Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 37-43

# A BIPHASIC EFFECT OF EXOGENOUS OLEATE ON THE RATE OF CHOLESTEROL BIOSYNTHESIS BY RAT HEPATOCYTES.

by

CLIVE R. PULLINGER and GEOFFREY F. GIBBONS

Medical Research Council Lipid Metabolism Unit,
Hammersmith Hospital, London W12 0HS, U.K.

Received December 19, 1980

SUMMARY

Short-term (0-1 h) incubations of rat hepatocytes with oleate (2 mM) resulted in a decrease in the rate of cholesterol synthesis compared to controls incubated in the absence of fatty acid. However, during this period the activity of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase was higher in the oleate-incubated cells. After longer incubation periods in the presence of oleate there was a higher rate of cholesterol synthesis than in the corresponding non-oleate controls and HMG-CoA reductase activity remained elevated. This biphasic effect provides an explanation for previous contradictory reports concerning the effect of exogenous fatty acids on the rate of cholesterol synthesis in liver. The present studies also suggest that in some physiological situations, the rate of cholesterol synthesis is determined by substrate supply rather than by HMG-CoA reductase activity.

Exogenous free fatty acids (or their CoA derivatives) regulate hepatic fatty acid biosynthesis partly by controlling the activity of acetyl-CoA carboxylase (EC 6.4.1.2.) the enzyme which commits acetyl-CoA exclusively to fatty acid biosynthesis (1,2). In addition, the supply of cytosolic acetyl-CoA itself is also decreased by the fatty acid-induced inhibition of pyruvate dehydrogenase (EC 1.2.2.2.) (3,4) and ATP citrate lyase (EC 4.1.3.8.) (5,6). However, cytosolic acetyl-CoA is also the precursor of cholesterol and any decrease in its rate of supply might be expected to influence the rate of hepatic cholesterogenesis. In fact, it has been reported on several occasions that free fatty acids decrease the rate of cholesterol synthesis in liver *in vitro* (7-9). Whether this inhibition arises solely as a result of substrate withdrawal or whether exogenous fatty acid also inhibits hydroxymethylglutaryl CoA reductase (HMG-CoA reductase) (EC 1.1.1.34.), generally considered to be the rate limiting enzyme for cholesterol synthesis, is presently unknown. In other studies, however, exogenous oleate was reported to increase

0006-291X/81/050037-07$01.00/0

the rate of cholesterol biosynthesis and the activity of HMG-CoA reductase in liver (10-12). The present work was undertaken partly in order to explain these apparently contradictory effects of fatty acid and also to investigate the precise effects of exogenous oleate on the availability of substrate for cholesterol biosynthesis.

## MATERIALS AND METHODS

The housing and treatment of animals, preparation of isolated hepatocyte suspensions and incubation procedures have been described previously (13,14). The final cell preparation was suspended in a solution containing glucose (11.1 mM), amino acids (15) and bovine serum albumin (1.1%) in Krebs-Henseleit (16) calcium and bicarbonate buffer (pH 7.4). After addition of triparanol (final concentration 2 μM) this was the standard incubation medium. Solutions of albumin-bound sodium oleate were prepared according to the method of Van Harken et al. (17) and added to the suspended hepatocytes such that the final oleate concentration was 2.0 mM.

The absolute rate of sterol synthesis in the cells was determined by measuring the weight of desmosterol (cholesta-5,24-dien-3β-ol) which accumulates in the cells over a given period of time in the presence of triparanol as described previously (13,14). Manipulative losses incurred during the extraction and purification of desmosterol (isolated as desmostenone, cholesta-4,24-dien-3-one) were accounted for by the addition of [26,27-$^{14}$C]desmosterol (10,000 dpm) as internal standard. The relatively low mass (12-15 ng) associated with this added label was subtracted from the total mass of the desmosterol derivative measured by gas-chromatography.

The activity of HMG-CoA reductase in the cells was determined using the whole cell homogenate (18). Before assay the cell homogenate was preincubated for 20 min at 37°C in the absence of cofactors and substrate. The rate of fatty acid synthesis by the hepatocytes was determined by measurement of incorporation of $^{3}$H into the fatty acid fraction isolated after incubation in the presence of $^{3}H_2O$ (final specific radioactivity 146 dpm/nmol) (19).

