Cholesterol Metabolism in Relation to Aging and Dietary Fat in Rats and Humans[1]

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ABSTRACT

A review of research in the authors' laboratories regarding effects of dietary fat polyunsaturation upon longevity in rats and some aspects of the regulation of cholesterol metabolism with regard to age of rats and humans is presented. The longevity of the rat was found to be enhanced by consumption of dietary fat providing a polyunsaturated to saturated fatty acid (P/S) ratio of 0.3 to 1, corresponding to about 5–12% of energy (en%) as linoleate, compared with less or more polyunsaturated fat. Mechanisms of the effects of the fats upon cholesterol metabolism were studied. With advancing age, there seems to be a decline in the rate of catabolism of cholesterol, resulting in longer retention in the body of the rat. In the human, there seems to be a decline in regulation of uptake of cholesterol by leukocytes and, therefore, perhaps other tissues, resulting in increased synthesis of cholesterol by the peripheral tissues. Moderate rather than high dietary consumption of polyunsaturated fat seems to be favorable to metabolic processes contributing to longevity. *Lipids* 20:825–833, 1985.

INTRODUCTION

There has been a continuing quest for sufficient information to know exactly how much and what kind of fat in the diet are most conducive to good health and long life. This review will discuss studies conducted in our laboratories on aging (1–3), which have been designed to determine an optimum dietary intake of essential fatty acids. Studies that have been conducted to determine possible mechanisms of dietary fat effects upon cholesterol and bile acid metabolism are included (4–6). Methods for those studies have been published in the papers cited.

For further insight into mechanisms of regulation of cholesterol metabolism, we have utilized the leukocyte as a model for metabolism in peripheral tissues. The original research conducted to verify the applicability of the leukocyte model is reported herein. Application of the use of the leukocyte was made in a human study of aging (7), and those results are included in this review.

MATERIALS AND METHODS

Animals

Male rats of the Sprague Dawley strain, weighing from 200–250 g initially, were used in the rat leukocyte experiments (SASCO, NE or ARS, Madison, Wisconsin). Lighting of the rat rooms was set so the dark phase occurred between 6:00 and 18:00 hr, and the light phase occurred between 18:00 and 6:00 hr, or vice versa. This facilitated experimentation with the highest diurnal enzyme activity and the lowest at the same time. All rats were fed ad libitum and given deionized water.

Diets

The composition of the four purified treatment diets is listed in Table 1. All diets contained 40% of energy (en%) as fat; however, the

TABLE 1

Composition of Diets used in the Rat Leukocyte Study

	BT	BT + CHOL	SO	SO + CHOL
	(Weight percent)			
Lactalbumin	19.00	19.00	19.00	19.00
Cornstarch	25.30	22.30	25.30	22.30
Sucrose	25.00	25.00	25.00	25.00
Cellulose	2.50	2.50	2.50	2.50
Vitamin mix[a]	2.00	2.00	2.00	2.00
Mineral mix[a]	5.00	5.00	5.00	5.00
Soybean oil (SO)[b]	4.29	4.29	20.52	20.52
Beef tallow (BT)[c]	16.19	16.19	0.68	0.68
Cholesterol (CHOL)[d]	0	3.00	0	3.00
P/S ratio	0.34	0.34	3.81	3.81

[a]Ref. 2.
[b]Nobel Supreme Salad Oil, Denver, CO.
[c]Litwak Rendering Co., Denver, CO.
[d]ICN Pharmaceuticals, Inc., Cleveland, OH.

[1]Presented at the AOCS meeting in Chicago, May 1983.

type of fat used in each diet was varied to yield a polyunsaturated to saturated (P/S) ratio as follows: predominantly beef tallow (BT), P/S = 0.34, and predominantly soybean oil (SO), P/S = 3.81. Half of the diets were made hypercholesterogenic (CHOL) by substitution of 3% cornstarch with cholesterol by weight. Addition of cholesterol to the diets was accomplished by first melting the appropriate fat and then dissolving the cholesterol in the melted fat (8). After cooling, the fat-cholesterol mixture was added to the diet. Each diet was kept frozen until it was fed to the rats.

