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Effect of Dietary Fat on the Release Rate of Cholesterol from Swine Erythrocytes (38077)

SHU-JEN CHANG YEH, TAKANOBU MIZUGUCHI, AND FRED A. KUMMEROW
The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois 61801

It has been shown that there is a rapid exchange of cholesterol between lipoprotein and erythrocytes (1–5) or between plasma and erythrocytes (5–7). Numerous reports have shown that the cholesterol content and fatty acid composition of plasma were altered by dietary fat. Furthermore, the fatty acid composition of the erythrocyte lipids was significantly influenced by the dietary fat (8–13). Since lipids are involved in the structure of the cell, it is possible that dietary factors may exert some influence on the function of erythrocyte membranes (13, 14). However, whether dietary fat influences the cholesterol exchange between plasma and erythrocytes is still unknown. The purpose of the present study was to evaluate: (1) the effect of dietary fat on the lipid composition of plasma and erythrocytes, and (2) the effect of dietary fat on the release rate of cholesterol from erythrocytes.

Materials and Methods. Six-month-old swine were divided into three groups of 12 each and fed different dietary fats for a period of 8 mo. The basal diet which contained 14.3% protein, 3.0% fat, and 72.7% carbohydrate was composed of ground yellow corn, solvent extracted soybean meal, and a mineral–vitamin premix. The corn oil group was fed the basal diet mixed with 17% corn oil; the hydrogenated fat group was fed the basal diet with 17% partially hydrogenated soybean oil which contained approximately 50% *trans* fatty acids (margarine base stock). Blood was obtained from the jugular vein by heparinized syringe. The plasma and erythrocytes were separated by centrifugation and the buffy coat was discarded. The erythrocytes were washed three times with saline solution (0.9% NaCl). Total lipids from the plasma and erythrocytes were extracted by the Folch procedure (15) using chloroform–methanol (2:1, v/v). Free and esterified cholesterol were determined on appropriate aliquots of the total lipid extracts by the method of Searcy and Bergquist (16). The fatty acid composition of the lipid moiety of the plasma and erythrocytes was determined by means of gas liquid chromatography (17). *Trans* fatty acids were also measured quantitatively by infrared absorption spectrometry of their methyl esters (18).

In order to obtain [^{3}H]-cholesterol-labeled erythrocytes, equal volumes of plasma and erythrocytes were mixed and incubated with ^{3}H-1,2α-cholesterol (0.2 μCi/ml blood) at 37°C in a metabolic shaker. The aqueous solution of cholesterol was prepared using Tween 80 according to the method of Meier *et al.* (19). After obtaining the [^{3}H]-cholesterol-labeled erythrocytes, these labeled erythrocytes were incubated with their own unlabeled plasma. Samples were withdrawn at each time interval and centrifuged to obtain plasma and erythrocytes. The erythrocytes were washed four times with cold saline solution (0.9% NaCl), the lipids extracted from both plasma and erythrocytes and the cholesterol content and its radioactivity determined. The release rate of cholesterol from the erythrocytes obtained from swine fed the different dietary fats were compared from the specific activity changes vs time.

Results. Both the free and esterified cholesterol content in the plasma were higher in those animals fed hydrogenated fat which

TABLE I. Effect of Dietary Fat on Cholesterol Content in Plasma and Erythrocytes.

Cholesterol content mg/100 ml	Corn oil	Basal	Hydrogenated fat
Plasma (total)	96 ± 5[a]	99 ± 8	129 ± 7
Plasma (free)	23 ± 1	24 ± 2	36 ± 3
Plasma (esterified)	74 ± 6	75 ± 6	94 ± 4
Erythrocytes	121 ± 5	122 ± 7	119 ± 3

[a] Mean for three samples ± SEM, each containing pooled blood from four swine.

contained *trans* fatty acids as compared to those either fed corn oil or the basal diet (Table I). However, the quantity of free cholesterol within the erythrocytes appeared to be constant regardless of the dietary fat. It is noteworthy that the cholesterol present in the erythrocytes was entirely in the unesterified form. The two different dietary fats stimulated considerable differences in the total fatty acid patterns of the plasma and erythrocyte lipids (Table II). Percentage of the 18:1 and 18:2 were changed most drastically in both the plasma and erythrocyte. The *trans* fatty acid content in the plasma from the swine fed hydrogenated fat were approximately 12% and was present mainly in the triglyceride and the cholesterol ester fractions. The amount of *trans* fatty acids in plasma from the swine fed corn oil or the basal diet was negligible. The content of the *trans* fatty acids in the erythrocytes from the swine fed hydrogenated fat was about 2%, none was present in the erythrocytes from the swine fed corn oil or the basal diet.

