JCI The Journal of Clinical Investigation

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E. P. Madhava Bhattathiry, Marvin D. Siperstein

J Clin Invest. 1963;42(10):1613-1618. https://doi.org/10.1172/JCI104846.

Research Article

Journal of Clinical Investigation
Vol. 42, No. 10, 1963

FEEDBACK CONTROL OF CHOLESTEROL SYNTHESIS IN MAN *

By E. P. MADHAVA BHATTATHIRY † and MARVIN D. SIPERSTEIN ‡

(From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas)

(Submitted for publication December 3, 1962; accepted June 27, 1963)

The ingenious balance studies of Schoenheimer and Breusch (1) first demonstrated that cholesterol metabolism in animals is under homeostatic control. It remained, however, for the isotopic experiments of Gould (2), Frantz, Schneider, and Hinkelman (3), and Tomkins, Sheppard, and Chaikoff (4) to prove conclusively that the feeding of cholesterol promptly suppresses hepatic cholesterol synthesis. The mechanism of this inhibition of cholesterol synthesis by dietary cholesterol has subsequently been studied extensively (5, 6) and has been shown to possess the characteristics of a negative feedback control system. Thus, in the sequence acetyl CoA → β-hydroxy-β-methylglutaryl CoA → mevalonic acid → cholesterol, a single reaction, the reduction of β-hydroxy-β-methylglutaryl CoA to mevalonic acid, has been shown to be the primary site of the depression in cholesterogenesis produced by exogenous cholesterol (7, 8).

It has also been demonstrated that this feedback system is operative in the livers of every species of experimental animal so far examined (2, 9, 10). Although it would thus seem likely that such a homeostatic mechanism would be present in human liver as well, no evidence for its existence in man has so far been reported. Furthermore, the only previous *in vitro* study of cholesterol synthesis in man (11) led to the unexpected conclusion that hepatic cholesterol synthesis is normally so low that such a feedback system in human liver could not play an important role in compensating for variations in dietary cholesterol. Because of certain objections to the conditions employed in the latter experiments and because of the physiological importance that such a feedback mechanism, if present, would have in controlling cholesterol synthesis in man, this problem seemed to warrant further study.

The present investigation was therefore carried out to determine the effect of dietary cholesterol on the rates of cholesterol synthesis in liver specimens obtained by biopsy from well-fed, unanesthetized human beings. The results clearly demonstrate that under the physiological conditions employed in this study, human liver synthesizes cholesterol at rates comparable to those seen in other animal species. Furthermore, we conclude that human liver does possess a negative feedback system for the control of cholesterol synthesis and that this mechanism is at least as sensitive as that previously noted in experimental animals.

METHODS

The subjects whose tissues were used in these studies were five women and eight men who were patients on the medical service of Parkland Memorial Hospital. Only patients from whom liver biopsies were to be obtained for diagnostic purposes were employed in this study, since we did not feel justified in obtaining such biopsies purely for experimental purposes. A variety of diseases had been diagnosed in these patients before biopsy (Table II), but six of the biopsy specimens were subsequently found to be histologically normal. Only patients who were in a good nutritional state and were eating the routine hospital diet were included in the study. Dietary records were kept of the food intake for 3 days before biopsy to insure that food consumption was adequate. One day's intake of the normal hospital diet was estimated to contain an average of 616 mg (range, 363 to 904 mg) of cholesterol. The high-cholesterol diet consisted of the regular hospital diet and three to four eggs per meal; the estimated cholesterol consumption of such patients was between 3 and 4 g per day. The low-cholesterol regimen consisted of a balanced diet that except for one lean, 90-g steak a day (68 mg cholesterol) was devoid of high-cholesterol foods. Patients on this diet consumed approximately 103 mg of cholesterol per day.

At the end of 3 days on one of the three diets, a percutaneous liver biopsy was performed with a Menghini

* This study was supported by grants from Dallas Heart Association, Texas Heart Association, and American Heart Association.

† Work done as U. S. Public Health Service trainee of the National Institutes of Health. Present address: Department of Biochemistry, Medical College, University of Saskatchewan, Saskatoon, Sask., Canada.