TABLE 2

Cholesterol (CHOL) and Triglyceride (TG) Content of Diluted Sera used for Incubation of Leukocytes

	Lipid-full		Lipid-poor	
Diet[a]	CHOL	TG	CHOL	TG
	(mg/dl)			
BT	55	59	22	22
BT + CHOL	72	83	28	34
SO	55	72	26	32
SO + CHOL	58	83	24	32

[a]BT, diet containing 40 en% beef tallow and 2 en% soybean oil; SO, diet containing 42 en% soybean oil.

Preparation of Lipid-Poor and Lipid-Full Serum

Rats designated for serum collection were fed one of the treatment diets for five weeks. During the mid-dark phase of the lighting cycle, unfasted rats were anesthetized lightly with ether, and blood was drawn from the exposed jugular vein. Blood from three rats of the same diet treatment was pooled and allowed to clot. The serum was drawn off through siliconized Pasteur pipettes and frozen in siliconized glass test tubes. Both lipid-full and lipid-poor serum were prepared by modification of the procedure used by Fogelman et al. (9). The serum was thawed, then heated at 56 C for 30 min, to inactivate complement. The serum was then centrifuged at 10,000 *g* for 1 hr. Next, the serum was filtered with suction through Whatman No. 40 paper, and the filtered serum was divided into two parts. Half was designated lipid-full, and the other half was delipidated (designated lipid-poor) by the method of McFarlane (10) as modified by Popjak and McCarthy (11). The filtered serum was shaken with 0.25 volumes of diethyl ether, frozen in dry ice (−70 C), then thawed slowly at room temperature. The ether layer was removed and the extracted serum stirred. The extraction was repeated four times. Any ether remaining in the serum was removed by bubbling the serum with nitrogen gas for 1 hr at 4 C. Cholesterol and triglyceride contents of the diluted lipid-full and lipid-poor sera were analyzed (Table 2) by using a Technicon Autoanalyzer II Continuous Flow instrument.

Leukocyte Isolation and Incubation

At the appropriate time in the light cycle, a rat weighing from 375 to 425 g was anesthetized lightly with diethyl ether. The intestinal cavity was opened, and blood was drawn from the aorta into a plastic syringe containing 1.0 ml of 50 mM Na_2EDTA in 50 mM K_2HPO_4, pH 6.5. Then syringes were rocked gently to mix the anticoagulant with the blood, and a sample was taken by using a Unopette capillary pipette (Becton Dickinson) for the counting of leukocytes in whole blood by using a Neubauer hemocytometer. Following the method employed by Young and Rodwell (12), leukocytes were isolated by sedimenting the red cells in a starch polymer and centrifuging the leukocyte-rich supernatant. The leukocyte pellet was resuspended in an original volume of ice-cold Krebs-Henseleit buffer (K-H) or 90.2 volume K-H without bicarbonate when the incubation was performed in presence of 4 ml lipid-full or lipid-poor serum. The final volume was 5 ml and contained 20 mM [U-^{14}C] alanine or [1-^{14}C] acetate (approximately 6 μCi each, New England Nuclear, Boston, Massachusetts). The gas phase was 95% O_2:5% CO_2, and the incubation was conducted for 360 min at 37 C, in a gyrotory water bath shaker oscillating at 110 rpm (12).

Liver Slice Preparation and Incubation

Immediately after blood was drawn, the medial lobe of the liver was excised and placed in ice-cold 0.9% NaCl. Slices of 200 ± 50 mg were cut by using a Stadie-Riggs microtome. Each slice then was incubated under conditions identical to those of the leukocyte incubation, except the duration was 120 min.

Cholesterol Incorporation Rate

[^{14}C] Cholesterol was isolated as the digitonide from the liver slices or the total leukocyte incubation medium by previously published procedures (13). Rate of incorporation was corrected for boiled blanks and known losses of label during cholesterogenesis.

