cocoa butter for 12 months. It would appear that the important factors in the production of minimal atherosclerosis in rabbits given a natural type of diet with added fat are the amount of fat given and the duration of the feeding. Whether the fat is largely saturated (our studies) or more unsaturated (peanut oil) would seem to be of little importance.

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