Incubation of ^{3}H-1,2α-cholesterol with whole blood resulted in a decrease in the specific activity of the free cholesterol in the plasma and the concomitant increase of specific activity of the erythrocyte cholesterol. However, the specific activity of plasma esterified cholesterol showed no change throughout the 8 hr incubation (Fig. 1). On the basis of these data, 4 hr incubations were carried out in subsequent experiments to obtain the [^{3}H]-cholesterol-labeled erythrocytes. After obtaining the [^{3}H]-cholesterol-labeled erythrocytes, they were incubated with their own unlabeled plasma. The data are indicated in Fig. 2. The specific activity of cholesterol in the erythrocytes decreased as the incubation time was increased, while the specific activity in the free and esterified cholesterol in the plasma increased. The free cholesterol of plasma readily exchanged with erythrocyte cholesterol. However, the esterified cholesterol exchange between erythrocytes and plasma was negligible. As the curve in Fig. 2 appears to be a first order reaction, the data which show the degree of equilibration between erythrocytes and plasma at each

TABLE II. Effect of Dietary Fat on Fatty Acid Composition in Plasma and Erythrocyte Lipids.[a]

Fatty acid[b]	Plasma			Erythrocyte		
	Corn oil	Basal	Hydrogenated fat	Corn oil	Basal	Hydrogenated fat
14:0	0.3 ± 0.0[c]	0.6 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	1.3 ± 0.4	2.3 ± 1.9
16:0	17.2 ± 0.5	22.4 ± 1.6	17.8 ± 0.1	30.6 ± 0.8	35.5 ± 2.9	30.6 ± 0.6
16:1	1.5 ± 0.1	2.7 ± 0.1	2.8 ± 0.2	trace	trace	trace
18:0	12.7 ± 0.8	12.4 ± 0.3	11.4 ± 0.3	15.0 ± 0.3	12.9 ± 0.2	12.0 ± 0.7
18:1	14.3 ± 0.6	21.7 ± 0.6	35.0 ± 1.0	24.5 ± 0.1	26.6 ± 1.9	35.4 ± 1.1
18:2	47.0 ± 1.2	33.1 ± 0.5	26.6 ± 1.0	26.0 ± 1.0	19.6 ± 0.2	16.5 ± 0.6
20:4	7.0 ± 0.3	7.1 ± 0.3	5.7 ± 0.1	3.5 ± 0.2	4.2 ± 0.5	3.0 ± 0.5

[a] Expressed as a percentage of the total fatty acids.

[b] Numbers before and after colon represent carbon chain length and number of double bonds, respectively.

[c] Mean for three samples ± SEM, each containing pooled blood from four swine.

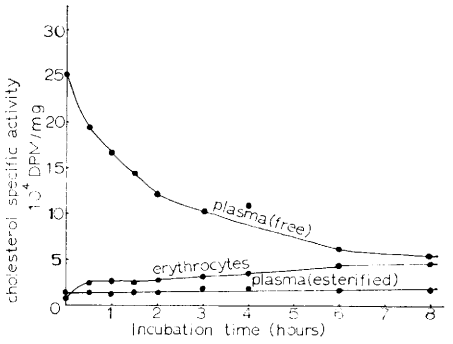

FIG. 1. Changes in specific activities of cholesterol in plasma and erythrocyte during incubation of [^{3}H]-cholesterol with whole blood. Each point represents the average of three determinations.

TABLE III. The Degree of Equilibration of [^{3}H]-Cholesterol Between Erythrocytes and Plasma vs Time.

Time (hr)	Corn oil	Basal	Hydrogenated fat
½	1.94 ± 0.09[a]	2.02 ± 0.09	1.76 ± 0.00
1	2.42 ± 0.07	2.69 ± 0.18	2.26 ± 0.06
2	4.53 ± 0.25	4.47 ± 0.46	3.14 ± 0.34
3	7.65 ± 1.07	7.60 ± 0.52	4.28 ± 0.35
4	—	—	6.22 ± 0.61

[a] Mean for three samples ± SEM. The degree of equilibration expressed as a/ (a − x) , "a" being the final equilibrium specific activity for each experiment and "x" being the specific activity at time "t."

time interval (Table III) can be plotted in exponential form. Plotting the degree of equilibration against incubation time on semilogarithmic graph paper was shown in Fig. 3. The release rate of labeled cholesterol from erythrocytes obtained from animals fed the different dietary fats can be compared. The calculated regression equations for equilibration between the erythrocytes and plasma from animals fed corn oil, basal and hydrogenated fat were $Y = 0.1574 + 0.2434x$, $Y = 0.1951 + 0.2286x$, and $Y = 0.1856 + 0.1518x$, respectively. The time required to reach 50% equilibration for the erythrocytes and plasma from those fed corn oil, basal diet, and hydrogenated fat was; 1.24, 1.32, and 1.98 hr, respectively.