The sources of materials used in the present work have been described elsewhere (13,14). In addition, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, NADP, sodium oleate and fatty acid-free bovine serum albumin were obtained from the Sigma Chemical Co, St. Louis, MO, USA. [26,27-$^{14}$C]desmosterol, 3RS-[3-$^{14}$C]hydroxymethyl-glutaryl-CoA and 3RS-[2-$^{3}$H]mevalonic acid were obtained from The Radiochemical Centre, Amersham, Bucks, UK.

## RESULTS AND DISCUSSION

Liver cells were incubated in the standard medium either in the presence or absence of oleic acid (2 mM). Aliquots (1.0 ml) of the cell suspensions were removed at hourly intervals for a total of 4 h and the mass of newly synthesised sterol was measured at each time interval. The results are shown in Figure 1. In both types of incubation, the rates of synthesis during the first hour were lower than those observed at subsequent hourly intervals. In addition, in the

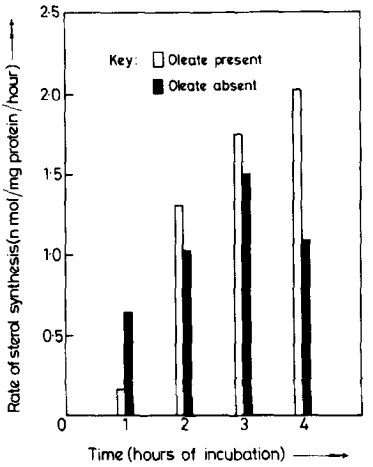


Fig. 1. Effects of oleate and time on the rate of sterol biosynthesis. Hepatocyte suspensions (6.9 mg protein/ml, total volume 15.0 ml) were incubated in the standard medium either in the absence or presence of oleate (2 mM). Portions (1.0 ml) of the cell suspensions were removed at hourly intervals and the rate of sterol synthesis was determined.

presence of oleate, during the first hour of incubation, sterol synthesis was suppressed to a level only 25% of that which occurred in its absence. During the second, third and fourth hours of incubation, however, sterol synthesis was stimulated to a greater extent in the presence of oleate so that at longer time periods the rate exceeded that observed in the corresponding control incubations.

In a similar experiment, hepatocytes were incubated in the presence of $^{3}H_{2}O$ and samples were removed at hourly intervals to determine incorporation of $^{3}H$ into fatty acids. The results are presented in Table 1 and show a continuing suppression of fatty acid synthesis by oleate for at least 4 h of incubation.

Other, similar experiments were carried out in which both the activity of HMG-CoA reductase and the rate of sterol synthesis were measured at hourly intervals in the presence or absence of oleate (2 mM). The results of these experiments are summarised in Figure 2. Changes in the rate of sterol synthesis observed in the presence of oleate during a particular hourly period are expressed as weights of sterol produced as a percentage of that formed during the same period in the absence of exogenous fatty acid. Changes in the activity of HMG-CoA reductase are expressed in a similar way.

Table 1. Effect of oleate on fatty acid biosynthesis.

Suspensions of hepatocytes were incubated in the standard medium containing $^3H_2O$ (146 dpm/nmol) either in the absence or presence of oleate (2 mM). At hourly intervals, portions (1.0 ml) of the cell suspensions were removed and the incorporation of $^3H_2O$ into fatty acids was determined. The total volume of each incubation was 15.0 ml containing 4.0 mg of cellular protein per ml. The incubations were conducted for a maximum of 4 h at 37°C with shaking ( ~70 oscillations per min).

| Incubation time (h) | Accumulated incorporation of $^3H_2O$ (nmol) into fatty acids | | Oleate present / Oleate absent (%) |
|---|---|---|---|
| | Oleate absent | Oleate present | |
| 1 | 163.8 | 16.5 | 10.1 |
| 2 | 432.5 | 116.5 | 26.9 |
| 3 | 710.1 | 111.0 | 15.6 |
| 4 | 879.3 | 179.5 | 20.4 |

The most striking feature of Figure 2 is the biphasic effect of oleate on the rate of sterol biosynthesis. This confirms the results expressed in Figure 1 and provides evidence for a significant decrease in the rate during the first hour of incubation, whilst during the fourth hour of incubation the rate was increased compared to the non-oleate controls. This initial inhibitory effect of oleate is in agreement with previous reports concerning the effects of fatty acids on sterol synthesis in perfused liver (8), liver slices (9) and liver cells (7). The present results also show, for the first time, that this decrease in the rate of sterol synthesis is not accompanied by a corresponding decline in the activity of HMG-CoA reductase. It is probable therefore that during this period the rate of sterol synthesis is regulated exclusively by the supply of pre-mevalonate substrates. In this respect, the rate of production of cytosolic acetyl-CoA, the precursor of both fatty acids and sterols is decreased by exogenous fatty acids or their metabolic products. This is due to inhibition of pyruvate dehydrogenase (3,4) and ATP-citrate lyase (5,6) and is part of the complex process by which dietary fatty acids regulate endogenous hepatic fatty acid biosynthesis. Although the initial decrease in sterol synthesis could be explained in these terms alone, the effect of fatty acids on the activities of other enzymes which supply HMG-CoA reductase with substrate, namely HMG-CoA synthase and acetoacetyl-CoA thiolase,