Statistical Procedures

Analysis of variance and planned com-

parisons of treatment means by the t-statistic were analyzed in each experiment by use of the Statistical Package for the Social Sciences (14) and Minitab (15) computer programs.

RESULTS AND DISCUSSION

Rat Aging Studies

We have conducted three rat aging studies comparing food fats differing primarily in polyunsaturation of the fatty acids. The three studies had different objectives and designs, and the only common parameter for comparison was mortality at 15 mo (Table 3). The study published in 1972 (1) showed that minimal linoleate (1–2% of energy, en%) in the diet was more conducive to early death than linoleate (from corn oil) at about 20 en% ($P < 0.05$ for both sexes; males greater than females $P < 0.05$). In the 1978 study (2), very few of those rats fed 3 or 11% linoleate (beef tallow plus soybean oil) had died by 15 mo, but excessive linoleate (safflower oil, 30 en%) was extremely deleterious ($P < 0.001$). With advancing age beyond 15 mo, the death rate for males, with 3 en% linoleate, exceeded that of those fed 11 en% in males (2).

A later study (Dupont, J., Mathias, M. M., Connally, P. T., and Bowen, R. A., unpublished data) again showed that moderate polyunsaturation (soybean oil plus beef tallow) was more conducive to longevity than high polyunsaturation. Those rats had a definite increase in mortality when linoleate was 20 en% (from corn oil) or 27 en% (from safflower oil) (Fig. 1). The net results suggest that extremes of availability of linoleate have a propensity to contribute to increased mortality.

Tissue Bile Acids

In the 1972 study, cholesterol retention in the carcass from a tracer dose was determined 10 days after injection (1). No striking effects were

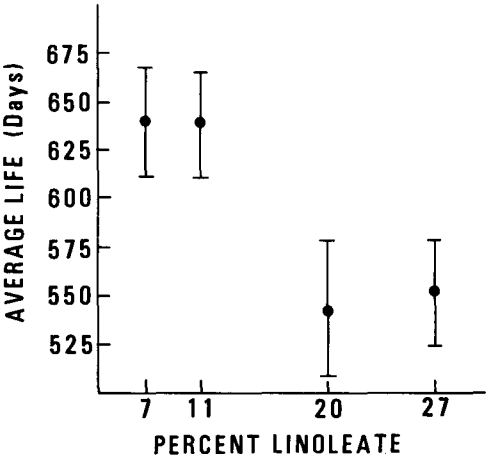

FIG. 1. Average life of male rats fed diets (details in Ref. 3) containing various concentrations of linoleate in mixtures of corn oil and beef tallow (linoleate = 7, 11 and 20 en%) or safflower oil (linoleate 27 en%).

TABLE 3

Mortality at 15 mo of Rats Fed Diets Containing Various Amounts and Kinds of Fat with Approximate Energy Content (en%) from Linoleate

		Linoleate (en%)						
		Low fat (2 en%)	High fat (40 en%)					
Study	Sex	1	2	3	7	11	20	27
		Percent dying spontaneously						
1972	M	46.2[a]	69.2[b]	—	—	—	16.7[a]	—
	F	0	36.4[b]	—	—	—	8.3[a]	—
1978	M	—	—	10.3[c]	—	7.0[c]	—	93.9[e]
	F	—	—	7.1[c]	—	0[c]	—	94.7[e]
1980	M	—	—	—	13.3[d]	6.7[d]	36.7[d]	42.1[e]

[a]Corn oil.
[b]Beef tallow + corn oil.
[c]Beef tallow + safflower oil.
[d]Beef tallow + soybean oil.
[e]Safflower oil.

seen in relation to diet, but an interesting finding was the retention of acidic steroids in nonhepatic or gastrointestinal tissues. From 15 to 43% of injected 4-^{14}C-cholesterol was recovered in an acidic lipid fraction of carcass. Additional measurements of that fraction were made in pigs and rats, and acidic steroid was found to be retained in many tissues. This led us to examine the composition of the acidic fraction. Thin layer chromatography (TLC) was the first method used, and several tissues were observed to have compounds similar to bile acids of liver and comparable to known standards (4). Samples from a number of tissues were analyzed by gas liquid chromatography (GLC) after deconjugation and derivatization to give quantitative data, and the compounds were shown by mass spectrometry to be cholanoic acids (5).

