Stimulatory effect of dietary lipid and cholestyramine on hepatic HMG CoA reductase

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Abstract The diurnal cycle of hepatic HMG CoA reductase activity was studied under conditions of controlled feeding where the percentage of dietary lipid, alone or in combination with 2% cholestyramine, was varied. Cholestyramine caused an increase in HMG CoA reductase activity that began soon after feeding started and peaked 6 hr later. In contrast, a diet containing 20% corn oil was a much weaker inducer of the enzyme but caused a prolonged elevation that began late in the fasting part of the cycle. These patterns suggest two different mechanisms of action.

Supplementary key words hepatic cholesterogenesis · dietary corn oil · cyclic feeding · diurnal cycles · feeding patterns

The stimulation of hepatic cholesterogenesis by dietary lipid has been reported by several investigators (1–3), but the biochemical events leading to the increased synthesis are not understood. Since tube feeding corn oil to rats results in a rapid decrease in fatty acid synthesis within 3 hr but an increase in cholesterol synthesis that begins only after 12 hr (2, 4), the increase cannot be a simple mass action effect due to an increased pool of acetyl CoA, but is more likely to be a result of enzyme activation or induction. The most probable level of enzymatic control is microsomal HMG CoA reductase (mevalonate:NADP oxidoreductase [acylating CoA], EC 1.1.1.34). This enzyme, which mediates the first biochemical transformation that is unique to cholesterol synthesis, is rate-controlling for cholesterogenesis under a variety of conditions, including the decreases seen after cholesterol feeding (5) and fasting (6) and the increases seen after Triton injection (6) and X-irradiation (7).

Feeding of cholestyramine also causes increased hepatic cholesterogenesis (8). But in this case, the cause of the elevation is at least partially understood; it is primarily a consequence of the bile acid sequestering action of the resin (8), which acts to release feedback inhibition or repression of HMG CoA reductase mediated by recirculating bile acid or cholesterol (9). Because the hepatic enzyme undergoes marked diurnal variation (10, 11), it is possible that stimulatory or inhibitory treatments might vary in the duration of their effects. We have, therefore, examined the rhythmic enzymatic changes in rats adapted to carefully controlled feeding schedules and killed at intervals during a 24-hr period. Our studies indicate that although dietary lipid and cholestyramine both cause an elevation of activity of this rate-controlling enzyme, the two effects appear to be mediated by different mechanisms.

MATERIALS AND METHODS

Male Holtzman rats weighing 120–140 g were used in all of these studies; they were housed one to a cage in environmentally controlled rooms where the lights were automatically regulated to go off at 8 AM and on at 8 PM daily. The rats were fed according to an "8–16" feeding schedule (12): semisynthetic diets were placed in the cages at 8 AM daily and removed at 4 PM daily. The rats were given water ad lib. Six different diets of equal caloric value per gram (Table 1) were fed to different groups of rats for 10 days before the animals were killed. The casein and vitamin fortification mixture were from General Biochemicals, Chagrin Falls, Ohio. Salt mixture P-H and cellulose (Alphacel) were from Nutritional Biochemicals, Cleveland, Ohio. Cholestyramine (Cuemid) was from Merck Sharp and Dohme, West Point, Pa.

All rats gained weight on the diets and those killed during or just after the feeding part of the cycle had large

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl.

TABLE 1. Compositions of diets

Diet[a]	Dextrose	Cellulose	Corn Oil	Cholestyramine
		%		
Basal	75			
5% Corn oil	65	5	5	
20% Corn oil	31	24	20	
Cholestyramine	73			2
5% Corn oil + cholestyramine	65	3	5	2
20% Corn oil + cholestyramine	31	22	20	2

[a] All diets contained 20% casein, 4% salt mixture P-H (salt mixture IV [13] + cobalt), and 1% vitamin fortification mixture.

