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Relationship Between Utilization of Fat and Synthesis of Cholesterol and Total Lipid in Young Female Rats[1]

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Abstract

Young adult female rats were fed diets containing 2% of calories from corn oil plus 20, 40, 60 or 80% of calories as beef tallow or diets containing 2% corn oil and the calorie allowance restricted to 80, 60, 40 or 20% of ad libitum consumption. Incorporation of C^{14}-acetate into cholesterol and total fat was determined as an indication of rate of synthesis.

As dietary fat was increased there was a linear increase in cholesterol radioactivity, as measured in serum, liver and carcass. As calories were decreased there were small but significant increases in cholesterol radioactivity. There was a highly significant decrease in incorporation of acetate into total fat as dietary fat increased, and a decrease in total fat radioactivity when calorie intake was restricted. The differences in rate of cholesterol biosynthesis were not accompanied by differences in total quantity of cholesterol. The conclusion reached was that utilization of fat for energy results in accelerated cholesterol biosynthesis.

Introduction

WILSON AND SIPERSTEIN (1) have reported that excretion of cholesterol in the feces of rats was greater with 20% fat in the diet than with no fat, whether the fat was corn oil or lard; 30% fat resulted in even greater excretion of cholesterol. Polyunsaturated fats seemed to enhance excretion to a greater extent than saturated fats. The enhancement of cholesterol excretion by feeding fat to humans was reported by Haust and Beveridge (2), but corn oil was the only kind of fat fed. Unsaturated fat also has been reported to enhance synthesis of cholesterol to a greater extent than does saturated fat (3,4). It has been observed many times that unsaturated fat in the diet for a prolonged period results in lowered serum cholesterol in hypercholesteremic subjects, and Wilson (5) observed that feeding linoleic acid to rats resulted in slight lowering of carcass cholesterol. Apparently cholesterol excretion is enhanced more by unsaturated fat than is synthesis. Why cholesterol is synthesized and excreted in such large quantities has not been determined.

In a report by Dupont and Lewis (6) it was suggested that increasing utilization of fat, either from the diet or from body stores as necessitated by reduction of calorie intake, caused increased biosynthesis of cholesterol by young female rats. Corn oil and a lard-butter mixture had similar effects and the total amount of cholesterol in serum and liver did not increase.

The present study is an attempt to determine whether graded increments in dietary levels of beef tallow or calorie restriction of increasing degree result in proportional increases in synthesis of cholesterol in young female rats.

Experimental

Four groups of 30, 3-month-old, Specific-Pathogen-Free, female rats were obtained from Carworth Farms at 2-week intervals. On arrival the rats were caged singly and fed pathogen-free diet biscuits (D and G Research Animal Laboratory Diet, Dietrich and Gambrill, Frederick, Maryland) for 24 hr, then weighed and distributed into 10 groups of 3, such that body weight range was roughly similar in all groups. Four replications resulted in 10 groups containing 12 rats each. Three rats were lost for reasons unrelated to the experiment.

The rats were fed ad libitum diets containing 2 to 80% of the calories as fat for 4 weeks. At the end of 4 weeks groups 7 through 10 were transferred to restricted calorie diets and groups 1 through 6 were continued ad libitum on the same diets fed them for the first 4 weeks. Calorie intake of animals in groups 1–6 was not limited to a similar level because the effect of partial starvation upon some rats would have made it more difficult to interpret the data than does variation in calorie intake.

Composition of diets formulated to contain the same amounts of all nutrients except fat and carbohydrate per 100 calories is given in Table I. Factors of 4 calories per gram of carbohydrate and protein and 9 calories per gram of fat were used. Diets for groups 5 and 6 were exactly the same, providing 2 control groups.

Every 50 calories supplied by diets 1 through 5 and the daily average food allowance for groups 7 through 10 supplied 2 g protein, 262 mg cellulose flour, and 262 mg of vitamin mix per rat. Diets 1 through 9 supplied 0.50 g of salt mix and diet 10, 0.34 g. The lower amount in diet 10 was necessary to avoid an excessive concentration of salts in that diet. All diets supplied 60–70 mg of linoleic acid (calculated) daily, on the average. The corn oil was fed only as an approximate minimum source of essential fatty acids and was assumed to have little bearing upon metabolism of beef tallow, or body fat.