‡ Recipient of Research Career Award, National Heart Institute.

needle, with 1% Xylocaine (2-diethylamino-2',6'-acetoxylidide) as a local anesthetic. The patients ate their respective diets on the day of the biopsy. Between 1.8 and 10.9 mg of the liver tissue obtained was placed in cold Krebs-Ringer bicarbonate buffer at pH 7.4 for the determination of the rates of cholesterol synthesis. The remainder of the biopsy sample was cut in sections for diagnostic purposes.

In most cases, blood cholesterol levels were determined by the method of Sperry and Webb (12) at the time of liver biopsy, and in the case of the patients on the high-cholesterol diet, control blood cholesterol levels were also obtained before the initiation of the experimental diet.

Incubation procedure. In order to evaluate the rates of cholesterol synthesis on samples of liver weighing less than 11 mg, the usual *in vitro* procedure of this laboratory (13) was performed in miniature. All incubations were carried out in 12-ml glass-stoppered centrifuge tubes to which was added 0.4 ml of Krebs-Ringer bicarbonate buffer at pH 7.4. The liver tissue without further preparation was transferred to the centrifuge tubes, and 2 μmoles of sodium acetate containing 4 μc of acetate-1-C^{14} was added in 0.1 ml of water. The tubes were treated with 95% oxygen and 5% carbon dioxide and sealed with rubber serum caps. Two-ml capacity tubes made by cutting off the tops of 5-ml cellulose nitrate tubes [1] were suspended from the serum caps to facilitate the later collection of the $C^{14}O_2$ produced. The centrifuge tubes were then incubated at a 30° angle for 2 hours at 37° C in a Dubnoff metabolic shaker.[2]

Method of analysis. At the completion of incubation, 1 ml of 1 N sodium hydroxide was injected with a syringe through the serum cap into the plastic tubes, and 0.25 ml of 10 N sulfuric acid was injected into the incubation medium. The tubes were then shaken at 0° C for 30 minutes, the serum caps removed, and the sodium hydroxide transferred quantitatively from the plastic tubes to 10-ml volumetric flasks. After dilution to 10 ml, 1 ml of the sodium hydroxide solution was added to 15 ml of the scintillation solution described by Bray (14), and the C^{14} content was assayed in a Packard Tri-Carb liquid scintillation spectrometer.

For assay of cholesterol-C^{14}, 1 mg of unlabeled cholesterol was next added to each of the incubation tubes, and the cholesterol esters were saponified by addition of 0.75 ml of 90% (90 g per 100 ml solution) potassium hydroxide and autoclaved at 120° C for 1 hour. The contents of the centrifuge tubes were then transferred to 250-ml Erlenmeyer flasks by rinsing with water and absolute alcohol to give a final volume of 10 ml of a 1:1 alcohol-water mixture. After extraction of the nonsaponifiable material three times with 50-ml portions of petroleum ether, the pooled petroleum ether extracts were dried under nitrogen, and the cholesterol was isolated as the digitonide by the procedure of Sperry and Webb (12). The cholesterol digitonide was then dissolved in 2.2 ml of absolute methyl alcohol, 1 ml was added to 15 ml of a 1% diphenoloxazone and 0.1% bisphenyloxazolylbenzene solution in toluene, and its carbon^{14} content was assayed in the liquid scintillation counter.

The reproducibility of the assay of cholesterol synthesis by this micro technique was determined by carrying out the entire procedure on repeated liver biopsies obtained from a normal mongrel dog.

[1] Ivan Sorvall, Inc., Norwalk, Conn.

[2] American Instrument Company, Silver Springs, Md.