amounts of food in their stomachs. However, since fasting for even brief periods is known to cause a decrease in hepatic microsomal HMG CoA reductase (14) and since food intake might vary with the level of dietary corn oil, a separate study of the feeding habits of the rats was also carried out. Individually caged rats, fed one of four diets, were studied using a device described by Spengler (15) and employing electromechanical balances (Viterra, Electronic Instruments, Wallisellen ZH, Switzerland) that support the feeding cups. When the tension in the balance is released by the rat removing food from the cup, a change in electrical resistance is amplified and continuously recorded (Fig. 1). With this device an accurate record of the feeding patterns of six simultaneously fed rats was obtained. Three rats fed 0% corn oil diet were compared with three rats fed a 20% corn oil diet; the same number of rats fed 0 or 20% corn oil diet with 2% cholestyramine were also compared. As in the enzyme studies, the rats were fed according to an "8–16" feeding schedule. Water was allowed ad lib., and total food intake and feeding records were obtained for 5 days after 10 days of adaptation to the device.

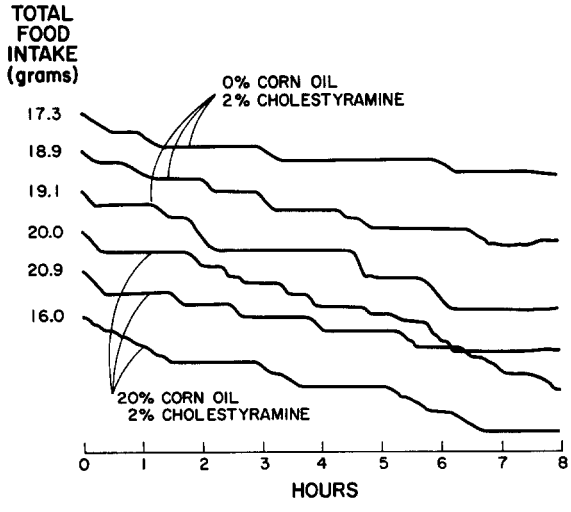

FIG. 1. Food intake and feeding records, during one 8-hr period beginning at 8 AM, of six rats fed either 0 or 20% corn oil diets with 2% added cholestyramine. The calibration for one unit of deflection per gram of diet, on the ordinate, is not identical for all the records. See text for additional details of experimental design.

Because the enzyme experiments on rats fed the basal diet or a corn oil diet without added cholestyramine (B-CO) were completed at an earlier time than those on rats fed cholestyramine with or without corn oil (Ch), they are described separately when differences in methodology exist. On day 10 of cyclic feeding, groups of five to seven Ch rats were killed by decapitation at 8 AM, 2 PM, 5 PM, and 12 PM. B-CO rats, three to five in a group, were killed at the same times and in addition at 11 AM. An additional group of six rats was fed a diet containing 20% corn oil and 2% cholestyramine for 10 days, fasted on day 11, and killed at 2 PM. In all experiments, homogenization, separation of microsomes, and assay of HMG CoA reductase were carried out as previously described (16). Livers were rapidly removed to ice cold beakers, and homogenizing solution (4 times the liver weight) was added to each beaker. Homogenizing solution for B-CO studies consisted of a buffer containing 0.05 M triethanolamine HCl, 0.25 M sucrose, 0.02 M Na_2-EDTA, and 0.01 M 2-mercaptoethanol, pH 7.3. Microsomal proteins were determined in this group by the previously described modified biuret determination (16). Assays were carried out on 0.5 and 1 mg of microsomal protein in 1 ml of incubating solution containing 0.15 μmole of DL-[3-^{14}C]HMG CoA (sp act 202,000 dpm/μmole), 30 μmoles of glucose-6-phosphate, 2 IU of glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.), and 1 mg of bovine serum albumin in a 0.1 M triethanolamine HCl buffer containing 0.02 M Na_2EDTA and 0.01 M 2-mercaptoethanol, pH 7.3. For the later studies of the enzyme activity in Ch rats, homogenization and centrifugation of liver was carried out in buffer containing 0.02 M K_2HPO_4, 0.25 M sucrose, 0.01 M Na_2EDTA, and 0.001 M dithiothreitol, pH 7.3. In these experiments, microsomal protein was determined by the method of Lowry et al. (17), and assays were carried out at levels of 0.25 and 0.5 mg of microsomal protein/ml of incubation solution. Incubation mixtures contained 0.06 μmole of DL-[3-^{14}C]HMG CoA (sp act 454,000 dpm/μmole), 10 μmoles of glucose-6-phosphate, 1 IU of glucose-6-phosphate dehydrogenase in a buffer containing 0.02 M K_2HPO_4, 0.07 M NaCl, 0.02 M Na_2EDTA, and 0.001 M dithiothreitol, pH 7.3.