In recent years, we have repeated some of the earlier work by using high performance liquid chromatography (HPLC) (7). For these analyses, it is not necessary to deconjugate or derivatize the compounds, so the analyses are more likely to indicate the physiological state of the compound. The values for rat tissues are remarkably similar in total to those that we obtained by GLC analysis (Table 4).

In the 1978 aging study, we again measured retention of ^{14}C-cholesterol (3). Again, we saw an age effect and substantial acidic steroid in carcasses. Figure 2 illustrates the greater retention of cholesterol in older rats 28 days after a tracer dose. Figure 3 shows that, in rats 15 mo and older, 4–10% of the original dose was present in the carcass in an acidic form 28 days after administration. In the females, there was no effect of dietary linoleate concentration. In males, cholesterol retention was similar to that of females, but the higher linoleate diet resulted in less retention of acidic metabolites.

The next step was to investigate the possibility of local tissue synthesis of cholanoic acids rather than absorption from blood. We found 7α-hydroxylase activity in all the rat tissues examined (Table 5), indicating that the first necessary enzymic step was possible in the peripheral tissues.

The aim of the next experiment was to com-

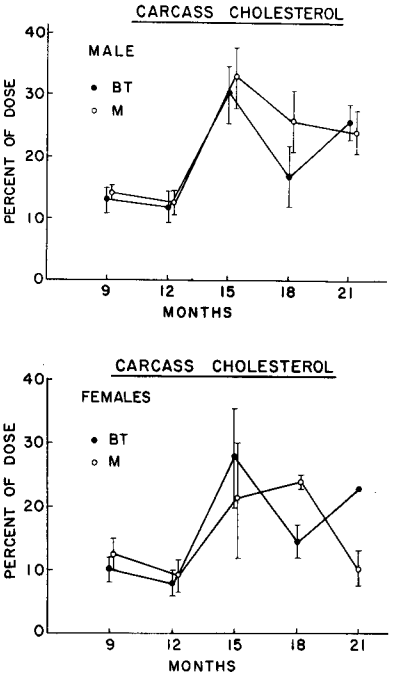

FIG. 2. Retention of 4-^{14}C-cholesterol in carcass cholesterol of aging rats (4). BT = diet containing 3% linoleate from beef tallow and safflower oil, and M = diet containing 11% linoleate from a mixture of the same fats.

TABLE 4

HPLC Quantitation of Cholanoic Acids[a] in Hepatic and Extrahepatic Tissues (7)

Tissue	Total	TC	TCDC	TDC	GC	GCDC	GDC
Rat (μg/g tissue)							
Liver	165	115	49.5	ND[b]	ND	ND	ND
Heart	13.9	9.7	4.2	ND	ND	ND	ND
Lung	11.8	4.4	7.4	ND	ND	ND	ND
Kidney	32.3	15.4	11.3	3.4	2.2	ND	ND
Adipose	5.2	2.4	3.4	2.4	ND	ND	ND
Human (μg/ml blood)							
Mixed leukocytes	1.5	0.49	0.13	ND	0.57	0.31	0.04

[a]TC, taurocholic; TDC, taurochenodeoxycholic; TDC, taurodeoxycholic; GC, glycocholic; GCDC, glycochenodeoxycholic; GDC, glycodeoxycholic acids.

[b]ND = Not detectable.

