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Regulation of Hepatic Cholesterol Biosynthesis by Fatty Acids: Effect of Feeding Olive Oil on Cytoplasmic Acetoacetyl-Coenzyme A Thiolase, β-hydroxy-β-methylglutaryl-CoA Synthase, and Acetoacetyl-Coenzyme A Ligase[1]

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We reported previously that, in the perfused rat liver, oleic acid increased the specific activity of cytosolic enzymes of cholesterol biosynthesis. In this study, we examined the effects of oral administration of olive oil on the activities of HMG-CoA synthase, AcAc-CoA thiolase, AcAc-CoA ligase and HMG-CoA reductase. Olive oil feeding increased the specific activity of hepatic HMG-CoA synthase by 50%, AcAc-CoA thiolase by 2-fold, and AcAc-CoA ligase by 3-fold. Olive oil had no effect on HMG-CoA reductase activity. These data suggest that the enzymes that supply the HMG-CoA required for hepatic cholesterogenesis are regulated in parallel by a physiological substrate, fatty acid, independent of HMG-CoA reductase under these conditions.
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AcAc-CoA thiolase[4] (EC 2.3.1.9) and HMG-CoA synthase (EC 4.1.3.5) in rat liver exist in distinct forms with different subcellular localization. The cytoplasmic forms catalyze the first two steps in the biosynthesis of cholesterol(1), and the mitochondrial forms catalyze the first two steps in the biosynthesis of ketone bodies (2). AcAc-CoA may also be generated in the cytoplasm by the action of AcAc-CoA ligase (EC 6.2.1.16). The rate of cholesterol synthesis, which generally is directly correlated with the activity of HMG-CoA

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4. Abbreviations used: Acetoacetyl coenzyme A, AcAc-CoA; Acetyl coenzyme A, AcCoA; β-hydroxy-β-methylglutaryl-CoA, HMG-CoA; Very low density lipoprotein, VLDL; Dithiothreitol, DTT

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reductase (3-6), may, under certain conditions, be limited by the rate of generation of HMG-CoA substrate (7-9). AcAc-CoA thiolase and HMG-CoA synthase, acting in sequence, generate HMG-CoA in the cytoplasm for hepatic cholesterogenesis (10-11). Along with HMG-CoA reductase, the activities of these enzymes in rat liver are suppressed by dietary cholesterol or fasting (12-13), and by exogenous sterol in cultured fibroblasts (14). Conversely, their activities are enhanced by addition of cholestyramine and/or lovastatin to the diet, presumably by depletion of the metabolic pool of cholesterol (13,15). In the isolated, perfused rat liver, fatty acids stimulate cholesterol secretion as a component of the VLDL (16), enhance the rate of cholesterogenesis (17,18), and increase the activities of cytosolic AcAc-CoA thiolase, AcAc-CoA ligase, HMG-CoA synthase and HMG-CoA reductase (19,20). We now report that the activities of the cytosolic enzymes of hepatic cholesterogenesis, but not that of microsomal HMG-CoA reductase, are increased in vivo by acute feeding of olive oil to fasted rats.

METHODS

Animal Treatments: Male, Sprague-Dawley rats (175-200g), obtained from Harlan Industries, Indianapolis, IN were housed with illumination from 8:00 hr to 17:00 hr. Animals were allowed access to standard laboratory chow and water ad libitum, and were deprived of food 24 hrs prior to sacrifice. Olive oil (oleic acid content approximately 75%) was administered (0.2 ml/100g body weight) by gastric intubation under light anesthesia with diethyl ether. Control animals were intubated with an equivalent volume of 0.9% NaCl. Animals were sacrificed and liver samples were removed for isolation of cytosols and microsomes for assays of enzyme activities.

Enzyme Assays: For determination of activities of cytosolic AcAcCoA-thiolase, AcAcCoA-ligase, and HMG-CoA synthase, livers were homogenized with four volumes of ice-cold 0.2 M Tris-HCl buffer, pH 8.2, containing 0.1M KCl and 0.5 mM dithiothreitol (DTT). The homogenate was centrifuged at 48,000 x g for 10 min, and the pellet discarded, after which the supernatant was centrifuged at 144,000 x g for 1 hr. The post-microsomal supernatant was stored at -70°C. The hepatic microsomal fraction (144,000 x g pellet) used for determination of HMG-CoA reductase activity was isolated the same way, with the exception that the original homogenization buffer was 50 mM postassium phosphate (pH7.4) containing 0.1 M KCl, 0.03 M EDTA, 2 mM DTT, and the initial centrifugation was at 16,000xg for 10 min. The fractions were maintained at -70°C until assayed.

The activity of AcAcCoA-ligase was determined spectrophotometrically by measurement of the disappearance of NADH (340 nM) in the presence of acetoacetate, CoASH, ATP, and L-(+)-hydroxyacyl-CoA dehydrogenase from pig heart (21). AcAc-CoA thiolase activity in the direction of formation of Ac-CoA was estimated by monitoring degradation of acetoacetyl-CoA (measured by the absorption of the enol form at 303 nm) caused by the addition of CoASH (1). HMG-CoA synthase activity was assayed by measurement of the incorporation of [1-^{14}C]acetyl-CoA into HMG-CoA in presence of 20 μM AcAc-CoA (22). HMG-CoA

reductase activity was determined radiochemically by reduction of DL[3-^{14}C]HMG-CoA to mevalonate (23). Cytoplasmic and microsomal protein was determined by the method of Lowry et al (24). Acetoacetyl-CoA for use in the measurement of thiolase and HMG-CoA synthase activity was prepared from diketene and CoASH (25).