RESULTS

Daily food intake of rats fed 0% corn oil diet (16.1 ± 0.8 g),[1] was no different from those fed 20% corn oil diet (16.8 ± 1.0 g). Similarly, there was no significant differ-

[1] Mean ± SE, this and subsequent values.

ence between the food intakes of those fed 2% cholestyramine with 0% corn oil (17.3 ± 0.6 g) and those fed cholestyramine with 20% corn oil (19.2 ± 0.7 g). Furthermore, although the feeding patterns of individual rats varied greatly from day to day, no differences were found among the different groups (Fig. 1). Except for an occasional more continuous nibbling pattern, rats from each group ate three to seven discrete meals daily, with one meal usually beginning immediately after the food was placed in the cages and the lights were turned off.

Fig. 2 shows the diurnal variation of hepatic microsomal HMG CoA reductase activity of rats fed diets with 0, 5, or 20% corn oil. A diurnal cycle was present for all three diets, with the highest level at about 2 PM and the lowest at about 12 PM. Increasing the corn oil content of the diet appeared to increase the level and duration of response of HMG CoA reductase. This responsiveness was most apparent when 0 and 20% corn oil diets were compared; there were statistically significant elevations with the 20% corn oil diet at 8 AM ($0.01 > P > 0.001$), 11 AM ($0.05 > P > 0.02$), 2 PM ($0.001 > P$), and 5 PM ($0.02 > P > 0.01$), but there was no difference at 12 PM. The increase in enzyme activity between 12 PM and 2 PM was fourfold for rats fed the 20% corn oil diet ($0.001 > P$), sixfold for rats on the 5% corn oil diet ($0.02 > P > 0.01$), and only twofold for rats fed the diet with no corn oil ($0.02 > P > 0.01$). HMG CoA reductase activity in rats fed the 20% corn oil diet began to rise during the fasting period and by 8 AM was already at a level 3.5 times greater than that at 12 PM ($0.01 > P > 0.001$).

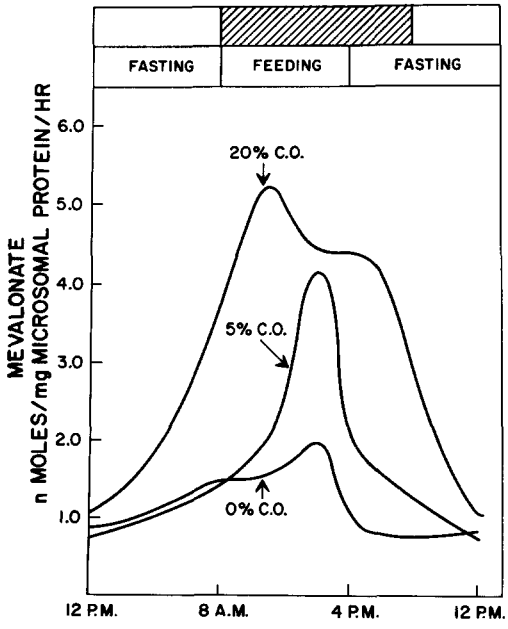

FIG. 2. HMG CoA reductase activity in hepatic microsomes of groups of rats fed the basal diet with 0, 5, or 20% corn oil and killed at 8 AM, 11 AM, 2 PM, 5 PM, and 12 PM.