Calorie intakes of groups 7 through 10 were restricted during the fifth week as indicated in Table I. The rats were fed their daily allowance in 2 portions,

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TABLE I

Description of Groups and Composition (wt. %) of Diets

	Diets fed to groups 1–6 ad libitum for 5 weeks and 7–10 ad libitum for 4 weeks					Diets fed to groups 7–10 in restricted amounts for week 5			
Group	1	2	3	4	5–10	7	8	9	10
% Fat calories	80	60	40	20	2	—	—	—	—
Calorie allowance (% of ad libitum)	100	100	100	100	100	80	60	40	20
Calories per g (calculated)	6.48	5.48	4.75	4.20	3.82	3.76	3.71	3.57	3.44
Casein [a]	18.0	15.0	12.0	11.2	10.0	13.0	17.0	24.0	43.0
Lactalbumin[a]	8.0	7.0	7.0	5.6	5.0	7.0	8.0	12.0	21.0
Cornstarch[b]	6.5	33.0	52.3	67.2	78.0	71.0	64.0	48.0	12.5
Cellulose flour[a]	3.4	2.8	2.5	2.2	2.0	2.6	3.2	4.6	8.4
Salt mixture[a]	6.7	5.7	5.0	4.4	4.0	5.4	6.5	8.9	10.9
Corn oil[c]	1.7	1.4	1.2	1.1	1.0	1.3	1.6	2.3	4.2
Beef tallow[d]	55.8	35.1	20.0	8.2	0	0	0	0	0
Vitamin mixture[e]	+	+	+	+	+	+	+	+	+

[a] Casein, lactalbumin, cellulose flour (alphacel), and Jones and Foster salt mixture obtained from Nutritional Biochemicals Company, Cleveland, Ohio.
[b] Corn Products Refining Company, Philadelphia, Pa.
[c] Mazola, obtained on local retail market.
[d] Beef kidney fat obtained from the Animal Husbandry Division and rendered by heating the ground material in a double boiler until the fat melted, then straining it through cheesecloth.
[e] Providing per 50 calories (approximate daily consumption) of diets 1–6 and per daily allowance of diets 7–10: (in μg) thiamine.HCl, riboflavin, pyridoxine, 100; niacin, Ca pantothenate, 500; 2-methyl-1,4-naphthoquinone, folic acid, 40; biotin, 2; vitamin B_{12}, 0.6; calciferol, 1; vitamin A acetate, 30; (in mg) p-amino-benzoic acid, 10; inositol, 20; choline-Cl, 40; α-tocopherol, 1. Ascorbic acid was added to the vitamin mixture in the amount of 100 mg/kg as an antioxidant.

to avoid starvation periods of 24 hr. The experimental feeding period for all groups was 5 weeks. At the end of the fifth week C^{14} labeled acetate was administered. On the evening before acetate administration, food was removed from the rats being fed ad libitum. The next morning, after a fast of 10 to 12 hr, food was offered to each rat for 1 hr. Rats of groups 1 through 5 were allowed food ad libitum and those of groups 7 through 10 were fed one half their regular daily allowance. All rats consumed some food assuring that the time of the final meal prior to acetate administration was the same for each rat, a condition necessary for valid interpretation of acetate incorporation data (7). At the end of the hour food was removed and the rat injected intraperitoneally with 6 μc/100 g body weight Na-1-C^{14}-acetate (2.0 mc/mM) in physiological saline.

After 4 hr exposure to the tracer each rat was anesthetized by intraperitoneal administration of amobarbital. A blood sample was obtained by heart puncture and the liver was excised. The gastrointestinal tract was discarded.