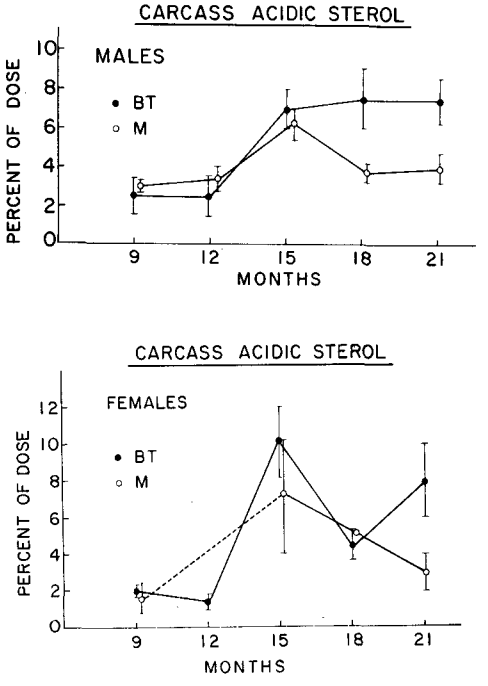

FIG. 3. Retention of 4-^{14}C-cholesterol in carcass acidic sterol of aging rats (4). BT = diet containing 3% linoleate from beef tallow and safflower oil, and M = diet containing 11% linoleate from a mixture of the same fats.

TABLE 5

Cholesterol 7α-hydroxylase Activity in Microsomes of Selected Rat Tissues (7)

Tissue	% Cholesterol conversion	nmoles/40 min
	(per mg microsomal protein)	
Liver	4.56	1.026
Heart	1.45	0.203
Lung	1.03	0.241
Kidney	1.34	0.378
Mixed leukocytes	0.247	0.113

TABLE 6

Incorporation of ^{14}C-labeled Alanine and Acetate into Cholesterol (Digitonin Precipitable Sterols) by Liver Slices and Leukocytes Taken from Rats Killed During the Mid-dark Phase (Means ±SEM)

Substrate	Liver (n = 8) (pm/min.mg)	Leukocyte (n = 5) (pm/min.10^{6}cells)
[U-^{14}C] alanine	1.32 ± .63	9.6 ± 1.4
[1-^{14}C] acetate	0.60 ± .09	5.5 ± 0.8

pare rat liver and leukocytes in regard to regulation of cholesterol synthesis. Preferred substrate, diurnal cycle, and effects of fasting, composition of dietary fats, and presence of lipid-full and lipid-poor serum were assessed. The ultimate goal was to characterize the regulation of leukocyte cholesterol metabolism to determine if it could be utilized to model human cholesterol metabolism in peripheral tissues.

Rat Leukocyte Study

Substrate preference. Table 6 illustrates the substrate incorporation rate into cholesterol (digitonin precipitable sterols) by rat liver and leukocytes. Both hepatic and leukocyte alanine incorporation exceeded acetate incorporation twofold. Results of this experiment advised use of alanine as the substrate of choice for the remaining physiological and dietary experiments.

Physiological variables. The rates of hepatic incorporation of alanine into cholesterol in response to lighting cycle and fasting are illustrated in Figure 4. Rats killed during the light phase demonstrated a 79% decrease in incorporation rate ($P < 0.005$) into cholesterol when compared with the dark-phase controls.

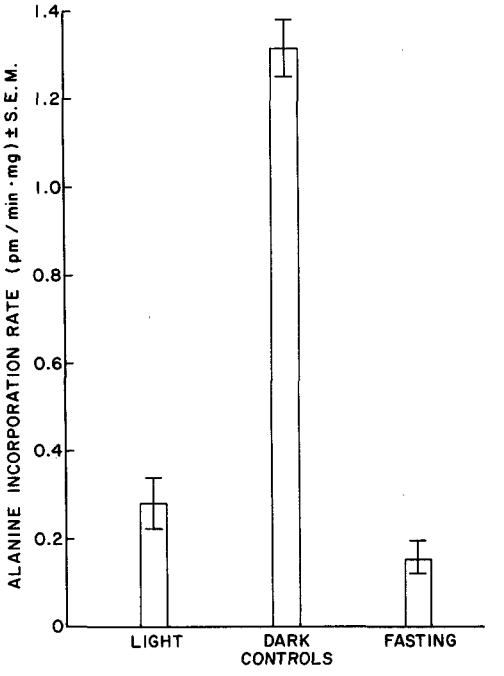

FIG. 4. Alanine incorporation into digitonin precipitable sterols by rat liver slices in response to light cycling and a 24-hr fast. Mean ±SEM (n = 8).