TABLE I

Synthesis of cholesterol in dog liver biopsy samples

Biopsy no.	Wt of tissue	Total cpm in isolated cholesterol	Cholesterol synthesis
	mg	*cpm*	*$\mu\mu$moles added acetate per mg tissue*
1	6.1	1,244	112
2	6.3	1,053	92
3	6.6	1,543	128
4	6.0	1,395	126
5	11.0	2,518	126

RESULTS

Reproducibility of the micro assay of cholesterol synthesis. The results of separate determinations of cholesterol synthesis in liver tissue obtained from five biopsies in a normal dog are shown in Table I. The rates of cholesterol synthesis in these biopsy samples averaged 116 $\mu\mu$moles of acetate-1-C^{14} converted to cholesterol per 1 mg liver and varied from 92 to 126 $\mu\mu$moles acetate per 1 mg liver. These values indicated a reproducibility considered to be very adequate for the purposes of this study, and the experiments on human liver biopsy samples were therefore undertaken.

Studies of cholesterol synthesis in liver biopsy samples from patients on a normal diet. As indicated in Table II, rates of cholesterol synthesis were measured on five patients who had eaten a balanced hospital diet for at least 3 days. Some indication of the reproducibility of the method of measurement of cholesterol synthesis in human liver is given by the two studies (Patients 1 and 2) in which duplicate samples of liver could be compared.[3] At this level of synthesis, a variation

[3] In the case of liver specimens from patients on the normal diet, the actual counts per minute found in the sample of synthesized cholesterol that was assayed averaged 777 cpm. Since the background count averaged 20 cpm and all samples were counted for 10 minutes, the

TABLE II

Negative feedback control of cholesterol biosynthesis in human liver

Patient	Age	Sex	Clinical diagnosis	Histologic diagnosis	Serum cholesterol: Before exp. diet	Serum cholesterol: At biopsy	Wt liver used for experiment	Conversion of acetate-1-C^{14} to: CO_2	Conversion of acetate-1-C^{14} to: Cholesterol
	years				*mg/100 ml*		*mg*	*μmoles added acetate/mg tissue*	
A. Normal diet									
1	56	♀	Primary biliary cirrhosis	Cirrhosis		335	6.7 6.2		216 218
2	39	♀	? Sarcoidosis	Normal		152	4.7 5.3		240 374
3	60	♀	Diabetes, hepatomegaly	Fatty metamorphosis		215	4.3	208	264
4	32	♂	Hodgkin's disease	Normal		137	3.2	752	308
5a	76	♂	Lymphoma	Normal		87	3.3	284	544
B. High-cholesterol diet									
5b	76	♂	Lymphoma	Normal	82	84	3.2	124	3
6	57	♂	Cirrhosis	Minimal focal inflammation	185	175	7.0	1,400	8
7	46	♂	Hodgkin's disease	Increased cellularity of sinusoids	175	179	10.9 7.9	1,104 872	4 4
8	52	♀	Cirrhosis	Slight fatty infiltration		235	4.1	200	4
9*	62	♂	Cirrhosis	Portal cirrhosis	90	92	2.4 3.6	1,000 888	26 46
C. Low-cholesterol diet									
10	58	♂	Chronic alcoholism, hepatomegaly	Fatty metamorphosis		178	2.4	660	1,016
11	73	♂	Alcoholism, hepatomegaly	Normal		126	1.8	516	744
12	45	♂	? Sarcoidosis	Normal		209	5.2 3.2	448 260	356 364
13	36	♀	Sarcoidosis	Granulomatous infiltration with normal liver cells			4.1	420	384

* Ate erratically.

of approximately 50% occurred in Patient 2, whereas in Patient 1 the two values agreed within 1%. Such variation probably partly represents sampling errors, since the amounts of tissue are so small that one portion of a liver lobule may predominate in one portion of the biopsy sample and a histologically different area of the lobule will predominate in another. Also, errors in determining the wet weight of such small samples of tissue are inevitable. For the purposes of this study, individual variations of these magnitudes do not influence the validity of the conclusions.

0.99 counting error of the determination will be 23 cpm (15). The system is, therefore, sufficiently active to allow accurate estimation of the cholesterol-C^{14} synthesized by these small samples of human liver. Cholesterol-C^{14} synthesis in the liver biopsy samples from the patients fed the high-cholesterol diet was, however, near the limit of significance of the counting procedure.