Fig. 3 shows the cyclic change in HMG CoA reductase activity in hepatic microsomes of rats fed diets containing 0, 5, or 20% corn oil and 2% cholestyramine. Again, as in the previous series, a diurnal rhythmic change in the enzyme activity was noted, but the addition of cholestyramine to the diet resulted in a 5–30-fold elevation of activity compared with results from enzyme preparations from rats fed corn oil diets without added cholestyramine. Furthermore, whereas peak values at 2 PM are a function of the percentage of dietary corn oil in B-CO rats, all rats fed 2% cholestyramine had uniformly high levels of enzyme at this time, 38–47 nmoles of mevalonate/mg of microsomal protein/hr. However, differences were apparent at other times, and especially at 8 AM, when rats fed the 20% corn oil and 2% cholestyramine diet had levels of microsomal enzyme activity five times higher ($0.001 > P$) than levels from rats fed diets containing 2% cholestyramine with no corn oil. In addition, an increment in enzyme activity occurred during the fasting period in rats fed the 20% corn oil–2% cholestyramine diet (Fig. 3), with a greater than twofold increase between 12 PM and 8 AM ($0.001 > P$). But the further increase to the peak 2 PM values required feeding on the day of killing, since the group that was fasted on the last day (dotted line, Fig. 3) had 2 PM values of 19.2 ± 0.8 nmoles of mevalonate/mg of microsomal protein/hr,

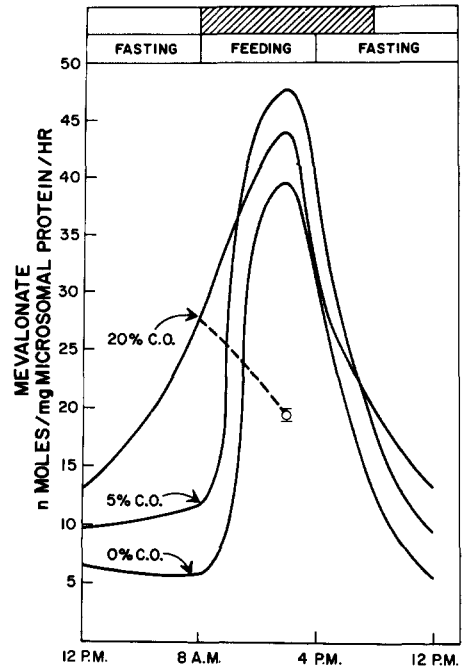

FIG. 3. HMG CoA reductase activity in hepatic microsomes of groups of rats fed 0, 5, or 20% corn oil diets with 2% added cholestyramine and killed at 8 AM, 2 PM, 5 PM, and 12 PM. The dotted line leads to the 2 PM value for a group of rats cyclically fed the 20% corn oil diet with 2% cholestyramine and fasted on the day of killing.

whereas fed rats killed at 2 PM had peak values of 44 ± 8.1 nmoles of mevalonate/mg of microsomal protein/hr. The 8 AM values, 27.6 ± 2.3 nmoles of mevalonate/mg of microsomal protein/hr were, in fact, 50% higher than the 2 PM fasting values.

DISCUSSION

The present studies indicate that the stimulation of cholesterogenesis by dietary lipid and cholestyramine results from increased activity of HMG CoA reductase in both cases. Cholestyramine was incorporated in the diets at the 2% level because it had previously been shown that when added to 15% corn oil diets it caused a 30-fold increase in bile acid excretion with only an average 8% greater loss of fat in the feces compared with rats fed the same diet without cholestyramine (18). Because the peak enzyme activity at 2 PM in rats fed a diet containing 2% cholestyramine with no corn oil is 20 times greater than the level in rats fed the same diet but without cholestyramine, it appears that the most important factor in the absolute increment is the rapid binding of bile salts by the resin in the gastrointestinal tract. Moreover, the peak activity in rats fed a 20% corn oil diet without cholestyramine is still only one-seventh of the highest activity obtained in rats fed the diet containing 2% cholestyramine and no corn oil, clearly indicating that dietary lipid is responsible for only a small absolute increase in enzyme activity. However, it also appears that some nondietary factor, probably a hormone, initiates the cyclic increase, since a diurnal cycle was noted with all the different diets used in this study and has been found in fasting rats (11). Our earlier observations (19) that microsomal HMG CoA reductase activity in two different Morris hepatomas does not increase after cholestyramine feeding nor decrease after cholesterol feeding, but does, however, show a diurnal rhythmicity in phase with the host liver, also points to a circulating mediator other than cholesterol or bile acids that serves a regulatory role. The recent suggestion that the diurnal cycle of HMG CoA reductase activity is mediated in some manner by adrenal hormones is of great interest (20). However, it is possible that the variation in HMG CoA reductase activity is itself a secondary response to varying bile acid formation triggered by a humoral or neurogenic mechanism. This speculation is put forward because cholesterol 7α-hydroxylase, the presumed rate-controlling enzyme for bile acid synthesis (21), also fluctuates diurnally in a manner that closely parallels the change in HMG CoA reductase (22). In addition, the rhythmic rise of the enzyme during the feeding period is abolished by adrenalectomy but is reelicited by dexamethasone injection (23). Thus, in response to increased bile acid formation, depletion of microsomal cholesterol or its derivative could release enzyme repression and account for the synthesis of HMG CoA reductase (24) that occurs during the rising phase of the diurnal cycle.