When rats were subjected to a 24-hr fast, substrate incorporation into cholesterol dropped significantly ($P < 0.005$) compared with the dark-phase controls (88%). Figure 5 shows the alanine incorporation by the leukocyte into cholesterol in response to light cycle and fasting. There were no significant differences ($P > 0.25$) in incorporation rates found for either physiological variable.

Serum and dietary incubation variables. The cholesterol and triglyceride contents of the lipid-full and lipid-poor sera, as used in the tissue incubations, are listed in Table 2. In the lipid-full sera, BT + CHOL had the highest cholesterol concentration (72 mg/dl), while the other sera contained the same amounts of cholesterol (55–58 mg/dl). The cholesterol concentration in the four lipid-poor sera were approximately equal (22–28 mg/dl). The triglyceride concentration averaged 74 mg/dl, and 60% was removed during preparation of the lipid-poor sera.

Figure 6 illustrates the effect of sera and dietary lipids on alanine incorporation into cholesterol by liver slices. In both the highly saturated fat (BT) and highly polyunsaturated fat (SO) diet groups, incubations of liver slices in lipid-full serum led to higher rates of incorporation than incubation in lipid-poor serum. Because only label appearing in hepatic sterols and not in the medium was determined, one explanation for these findings is that the lipid-poor serum enhanced release of cholesterol from the lipid slice into the medium. Dietary CHOL depressed hepatic cholesterol genesis as reflected by highly significant contrasts ($P < 0.001$) between groups incubated in lipid-full serum. There were no significant effects on the rate of incorporation by the saturation of fat in the diet.

Figure 7 illustrates results obtained from incubation of leukocytes under analogous variables, but in this case total sterol synthesis was assessed. There was a consistent pattern of increased ($P < 0.002$) alanine incorporation into cholesterol due to the presence of lipid-poor compared with lipid-full sera. This result is consistent with the known effects of feedback regulation of cholesterol synthesis. There was no effect of cholesterol feeding or saturation of fat on incorporation rates ($P > 0.25$).

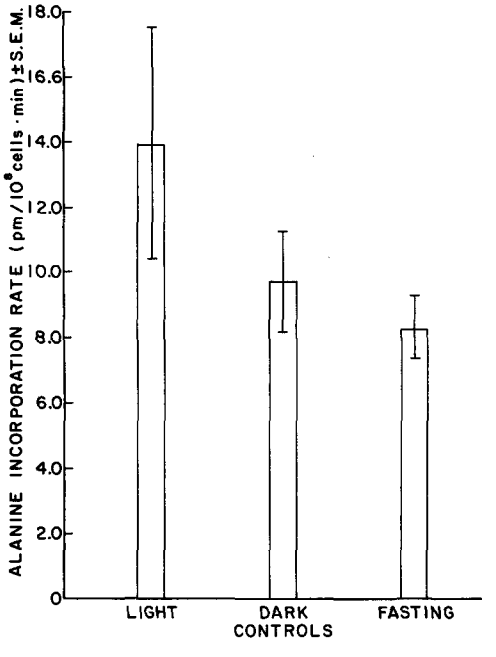

FIG. 5. Alanine incorporation into digitonin precipitable sterols by rat leukocytes in response to light cycling and 24-hr fast. Mean ±SEM (n = 5).