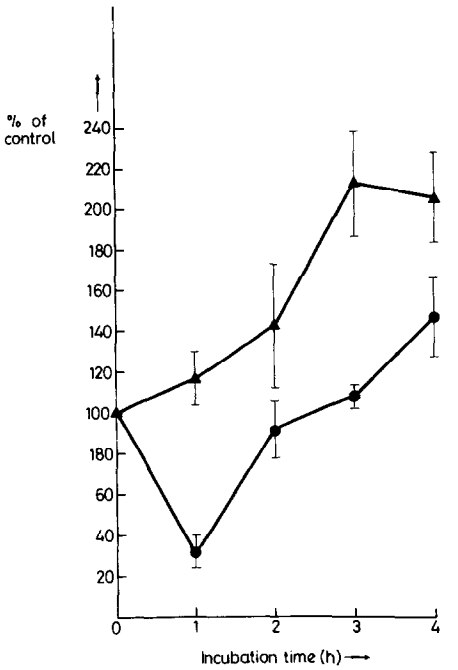


Fig. 2. Relative effects of oleate on the rate of sterol synthesis and HMG-CoA reductase activity after different periods of incubation. Hepatocyte suspensions (total volume, 15.0 ml) were incubated in the standard medium either in the absence or presence of oleate (2 mM). Portions of the cell suspensions were removed at hourly intervals and the rate of sterol synthesis and the activity of HMG-CoA reductase were determined. The activity of the reductase was also measured immediately before incubation (zero time). The results are expressed as rates of synthesis or enzymic activity in the presence of oleate as a percentage of the values observed at the same time in the absence of oleate. Each point represents the average of the results obtained from the liver cells of at least three separate animals together with the corresponding SEM. ▲——▲ HMG-CoA reductase ●——● Sterol synthesis

is currently unknown. The importance of these enzymes in the co-ordinated regulation of cholesterogenesis is becoming increasingly widely recognised (20-22).

The stimulatory effect of oleate on the activity of HMG-CoA reductase, even during periods of low cholesterol synthesis, is enigmatic. It resembles, in some aspects, the contrasting effects of the fungal metabolite ML-236B, also known as compactin (23), on the activity of HMG-CoA reductase and the overall rate of sterol biosynthesis. This compound is a potent inhibitor of HMG-CoA reductase (24,25) which results in a decreased rate of cellular sterol biosynthesis. However, in cells or animals pre-treated with compactin the levels of HMG-CoA reductase were dramatically elevated (26-28). This effect has been ascribed to an induction of

synthesis of HMG-CoA reductase protein in response to a deprivation of terpenoid and steroid products of mevalonic acid metabolism which results from enzyme inhibition in the intact cells. Upon cell disruption, the inhibitor is diluted out and this latent activity becomes apparent. In the present work, oleate also gave rise to a temporary decrease in the supply of substrate for terpenoid and steroid synthesis and this was apparent from the early decrease in the rates of cholesterol synthesis (Figures 1 and 2). It appears that HMG-CoA reductase responds to this deprivation of post-mevalonate metabolites in a way similar to that observed with compactin.

During longer incubation periods the short-term inhibitory effect of oleate on sterol synthesis was overcome to such an extent that during the fourth hour of incubation more sterol was synthesised in the presence of oleate than in its absence. This is consistent with those studies in which longer-term perfusions (upto 4 h) of rat liver with oleate gave rise to an increase in the rate of cholesterol synthesis and HMG-CoA reductase activity (10-12). Thus the precise effects of exogenous fatty acids upon the rate of sterol synthesis in liver depend upon the length of time during which the fatty acid is available to the liver cell.