Liver lipids were extracted by the Folch (8) procedure. Aliquots of the chloroform-methanol extract were dried and weighed to determine total fat, and C^{14} was determined in the total fat by counting in an automated beta detector (Sharp Wide-Beta, The Beckman Instrument Company). Self-absorption corrections were made. Cholesterol digitonide was isolated from the lipid extract by saponifying, extracting with petroleum ether (30–60C) and precipitating the digitonide by an adaptation of the Windaus method (9). Quantitative precipitation of sterols without excess digitonin precipitation was accomplished by dissolving the nonsaponifiable fraction in 90% ethanol at 60C and adding approximately a twice molar excess of digitonin dissolved in 50% ethanol. After allowing the samples to stand overnight at room temperature, the precipitate was deposited by suction on 3.2 cm circles of filter paper which had been washed with 90% ethanol, air-dried and weighed on a microbalance (Cahn Electrobalance). The precipitate was washed, dried and weighed in the same manner as the papers and the weight of digitonide multiplied by 0.24 to determine the weight of cholesterol. The digitonides plated on filter paper were then placed in aluminum planchets and C^{14} was counted as described above.

Carcasses were hydrolyzed by heating at 75–80C in 10% NaOH in 95% ethanol for 16 hr. The hydrolysate was made to a convenient volume with 50% ethanol and aliquots were extracted with petroleum ether. Cholesterol was determined in the same manner as in liver. Other aliquots were acidified with concentrated HCl and extracted 3 times with petroleum ether. Aliquots of the combined extracts were

TABLE II

Mean Calorie Intake, Carcass Weight and Fat, and Tissue Cholesterol in Relation to Dietary Variation

Group		Calorie intake[a]	Carcass		Cholesterol		
			Wt.	Fat	Serum	Liver	Carcass
	Dietary fat[b]		g	g	mg/100 ml	mg/liver	mg/carcass
1	80	52.9±1.8[d]	211±8	50.6	71± 3.2	13.6±0.72	189± 5.2
2	60	50.8±1.2	210±6	46.9	66± 5.9	13.4±1.26	181±10.2
3	40	45.3±1.1	206±3	44.6	61± 4.8	13.0±0.86	197±11.5
4	20	43.1±1.4	196±5	35.6	64± 4.5	11.6±0.94	210±11.8
5	2	43.0±1.3	197±3	38.9	74± 8.1	12.0±0.96	205± 7.4
	Calorie allowance[c]						
6	100	41.7±1.2	192±5	37.1	63± 5.2	12.7±0.78	197±12.5
7	80	37.2±0.8	193±4	33.4	64± 5.4	12.9±0.82	214± 9.7
8	60	28.7±0.6	184±3	28.8	46± 5.4	12.0±0.63	224±12.5
9	40	19.4±0.5	186±4	31.2	68±12.9	10.9±0.76	200± 8.1
10	20	9.8±0.2	177±4	27.6	53± 4.6	11.0±0.52	208±12.9

[a] Average daily, week 5.
[b] Percent of calories.
[c] Percent of ad libitum.
[d] Mean plus or minus standard error of the mean.

dried and counted and the remainder was dried and weighed to determine total fatty acids.

Serum was extracted by the Folch method and cholesterol digitonide was obtained in the same manner as for liver. The digitonides were counted for C^{14} but were not weighed because the gravimetric method is not accurate for microgram quantities. Cholesterol was determined with the Zak color reagent (10) using aliquots of the same sample prepared for digitonin precipitation.

Analysis of variance and regressions of various parameters upon dietary fat and calories were made using the least squares method of fitting constants. Results having a probability of less than 1% of being due to chance were considered significant.

Results

Cholesterol Method

Standard cholesterol precipitated with digitonin by the Windaus method and weighed under conditions of low humidity was quantitatively recovered (102–104%) with a similar degree of error at levels from 1.0 to 2.0 mg. Samples of less than 1.0 mg were less accurate. When humidity was higher than 25% it was necessary to use a controlled atmosphere apparatus (glove bag, Instruments for Research and Industry, Cheltenham, Pa.) to obtain quantitative recovery (94–97%).

General Effects of Diet

Effects of diet upon calorie intake, carcass weight and carcass fat are shown in Table II. The rats in groups 1–5 ate ad libitum and calorie intake was greater as concentration of fat in the diet increased. There was significant regression of carcass weight and carcass fat on calorie intake. Serum, liver and carcass cholesterol values are also shown in Table II; there were no significant differences related to diet among the 10 groups.