The stimulation of enzyme activity by diets containing corn oil appears to be mediated by a more complex mechanism than that elicited by cholestyramine administration. The distinctive effect most apparent when comparing rats fed 0 or 20% corn oil diet was an enzyme increase that began late in the fasting period and that appeared to be triggered by the previous day's feeding. The effect was also apparent when cholestyramine was incorporated in the diet, most prominently at 8 AM when the stimulation by cholestyramine was minimal. In contrast, 6 hr after feeding began, the cholestyramine effect was maximal and overwhelmed any difference due to variation in corn oil. Since total food intake and feeding patterns were similar in the compared groups, the differences must be attributed to dietary lipid and not to a variation in the amount or rate of ingestion of calories. While the delayed enzyme increase might be related to slow absorption of lipid in the presence of cholestyramine, this reason cannot be invoked in rats fed a diet containing 20% corn oil without resin. Finally, it might be argued that the effect of the high lipid diets in stimulating HMG CoA reductase activity is not due to lipid but to a response to bile acid binding by the increased quantity of indigestible residue in these diets (25). This too is unlikely, since if it were true one would expect to see a rapid response similar to that induced by cholestyramine. The recent observation that dietary cellulose, per se, does not increase hepatic cholesterogenesis also argues against such an interpretation (26). Of probable importance in understanding the delayed postprandial increment is Chevallier's (27) finding that, under conditions where rats were adapted to 2 hr of feeding and 22 hr of fasting, a cyclic increase in the conversion of dietary [26-^{14}C]-cholesterol to $^{14}CO_2$ was noted prior to the feeding period. This increase, which most certainly reflects a diurnal fluctuation in bile acid synthesis, could indicate an adaptation whereby bile acids are synthesized in preparation for intake of diets containing lipid.