The mechanism by which cholesterol synthesis recovers over the longer term is obscure. That this is not due to a removal of the inhibitory effect by metabolism of the added oleate is clear from the continued suppression of fatty acid biosynthesis (Table 1). Since HMG-CoA reductase activity in the oleate-incubated cells is at all times higher than in the controls, the recovery appears to be due to an increase in the availability of pre-mevalonate substrates of sterol synthesis. This amount will be determined by the relative activities of enzymes such as pyruvate dehydrogenase, ATP citrate lyase and acetyl-CoA carboxylase and by the primary metabolic pathway by which the bulk of substrate is produced. Under normal circumstances, the liver derives much of its acetyl-CoA by glycogenolysis (29,30). In the presence of exogenous fatty acid, however, a large proportion of the cellular acetyl-CoA is derived by β-oxidation. Changes in the rate of supply of substrate for cholestero-

genesis may reflect the time required for the cell to adapt enzymically to the utilisation of fatty acids rather than carbohydrate for acetyl-CoA production.

REFERENCES


1. Bortz, W.M., and Lynen, F. (1963) Biochem. Z. 337, 505-509.
2. Goodridge, A.G. (1972) J. Biol. Chem. 247, 6946-6952.
3. Wieland, O.H., Patzelt, C., and Löffler, G. (1972) Eur. J. Biochem. 26, 426-433.
4. Patzelt, C., Löffler, G., and Wieland, O.H. (1973) Eur. J. Biochem. 33, 117-122.
5. Yeh, Y.Y., and Leveille, G.A. (1970) J. Nutr. 100, 1389-1397.
6. Yeh, Y.Y., and Leveille, G.A. (1971) J. Nutr. 101, 911-918.
7. Nilsson, Å., Sundler, R., and Åkesson, B. (1973) Eur. J. Biochem. 39, 613-620.
8. Brunengraber, H., Boutry, M., and Lowenstein, J.M. (1978) Eur. J. Biochem. 82, 373-384.
9. Andersen, J.M., and Dietschy, J.M. (1979) J. Lipid Res. 20, 740-752.
10. Goh, E.H., and Heimberg, M. (1973) Biochem. Biophys. Res. Commun. 55, 382-388.
11. Goh, E.H., and Heimberg, M. (1977) J. Biol. Chem. 252, 2822-2826.
12. Goh, E.H., and Heimberg, M. (1979) Biochem. J. 184, 1-6.
13. Gibbons, G.F., and Pullinger, C.R. (1977) Biochem. J. 161, 321-330.
14. Gibbons, G.F., and Pullinger, C.R. (1979) Biochem. J. 177, 255-263.
15. East, A.G., Louis, L.N., and Hoffenberg, R. (1973) Exp. Cell Res. 76, 41-46.
16. Krebs, H.A., and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33-66.
17. Van Harken, D.R., Dixon, C.W., and Heimberg, M. (1969) J. Biol. Chem. 244, 2278-2285.
18. Saucier, S.E., and Kandutsch, A.A. (1979) Biochim. Biophys. Acta. 572, 541-556.
19. Windmueller, H.G., and Spaeth, A.E. (1966) J. Biol. Chem. 241, 2891-2899.
20. Balasubramaniam, S., Goldstein, J.L., and Brown, M.S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 1421-1425.
21. Chang, T.-Y., and Limanek, J.S. (1980) J. Biol. Chem. 255, 7787-7795.
22. Ramachandran, C.K., Gray, S.L., and Melnykovych, G. (1980) Biochim. Biophys. Acta 618, 439-448.
23. Brown, A.G., Smale, T.C., King, T.J., Hasenkamp, R., and Thompson, R.H. (1976) J. Chem. Soc. Perkin I, 1165-1170.
24. Endo, A., Kuroda, M., and Tsujita, Y. (1976) J. Antibiot. 29, 1346-1348.
25. Endo, A., Kuroda, M., and Tanzawa, K. (1976) FEBS Lett. 72, 323-326.
26. Brown, M.S., Faust, J.R., Goldstein, J.L., Kaneko, I., and Endo, A. (1978) J. Biol. Chem. 253, 1121-1128.
27. Endo, A., Tsujita, Y., Kuroda, M., and Tanzawa, K. (1979) Biochim. Biophys. Acta 575, 266-276.
28. Brown, M.S., and Goldstein, J.L. (1980) J. Lipid Res. 21, 505-517.
29. Salmon, D.M.W., Bowen, N.L., and Hems, D.A. (1974) Biochem. J. 142, 611-618.
30. Clark, D.G., Rognstad, R., and Katz, J. (1974) J. Biol. Chem. 249, 2028-2036.