Discussion. The present data indicate that the plasma cholesterol level was increased in swine fed hydrogenated fat which contained *trans* fatty acids. This effect was consistent with a previous report (20) in which rats fed *trans* fatty acids had higher serum cholesterol levels than those fed the corresponding *cis* fatty acids. McMillan *et al.* (21) also found that feeding elaidinized oleic acid to rabbits caused a greater concentration of total, free, and esterified cholesterol in the serum than did the feeding of natural oleic acid. However, the reason for

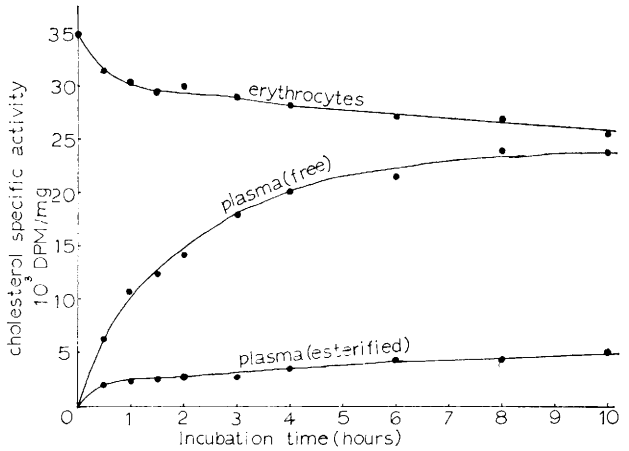

FIG. 2. Changes in specific activities of cholesterol in plasma and erythrocyte during incubation of [^{3}H]-cholesterol labeled erythrocytes with unlabeled plasma. Each point represents the average of three determinations.

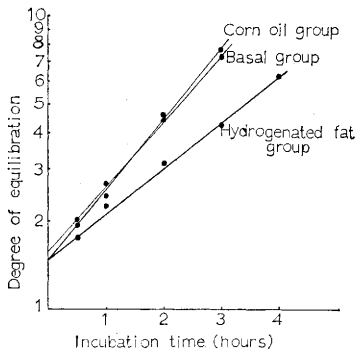

FIG. 3. The degree of equilibration of [^{3}H]-cholesterol between erythrocytes and plasma expressed as log a/a-x vs time. Each point represents the average of three samples for each time interval.

increasing plasma cholesterol levels in those fed *trans* fatty acids is still unknown. The amount of cholesterol in the erythrocytes was not subject to the influence of dietary fat as has been shown by Monsen *et al.* (22) and Walker and Kummerow (23). This was also true for the present study which indicated that swine fed different dietary fats had the same amount of cholesterol in the erythrocytes. However, the fatty acid composition of the erythrocytes was influenced by the dietary fat content.

It is generally assumed that all of the unesterified cholesterol of plasma can exchange with that of the erythrocytes. Bell and Schwartz (4) found that in swine the maximum exchange erythrocytes and lipoproteins, *in vitro*, occurred between 6–8 hr. Sardet *et al.* (5) have shown that the plasma cholesterol content influenced the interchange rate of plasma and erythrocytes in the guinea pig. It is possible that the higher plasma cholesterol level in those fed hydrogenated fat which contained *trans* fatty acids may cause a slower release rate of cholesterol from the erythrocytes. Differences in geometric configuration, in the chain length, and the degree of saturation of the fatty acid constituents in the erythrocytes may play a role in the functional mechanism of erythrocyte membrane. The *trans* fatty acids that are produced during hydrogenation are deposited in the tissue (24). Decker and Mertz (25) have reported the incorporation of dietary elaidic acid into lipids of rat erythrocyte stroma. The present study indicated that the *trans* fatty acid content in the erythrocyte obtained from swine fed hydrogenated fat was only 2% of the total lipids. However, the straighter spatial configuration of the *trans*-isomer in the erythrocyte membrane, although the amount was small may alter the permeability characteristics of the membrane.

The release rate of cholesterol from erythrocyte may depend on (1) the amount of cholesterol in the plasma, (2) the fatty acid composition of the erythrocyte, and (3) the presence of *trans* fatty acid in the erythrocyte membrane. These three factors may play a role in the slower release rate of cholesterol from the erythrocyte in swine fed hydrogenated fat which contained *trans* fatty acids.

Summary. Three groups of swine were fed for 8 mo the basal ration; the basal ration plus 17% corn oil or the basal ration plus 17% hydrogenated fat which contained 50% *trans* fatty acids (margarine base stock). The cholesterol content in the plasma was higher in those fed hydrogenated fat diet as compared to those fed the basal or corn oil. However, the cholesterol content in the erythrocytes appeared to be constant regardless of dietary fat. Different dietary fats resulted in considerable differences in the total fatty acid patterns of the plasma and erythrocyte lipids. The release rate of cholesterol from the erythrocytes obtained from swine fed the different dietary fats was compared from cholesterol specific activity changes vs time. The results indicate that the time required to reach 50% equilibration for the erythrocytes and plasma from those fed corn oil, basal diet, and hydrogenated fat was; 1.24, 1.32, and 1.98 hr; respectively. A possible explanation for the slower release rate of cholesterol in those fed hydrogenated fat was given.

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