Cholesterol Biosynthesis

The effects of dietary fat and calorie restriction upon incorporation of acetate into cholesterol are shown in Table III and Figures 1 and 2. The biosynthesis of cholesterol was related to concentration of dietary calories from fat as shown by incorporation of C^{14}-acetate into cholesterol isolated from serum, liver and carcass of groups 1 through 5. The regression of total activity (per cent of injected dose) of cholesterol on grams of dietary fat per day was highly significant for all three tissues. The linear regression is illustrated for carcass cholesterol in Figure 1. Specific activity (total activity per gram cholesterol) followed a similar pattern, with regression on dietary fat significant for serum, liver and carcass cholesterol.

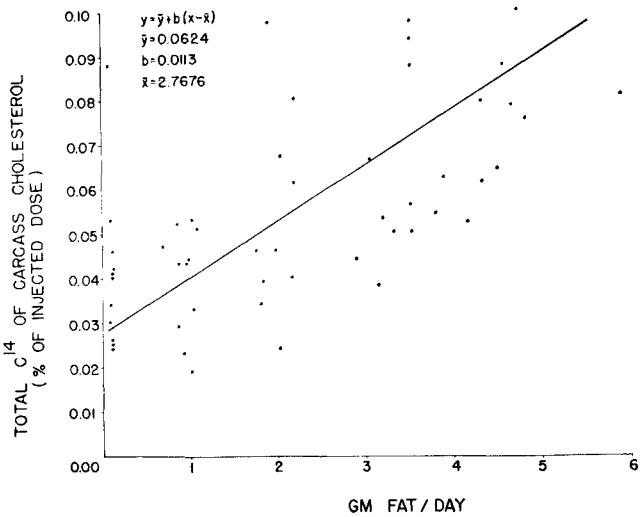

FIG. 1. Regression of total C^{14} of carcass cholesterol on dietary fat (groups 1–5).

As body fat was increasingly utilized for energy due to decreased calorie allowance (groups 6–10), acetate incorporation into cholesterol of carcass increased but did not increase in cholesterol of serum or liver. The inverse regression of total activity on calorie intake was significant for carcass cholesterol (Fig. 2) but not for liver or serum. The regression was slightly curvilinear with the greatest effect occurring in the group most severely restricted in calorie intake.

Total Lipid Biosynthesis

The incorporation of C^{14}-acetate into total lipids of liver and carcass was inversely related to amount of fat in the diet and directly related to calorie intake when calories were restricted (Table III). The regression of total activity of carcass fat on daily fat intake was highly significant and followed the curvilinear pattern shown in Figure 3. The regression for activity of liver fat was similar and also significant. It is apparent that the major effect of dietary fat was exerted between 0 and 2 g per day.

TABLE III

Acetate Incorporation into Cholesterol and Total Lipids in Serum, Liver and Carcass in Relation to Dietary Variations (Statistical analysis is shown in Figures 1–5)

Group		Cholesterol						Total lipid			
		Serum		Liver		Carcass		Liver		Carcass	
		TA[a]	SA[b]	TA[a]	SA[b]	TA[a]	SA[b]	TA[a]	SA[b]	TA[a]	SA[b]
	Dietary fat[c]	x10³		x10³		x10³					
1	80	2.4	3.5	52	3.8	80	0.43	0.26	0.66	0.91	0.019
2	60	3.1	5.2	51	4.0	75	0.43	0.49	1.30	1.09	0.025
3	40	1.2	2.0	30	2.4	51	0.27	0.38	1.10	1.70	0.040
4	20	0.68	1.0	20	1.8	44	0.20	1.59	5.18	6.28	0.169
5	2	0.82	1.2	22	1.7	41	0.20	2.80	9.46	9.30	0.300
	Calorie allowance[d]										
6	100	0.35	0.6	11	0.9	37	0.19	1.74	5.63	7.79	0.218
7	80	0.75	1.3	17	1.4	40	0.19	1.99	6.92	7.16	0.247
8	60	0.47	1.1	17	1.4	43	0.19	1.76	6.89	8.19	0.336
9	40	0.71	1.3	18	1.5	48	0.24	1.12	4.28	3.78	0.134
10	20	0.43	0.8	12	1.0	53	0.26	0.29	1.11	0.94	0.042

[a] Total activity (% of injected dose).
[b] Specific activity (total activity per gram).
[c] Percent of calories.
[d] Percent of ad libitum.