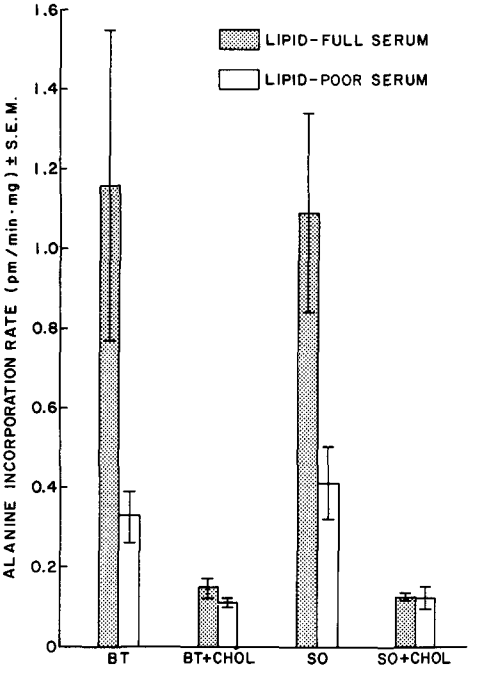

FIG. 6. Alanine incorporation into digitonin precipitable sterols of liver slices from rats of the four experimental diet groups. Liver slices were incubated for 2 hr in lipid-full and lipid-poor serum. Mean ±SEM (n = 8). BT, diet containing 19% by weight of beef tallow; CHOL, 3% by weight of cholesterol; SO, diet containing 19% by weight of safflower oil.

Human Studies

From the results with rat leukocytes, we concluded that leukocytes were more like peripheral tissues than like liver, and so chose them to study as a model for regulation of cholesterol metabolism in the human. We studied groups of healthy males of different ages (7). Their leukocytes contained bile acids (Table 7) and hydroxymethylglutaryl coenzyme A reductase (HMGR) and 7α-hydroxylase (COH) activities (Table 8). They also had bile acids in each lipoprotein fraction (Table 9). The men consumed similar diets (P/S = 0.4, cholesterol 300–400 mg/day) at all ages, so there were no dietary correlations. There were correlations of age with cholesterol metabolizing enzymes. Both enzyme activities increased with age and with LDL concentration. This finding in relation to LDL is contrary to the observations made in rats by using lipid-full compared with lipid-poor serum. Based upon present knowledge of mechanisms of LDL cholesterol metabolism, it seems that the rat leukocytes responded to decreased cholesterol by increasing their cholesterol synthesis. On the other hand, there may be a decrease in receptor activity in the human leukocytes with advancing age and, therefore, a failure in inhibition of cholesterol synthetic activity.

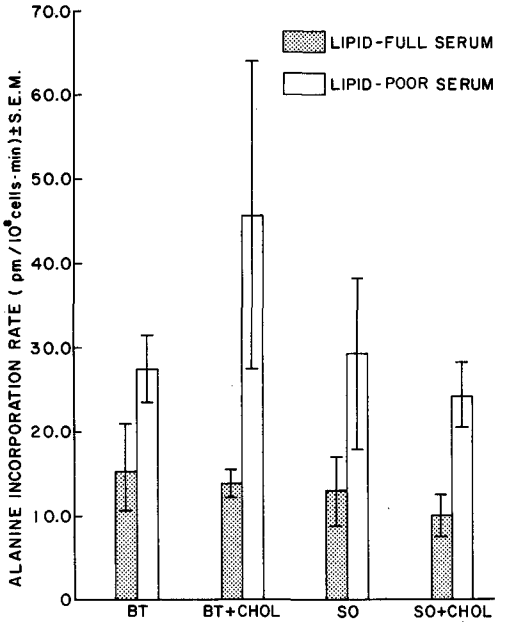

FIG. 7. Alanine incorporation into digitonin precipitable sterols by leukocytes from rats of the four experimental diet groups. Leukocytes were incubated for 2 hr in lipid-full and lipid-poor serum. Mean ±SEM (n = 8). BT, diet containing 19% by weight of beef tallow; CHOL, 3% by weight of cholesterol; SO, diet containing 19% by weight of safflower oil.

CONCLUSIONS

A dietary content of 3 to 11 en% of linoleate seems to be most favorable for longevity of rats, with males more susceptible than females to deleterious effects of lower levels.