In Table II, the rates of cholesterol synthesis by human livers are expressed as micromicromoles of added acetate converted to cholesterol in 2 hours per 1 mg of liver. The average rate of cholesterol synthesis in normal liver was 282 $\mu\mu$moles per mg liver, and the individual rates ranged from 216 to 544 $\mu\mu$moles per mg. In the more conventional form as percentage of added acetate converted to cholesterol per 500 mg of liver, the average is 6.8%. Although such an extrapolation from 1 mg of tissue can yield only an approximate value, this is at least as high as, and perhaps somewhat higher than, the rates of synthesis in 500-mg samples of rat or mouse liver (7, 13, 16). Three of these patients proved to have histologically normal liver biopsy samples; in the two abnormal livers, however, cholesterol synthesis was apparently not significantly affected by the disease process.

Effect of high-cholesterol diets on cholesterol synthesis. The effect of feeding 3 to 4 g cholesterol per day was studied in five patients (Patients 5b through 9). Patient 5 was initially employed as a control subject, and the first biopsy sample was therefore obtained while he was on a normal diet. This tissue was diagnostically unsatisfactory, and 15 days later a second biopsy sample was obtained after the patient had been on a high-cholesterol diet for 3 days. The patient was thus able to serve as his own control. After 3 days on a high-cholesterol diet, the ability of this patient's liver to synthesize cholesterol was practically completely suppressed, i.e., to less than 0.6% of the control value.

Similarly, the three other patients in this group who consumed their diets regularly showed markedly depressed hepatic synthesis of cholesterol. The average rate of cholesterogenesis in this group of four patients was 4.6 $\mu\mu$moles of added acetate converted to cholesterol per mg of tissue. When this is compared with a corresponding average of 282 $\mu\mu$moles in the control subjects, cholesterol feeding can be seen to have suppressed cholesterol synthesis to approximately 2% of normal.

One patient in this group, Patient 9, consumed his cholesterol diet only erratically, and his level of cholesterol synthesis, although definitely depressed, was significantly higher than that of the other subjects in this group. This finding suggests that intermediate degrees of suppression of cholesterol synthesis can be produced with smaller amounts of dietary cholesterol.

In none of the five patients did the consumption of these amounts of cholesterol for 3 days produce a significant rise in the level of the serum cholesterol.

Effect of low-cholesterol diets. An attempt was next made to determine whether hepatic cholesterol synthesis could be increased by placing patients on a diet containing approximately 100 mg of cholesterol per day. The first two subjects on this diet showed levels of cholesterol synthesis that were definitely elevated, and the last two patients synthesized cholesterol at rates well above the average of the patients on the normal diet, but still within the normal range. These data, although not definitive, suggest that under some circumstances a diet low in cholesterol may cause an increase in hepatic cholesterol synthesis.

DISCUSSION

The results of this study clearly demonstrate that the addition of cholesterol to the diet will produce a striking depression in cholesterol synthesis in the livers of human beings; indeed, in none of the five patients studied did we fail to find a markedly depressed rate of hepatic cholesterol synthesis when a high cholesterol diet was fed. It is therefore apparent that hepatic cholesterol synthesis in man, as in all other species so far studied, i.e., rat (3, 4), mouse (9), dog, rabbit (2), and chicken (10), is regulated by exogenous cholesterol. Although the mechanism of this feedback system was not specifically examined, presumably it is similar to that previously demonstrated in the detailed studies in rat liver (5–8).

The modification into miniature of the usual *in vitro* procedure for measuring cholesterol synthesis makes possible reasonably accurate estimates of hepatic cholesterol synthesis on as little as 2 mg of hepatic tissue. In this manner, hepatic cholesterol synthesis could be examined in biopsy samples by Menghini needle from patients who were able to eat their normal or experimental diets through the day of the experiment. Under such physiological conditions, it was possible to demonstrate levels of cholesterol synthesis per unit weight of human liver that were at least as high

as those previously observed in experimental animals (7, 10, 13, 16). Furthermore, the average rate of cholesterol synthesis in the livers of the subjects on a normal diet (282 $\mu\mu$moles of added acetate incorporated) was somewhat greater than that seen in the dog liver sampled by an identical technique (116 $\mu\mu$moles of added acetate incorporated). Like all other animals studied, then, human beings clearly are capable of very active hepatic cholesterol synthesis.