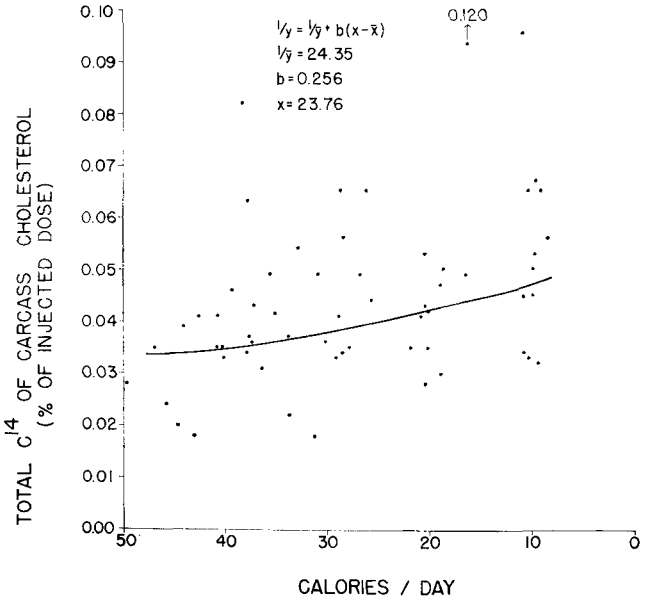

FIG. 2. Regression of total C^{14} of carcass cholesterol on calorie intake (groups 6–10).

Restriction of calories below ad libitum consumption resulted in decline of incorporation of acetate into total fat in carcass and liver at the lower levels of calorie intake. The regression of total activity of carcass fat on daily calorie intake is shown in Figure 4. The linear regression was highly significant but it is apparent that the portion associated with calorie intake of 50 to 30 is not actually linear. It appears that incorporation of acetate into carcass lipid bears little relation to calorie intake until restriction reaches approximately 50%.

Ratio of Cholesterol-C^{14} to Total Lipid-C^{14}

The use of the ratio of cholesterol-C^{14} to total lipid-C^{14} (total lipid includes cholesterol) as reported by Dupont and Lewis (6) eliminates much of the individual variation inherent in tracer studies using C^{14}-acetate. Presumably acetate for both pathways comes from the same pool and the calculation of the proportion going to the two products removes variation related to pool size, degree of mixing of the label and loss of labeled acetate during administration. A linear regression of this ratio upon dietary fat intake was found for carcass; 83% of the variance was accounted for by the regression. Liver and serum data were more variable than those for carcass. Figure 5 shows the regression of this ratio in liver upon dietary fat with confidence intervals. The comparable regression for serum was not calculated because of insufficient data for serum total lipid. It is apparent that data obtainable from liver or serum could not be used with confidence on an individual basis as indicators of lipid metabolism.

Discussion

It has been reported that rats fed diets containing fat synthesize more cholesterol than those fed diets containing little or no fat (3,6). The data presented herein showed that in young female rats there was a linear relationship between quantity of fat in the diet and synthesis of cholesterol. Decline in fatty acid biosynthesis as fat in the diet was increased has been reported (11, 12). The present data are in agreement with such observations and indicate, further, that the effect diminishes as dietary fat concentration in-