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REFERENCES

1. Linazasoro, J. M., R. Hill, F. Chevallier, and I. L. Chaikoff. 1958. Regulation of cholesterol synthesis in the liver: the influence of dietary fats. *J. Exp. Med.* **107:** 813–820.
2. Bortz, W. M. 1967. Fat feeding and cholesterol synthesis. *Biochim. Biophys. Acta.* **137:** 533–539.
3. Diller, E. R., and O. A. Harvey. 1964. Interrelationship of sterol and fatty acid biosynthesis in rat liver slices as related to dietary lipid. *Biochemistry.* **3:** 2004–2007.
4. Hill, R., W. W. Webster, J. M. Linazasoro, and I. L. Chaikoff. 1960. Time of occurrence of changes in the liver's capacity to utilize acetate for fatty acid and cholesterol synthesis after fat feeding. *J. Lipid Res.* **1:** 150–153.
5. Linn, T. C. 1967. The effect of cholesterol feeding and fasting upon β-hydroxy-β-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **242:** 990–993.
6. Bucher, N. L. R., P. Overath, and F. Lynen. 1960. β-Hydroxy-β-methyglutaryl coenzyme A reductase, cleavage and condensing enzymes in relation to cholesterol formation in rat liver. *Biochim. Biophys. Acta.* **40:** 491–501.
7. Berndt, J., and R. Gaumert. 1971. Stimulation of hydroxymethylglutaryl-coenzyme A reductase in mouse liver by X-rays. *FEBS Lett.* **13:** 49–52.
8. Huff, J. W., J. L. Gilfillan, and V. M. Hunt. 1963. Effect of cholestyramine, a bile acid binding polymer on plasma cholesterol and fecal bile acid excretion in the rat. *Proc. Soc. Exp. Biol. Med.* **114:** 352–355.
9. Goldfarb, S., and H. C. Pitot. 1971. Regulation of hepatic HMG-CoA reductase: diurnal rhythm and effect of fat and cholestyramine feeding. *Federation Proc.* **30:** 633.
10. Kandutsch, A. A., and S. E. Saucier. 1969. Prevention of cyclic and Triton-induced increases in hydroxymethylglutaryl coenzyme A reductase and sterol synthesis by puromycin. *J. Biol. Chem.* **244:** 2299–2305.
11. Hamprecht, B., C. Nüssler, and F. Lynen. 1969. Rhythmic changes of hydroxymethylglutaryl coenzyme A reductase activity in livers of fed and fasted rats. *FEBS Lett.* **4:** 117–121.
12. Scott, D. F., and V. R. Potter. 1970. Metabolic oscillations in lipid metabolism in rats on controlled feeding schedules. *Federation Proc.* **29:** 1553–1559.
13. Phillips, P. H., and E. B. Hart. 1935. The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. *J. Biol. Chem.* **109:** 657–663.
14. Regen, D., C. Riepertinger, B. Hamprecht, and F. Lynen. 1966. The measurement of β-hydroxy-β-methyl-glutaryl CoA reductase in rat liver; effects of fasting and refeeding. *Biochem. Z.* **346:** 78–84.
15. Spengler, J. 1960. Ein Apparat zur quantitativen fortlaufenden Registrierung der Nahrungs- und Wasseraufnahme von Ratten. *Helv. Physiol. Acta.* **18:** 50–55.
16. Goldfarb, S., and H. C. Pitot. 1971. Improved assay of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Lipid Res.* **12:** 512–515.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
18. Harkins, R. W., L. M. Hagerman, and H. P. Sarett. 1965. Absorption of dietary fats by the rat in cholestyramine-induced steatorrhea. *J. Nutr.* **87:** 85–92.
19. Goldfarb, S., and H. C. Pitot. 1971. The regulation of β-hydroxy-β-methylglutaryl coenzyme A reductase in Morris hepatomas 5123C, 7800, and 9618A. *Cancer Res.* **31:** 1879–1882.
20. Hickman, P. E., B. J. Horton, and J. R. Sabine. 1972. Effect of adrenalectomy on the diurnal variation of hepatic cholesterogenesis in the rat. *J. Lipid Res.* **13:** 17–22.
21. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1970. Biochemical site of regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **11:** 404–411.
22. Gielen, J., J. van Cantfort, B. Robaye, and J. Renson. 1969. Rythme circadien de la cholestérol-7-α-hydroxylase chez le rat. *C. R. Acad. Sci. Ser. D.* **269:** 731–732.
23. Gielen, J., B. Robaye, J. van Cantfort, and J. Renson. 1970. Facteurs endocriniens contrôlant le rythme circadien de la biosynthèse des acides biliaires. *Arch. Int. Pharmacodyn.* **183:** 403–405.
24. Higgins, M., T. Kawachi, and H. Rudney. 1971. The mechanism of the diurnal variation of hepatic HMG CoA reductase activity in the rat. *Biochem. Biophys. Res. Commun.* **45:** 138–144.
25. Portman, O. W., and P. Murphy. 1958. Excretion of bile acids and β-hydroxysterols by rats. *Arch. Biochem. Biophys.* **76:** 367–376.
26. Terrell, E. B., and D. M. Regen. 1971. Effects of simple diets on cholesterol synthesis in rat liver. *J. Nutr.* **101:** 437–444.
27. Chevallier, F. 1967. Dynamics of cholesterol in rats, studied by the isotopic equilibrium method. *Advan. Lipid Res.* **5:** 209–239.