This conclusion contrasts with that of Davis, Cox, Taylor, and Cross, who have reported that human liver biopsy samples synthesize cholesterol much more slowly than do livers of experimental animals (11). On the basis of their *in vitro* studies, these investigators have also questioned the ability of the human liver to supply the 1.5 to 2.0 g of cholesterol thought to be turned over each day in man. Without knowing the degree of dilution of intermediate pools, it is not possible on the basis of isotopic incorporation to estimate accurately the absolute amounts of cholesterol synthesized by an *in vitro* system; the validity of the quantitative calculations of Davis and associates is thus open to serious question. Nonetheless, the data of these investigators do indicate rates of incorporation of added acetate into cholesterol that are definitely lower than we have observed. This discrepancy may be explained by the fact that their studies were carried out on liver samples obtained during abdominal surgery. The patients were probably, therefore, under general anesthesia and were likely to have fasted immediately before surgery. There is good evidence to indicate that short periods of food restriction will greatly inhibit hepatic cholesterol synthesis (17, 6). Also, a prior dietary history was not reported, and the patients may have been on diets sufficiently high in cholesterol to produce further suppression of hepatic cholesterogenesis. Such differences in technique may account for the considerably higher rates of cholesterol synthesis in our studies in unanesthetized, nonfasting patients than in those of the previous investigators.

Since the present studies were carried out on portions of biopsy material obtained primarily for diagnostic purposes, in many cases pathologic changes of various types were found on subsequent histologic examination. In each of the experimental groups, at least one of the biopsy specimens studied proved to be histologically normal, and in it, cholesterol synthesis fell within the same range as in those in which pathologic changes were observed. Also, within experimental groups, no relationship between pathological abnormalities and rates of cholesterol synthesis was noted. We do not think, therefore, that such pathological changes, when present, significantly influenced the observed effects on feedback inhibition.

These studies were primarily designed to determine whether the hepatic cholesterol feedback system is present in man, and for this reason, large amounts of cholesterol were fed in the experimental diets. For practical purposes, complete suppression of cholesterol synthesis could be obtained with this amount of dietary cholesterol, and no attempt was made to define the lower limits of sensitivity of the feedback system. One patient, however, failed to eat his high-cholesterol diet consistently, and a partial suppression of cholesterol synthesis resulted. This finding suggests that the activity of the feedback system will vary with the levels of dietary cholesterol. Some support for this suggestion is provided by the fact that in at least two of the patients fed diets containing less than normal amounts of cholesterol, the rates of cholesterol synthesis were significantly increased above those seen in the livers of patients eating a normal diet.

Finally, there appeared to be no striking correlation between the levels of plasma cholesterol and the rates of hepatic cholesterol synthesis. On the one hand, even patients with plasma cholesterol concentrations in excess of 200 mg per 100 ml failed to show suppression of hepatic cholesterogenesis. On the other hand, the prompt inhibition of cholesterol synthesis produced by dietary cholesterol was not accompanied by an increase in plasma cholesterol. Since in the patients fed the normal or low-cholesterol diets, plasma cholesterol was primarily derived from endogenous sources, the dissociation between plasma cholesterol and cholesterogenesis offers some support for our previous suggestion that the cholesterol feedback system may be less sensitive to cholesterol of endogenous than of exogenous origin (10).

SUMMARY

The influence of dietary cholesterol on the synthesis of cholesterol by the liver has been studied in liver biopsy samples from well-fed, unanesthetized human beings. Dietary cholesterol in amounts of 3 to 4 g per day suppressed almost completely the synthesis of cholesterol by the human liver. We conclude that, like all other animals so far examined, man possesses a hepatic feedback mechanism for regulating the synthesis of cholesterol. Also, the human liver appears to be capable of synthesizing cholesterol at rates similar to those of other animal species. Finally, the rates of hepatic cholesterol synthesis appear to be unrelated to the levels of plasma cholesterol.

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. Burton Combes for making available the liver biopsy samples used in this study, and to Mrs. Harold I. Nachimson for her valuable advice and assistance in the dietary management of the patients.

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