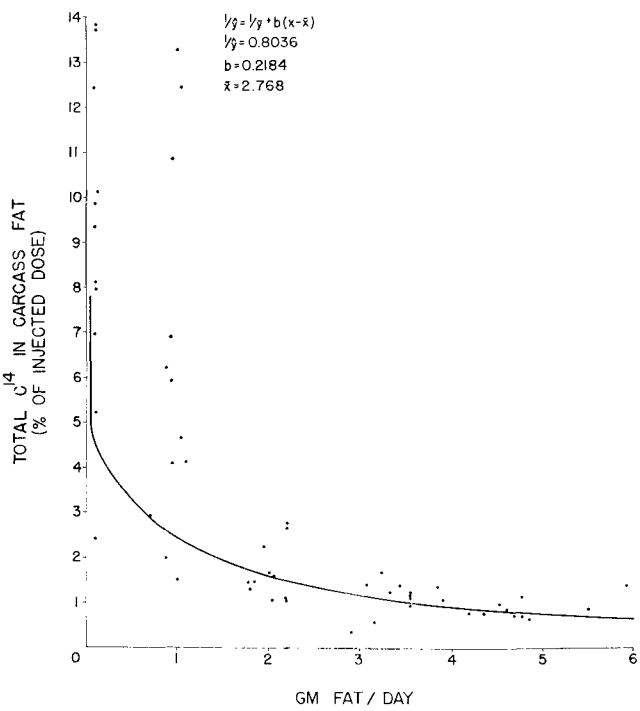

FIG. 3. Regression of total C^{14} of total carcass lipid on dietary fat (groups 1–5).

creases. The most pronounced difference occurred between the groups fed diets containing 2 and 20% fat calories.

The effects of calorie restriction on cholesterol biosynthesis found in this experiment (groups 7–10) differ from results reported by Hutchens et al. (13) where fasting for 18 to 240 hr was compared to ad libitum food consumption. In that study all fasted rats had less cholesterol radioactivity in the liver than did fed rats. In another study these investigators (14) found that cholesterol radioactivity from injected acetate declined in carcass for 72 hr of fasting, then began to rise and increased 25% between the 72nd and 120th hours of fasting. In the present experiment acetate incorporation into serum and liver cholesterol did not decrease significantly as calorie intake was decreased. It appears that utilization of body fat brought about an increase in cholesterol biosynthesis. Bloomfield (15) has reported a study from which it was concluded that cholesterol biosynthesis of rats fed ad libitum was proportional to calorie intake. Rats fed ad libitum in the present study had no significant regression of carcass cholesterol activity on calorie intake and when calories were reduced below ad libitum consumption the regression found was inverse. Fat content of the diet accounted for the regression in this study and could possibly have done so in Bloomfield's study.

Utilization of body fat affected fatty acid biosynthesis in a manner similar to the effect of dietary fat. Hill et al. (11) have shown that within an hour after dietary fat reached the liver, fatty acid synthesis was decreasing. Apparently lipid mobilized from body stores acts in the same manner as dietary lipid.

The question of acetate pool size must always be considered in interpreting effects of dietary treatment upon biosynthesis of any compound from acetate. Diller and Harvey (16) have recently reported a study in which safflower oil was fed to rats as 5, 10 or 20% of the weight of diet. They measured incorporation of acetate or mevalonate into cholesterol and

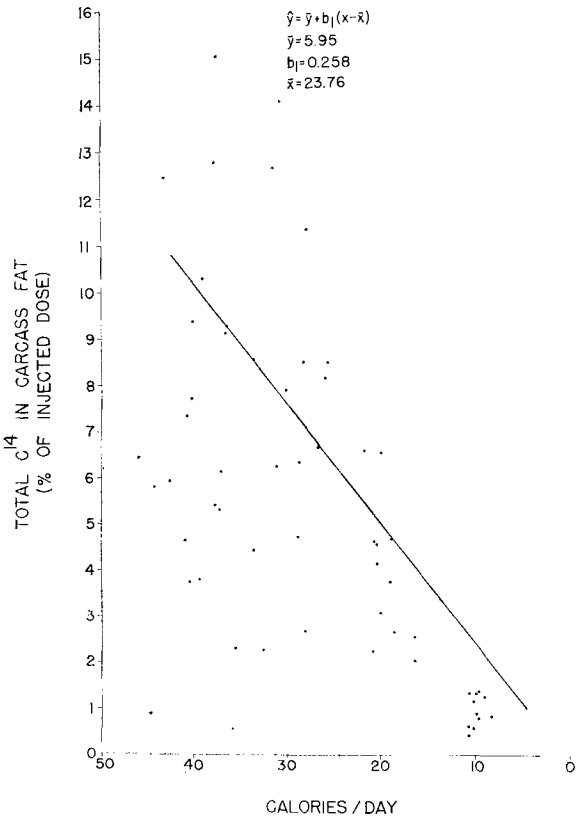

FIG. 4. Regression of C^{14} activity of total carcass lipid on calorie intake (groups 6–10).