Reagents: DL[3-^{14}C]HMG-CoA, D[5-^{3}H]Mevalonate, and [1-^{14}C]acetyl-CoA were purchased from New England Nuclear. Dithiothreitol, CoASH, acetic anhydride, diketene, acetoacetate, ATP, NADH, HMG-CoA, NADPH, glucose-6-P, glucose-6-P dehydrogenase and mevalonic acid lactone were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals were of analytical grade.

Statistics: Values in all tables are reported as means ± standard error (SE). Statistical comparisons were made using Student's unpaired t-test. Statistical significance was taken as $p<0.05$.

RESULTS AND DISCUSSION

Administration of olive oil (75% oleic acid) by gastric intubation to fasted rats 2 hr prior to sacrifice increased the activities of hepatic cytosolic AcAc-CoA thiolase, AcAc-CoA ligase and HMG-CoA synthase (Table 1). In a

TABLE 1. Effects of administration of olive oil to fasted rats on the activities of hepatic cytosolic HMG-CoA synthase, AcAc-CoA ligase, AcAc-CoA synthase and microsomal HMG-CoA reductase

	nmol/mg protein/min			pmol/mg protein/min
Group	HMG-CoA Synthase	AcAc-CoA Thiolase	AcAc-CoA Ligase	HMG-CoA Reductase
EXPERIMENT 1 (2 hrs)				
1. Control (No Oil)	6.80±0.40 (3)	140±16 (4)	4.15±0.85 (4)	17.2±3.5(3)
2. Experimental (With Oil)	9.70±0.52* (4)	308±47* (6)	13.40±3.0*(5)	12.5±1.4(3)
EXPERIMENT 2 (4 hrs)				
1. Control (No Oil)	5.52±0.23 (3)	148±22 (3)	ND	10.6±3.8(3)
2. Experimental (With Oil)	7.75±0.30*(3)	340±18*(3)	ND	9.5±2.7(3)

Olive oil was administered to fasted rats (0.2ml/100g animal wt.) by gastric intubation, and enzyme activities in the hepatic cytosolic or microsomal fractions were assayed 2 hrs or 4 hrs later as described in the text. All values are means ± SEM. Figures in parentheses indicate the number of observations.

ND - Not determined

*different from corresponding values in control animals. ($p<0.05$)

second experiment, activities of AcAc-CoA thiolase and HMG-CoA synthase were not different 4 hr after feeding olive oil from those observed after 2 hrs (Table 1). Activity of HMG-CoA reductase was unaffected by feeding of olive oil, after either 2 hrs or 4 hrs (Table 1).

Addition of an oleic acid-albumin complex to the assay mixture in vitro (final fatty acid concentration: 0.6 mM) did not stimulate the activity of AcAc-CoA thiolase or HMG-CoA synthase in hepatic cytosol and had a slight inhibitory effect on HMG-CoA reductase in hepatic microsomes. Moreover, oleyl-CoA (100 μM) added to the cytosolic preparations of the enzymes in vitro prior to assay also failed to stimulate the activities of AcAc-CoA thiolase, AcAc-CoA ligase or HMG-CoA synthase (data not shown). These data make it unlikely that the fatty acid or its primary metabolite could be augmenting activity by a direct interaction with the enzyme.

To assess possible contamination of the 144,000 x g supernatant with the mitochondrial forms of AcAc-CoA thiolase or HMG-CoA synthase (12,15), we measured the activity of a mitochondrial marker enzyme, citrate synthase, in hepatic cytosols from rats fed olive oil or vehicle. The cytosolic activity of citrate synthase was 5.8%±0.2 (N=3) and 6.1%±0.5 (N=3) for the control and olive oil groups, respectively, of the total activity in the crude homogenate.

At present, fatty acids are the only physiological substrates known to stimulate rates of hepatic cholesterol biosynthesis. The mechanism by which this stimulation occurs is not known, although it has been suggested by the work of this laboratory that the enhanced synthesis of cholesterol may be an indirect consequence of depletion of a metabolic pool of cholesterol required for the transport of triglyceride in the VLDL (19,20,27). However, the possibility cannot be ruled out at present that the fatty acids may have some direct cellular effect, independent of diminution of a metabolic pool of cholesterol, to stimulate synthesis of cholesterol and the activities of the enzymes discussed here. As shown in Table 1, oral administration of olive oil (which contains a high content of oleic acid) to fasted rats brought about, within 2 hours, an elevation in the activities of hepatic HMG-CoA synthase, AcAc-CoA

thiolase and AcAc-CoA ligase, while the activity of hepatic HMG-CoA reductase was unaffected. Hepatic HMG-CoA synthase, AcAc-CoA thiolase and AcAc-CoA ligase activities in the intact animal are therefore susceptible to acute stimulation by dietary fatty acid (as triglyceride), as are these enzymes in the isolated perfused liver by addition of fatty acid to the medium. Goldfarb and Pitot (28) reported that the addition of corn oil to a fat-free diet in rats increased the activity of hepatic HMG-CoA reductase after several weeks of feeding. It is conceivable, that under conditions of acute feeding of olive oil (e.g., 2-4 hrs), activity of HMG-CoA reductase is more responsive to inhibitory factors in vivo (e.g., cholesterol) than are AcAc-CoA thiolase, HMG-CoA synthase, and AcAc-CoA ligase.

The mechanism(s) by which olive oil promotes increases in the activities of these cytosolic enzymes of cholesterol biosynthesis are currently under investigation. The regulatory effects of dietary fatty acids on concentations of cholesterol in the plasma low density lipoprotein and VLDL fractions may be of special importance because of the associated risk between these lipoproteins and the development of atherosclerotic cardiovascular disease. We suggest that cholesterol is an absolute requirement for the secretion of the VLDL by the liver, a process stimulated by fatty acids 26).

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