Various reasons for differences in life span related to dietary fat have been discussed by others. Harmon (16,17) has studied and reviewed the interactions of polyunsaturated fatty acids and antioxidants in the diet. All the diets used in our studies had adequate quantities of α-tocopherol, so excessive free-radical

TABLE 7

Total and Differential Leukocyte Count and Cholesterol and Bile Acid Concentrations in Leukocytes of Healthy Men in Three Different Age Groups (Mean ±SEM) (7)

Variable	Age groups, yr		
	19–25 (n = 10)	40–50 (n = 9)	60–70 (n = 10)
Counts			
Total number/ml plasma	7194 ± 474	6494 ± 417	7816 ± 694
Leukocytes (%)	44 ± 2.7	40 ± 3.4	38 ± 2.9
Granulocytes (%)	56 ± 2.7	60 ± 3.4	62 ± 2.9
Cholesterol (μg per 10^8 cells)			
Total	101 ± 11.9	106 ± 14.3	135 ± 28.1[a]
Free cholesterol	78 ± 10.3	79 ± 10.8	100 ± 18.9[a]
Cholesteryl ester	23 ± 3.1	27 ± 3.8	35 ± 9.4
Bile acids (nmoles per 10^8 cells)	2.11 ± 0.16	2.16 ± 0.25	2.11 ± 0.18

[a]Significantly different ($P < 0.05$).

TABLE 8

Hydroxymethylglutaryl Coenzyme A Reductase (HMGR)[a] and Cholesterol 7α-hydroxylase (COH)[b] Activity in Mononuclear Leukocyte Microsomes of Healthy Men in Three Different Age Groups (Mean ±SEM) (7)

Enzyme activity expressed on basis of:	Age groups, yr[c]					
	19-25 (n = 10)		40-50 (n = 9)		60-70 (n = 10)	
	HMGR	COH	HMGR	COH	HMGR	COH
Per mg microsomal protein	10.5[a] ± 0.54	0.106[a] ± 0.0059	13.1[b] ± 0.81	0.107[a] ± 0.0051	14.4[b] ± 1.22	0.128[a] ± 0.0113
Per 10^7 mononuclear cells	30.4[a] ± 3.24	0.303[a] ± 0.0300	51.4[b] ± 6.52	0.419[ab] ± 0.0549	45.2[b] ± 4.10	0.401[b] ± 0.0386
Per ml blood	9.1[a] ± 0.49	0.091[a] ± 0.0054	12.2[b] ± 0.75	0.099[a] ± 0.0079	12.5[b] ± 0.77	0.110[a] ± 0.0069

[a]Enzyme activity was expressed as pmoles of mevalonate formed per hr.

[b]Enzyme activity was expressed as % of ^{14}C-cholesterol converted to ^{14}C-7α-hydroxycholesterol per 40 min.

[c]Means for each age group with different letter superscripts within the same row are significantly different ($P < 0.05$).

TABLE 9

Bile Acids[a] in Human Plasma Lipoproteins (7)

Lipoprtein	μg/dl	% of total steroid
VLDL	47.5	0.21
LDL	177.4	0.11
HDL	93.6	0.20

[a]Calculated on the basis of glycoholic acid mass.

formation does not seem to account for differences in longevity. Birt et al. (18) reported longer life span of Syrian hamsters when approximately 20 en% linoleate was fed compared with 10 or 5 en% (from corn oil). Other components of the diet also were varied. Possible involvement of eicosanoids has been discussed (2) and reviewed (19). A range from 2 to 15 en% linoleate should be tested more closely, but outside that range, combinations with other factors may result in reduction of life span, depending on other components of the diet.

We still do not understand the mechanisms of effects of dietary fat upon cholesterol metabolism. The regulation of cholesterol metabolism includes events in peripheral tissues that may include conversion of cholesterol to acidic steroids. Use of the leukocyte offers a good biopsy tissue for human studies. Responses of the young rat leukocyte to lipids in serum may be analogous to young humans. The decrease in ability of older men to respond to plasma cholesterol concentration may be an important mechanism for the rise in serum cholesterol with age (1,2,20,21).

The participation of bile acids in regulation of cholesterol metabolism in lipoproteins and peripheral tissues requires additional exploration. The coordination of HMGR and COH activities in leukocytes suggests a function for bile acids that could be analogous to its functions with cholesterol esterification enzymes (22,23).

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