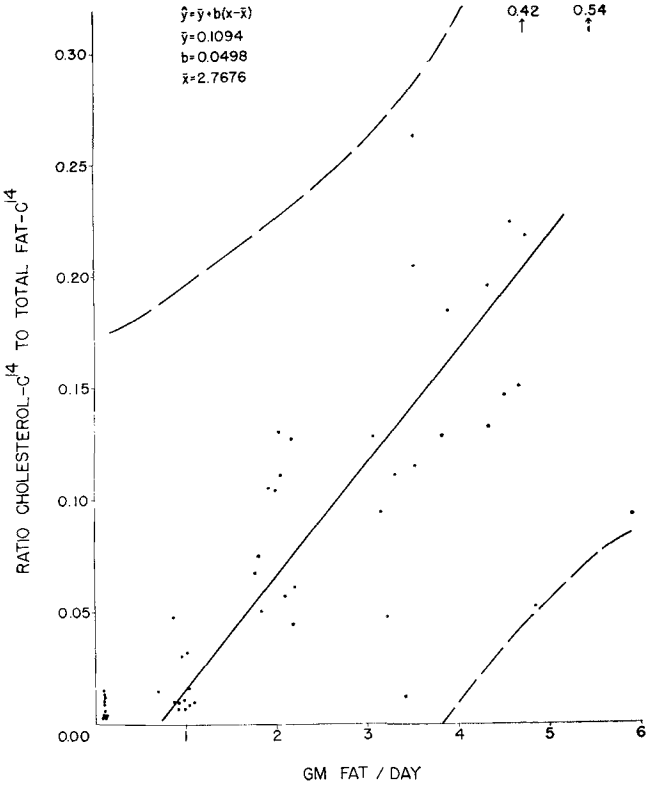

FIG. 5. Regression of the ratio of cholesterol-C^{14} to total lipid-C^{14} in liver upon dietary fat intake with 95% confidence intervals (groups 1–5).

fatty acids of liver slices from these rats. The results are in complete accord with those reported here, indicating that the results in the intact animal are a true indication of effects upon biosynthesis and not upon acetate pool size.

Factors other than dietary fat concentration have been reported to increase cholesterol biosynthesis. Diabetes (17) and pregnancy ketosis in guinea pigs (18) are two such factors. Both of those conditions involve utilization of fat for energy in lieu of carbohydrate and both result in ketone body formation and ketosis. Dietary fat utilization by the rat also results in ketosis (19). Apparently a relatively high level of serum ketones can be tolerated before ketonuria takes place (18,19). Williamson and Krebs (20) have reported that acetoacetate is preferred over glucose as a source of fuel for respiration in rat heart. Thus it appears that moderate ketosis is a normal part of the process of utilization of fat for energy.

Cholesterol biosynthesis may be related to utilization of fat for energy in the processes where the two have occurred concurrently. The only apparent connection between the two phenomena is the function of acetoacetyl-coenzyme A as a precursor to cholesterol. There is also a possibility that cholesterol has a function in utilization of lipids for energy. Maintenance of tissue cholesterol concentrations appears to be, normally, homeostatic in each individual and deviation from a constant concentration appears to be pathological. At present, it is difficult to define a normal range in parameters of lipid metabolism for individuals or population groups. Investigation of the metabolic functions associated with a possible relationship between cholesterol and utilization of lipids for energy is needed in order to establish definitions of normalcy in lipid metabolism. The responses to dietary and body fat utilization exhibited by the young female rats in this study would appear to be normal. If further studies show that under some conditions a point is reached where cholesterol biosynthesis does not vary with lipid utilization, it may be indicative of deranged cholesterol metabolism leading to increased concentration in serum. The variability between individuals in biosynthetic rates, however, makes it likely that only large differences in the rates can be interpreted as evidence of derangement.

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