Stimulation of hepatic cholesterol biosynthesis by fatty acids

Effects of oleate on cytoplasmic acetoacetyl-CoA thiolase, acetoacetyl-CoA synthetase and hydroxymethylglutaryl-CoA synthase

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The effects of oleic acid on the activities of cytosolic HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) synthase, AcAc-CoA (acetoacetyl-CoA) thiolase and AcAc-CoA synthetase, as well as microsomal HMG-CoA reductase, all enzymes in the pathway of cholesterol biosynthesis, were studied in the isolated perfused rat liver. Oleic acid bound to bovine serum albumin, or albumin alone, was infused for 4 h at a rate sufficient to sustain an average concentration of 0.61 ± 0.05 mM fatty acid during the perfusion. Hepatic cytosol and microsomal fractions were isolated at the termination of the perfusion. Oleic acid simultaneously increased the activities of the cytosolic cholesterol-biosynthetic enzymes 1.4–2.7-fold in livers from normal fed rats and from animals fasted for 24 h. These effects were accompanied by increased net secretion by the liver of cholesterol and triacylglycerol in the very-low-density lipoprotein (VLDL). We confirmed the observations reported previously from this laboratory of the stimulation by oleic acid of microsomal HMG-CoA reductase. In cytosols from perfused livers, the increase in AcAc-CoA thiolase activity was characterized by an increase in $V_{\max}$, without any change in the apparent K_m of the enzyme for AcAc-CoA. In contrast, oleic acid decreased the K_m of HMG-CoA synthase for Ac-CoA, without alteration of the $V_{\max}$ of the enzyme. The $V_{\max}$ of AcAc-CoA synthetase was increased by oleic acid, and there was a trend towards a small increase in the K_m of the enzyme for acetoacetate. These data allow us to conclude that the enzymes that supply the HMG-CoA required for hepatic cholesterologenesis are stimulated, as is HMG-CoA reductase, by a physiological substrate, fatty acid, that increases rates of hepatic cholesterol synthesis and cholesterol secretion. Furthermore, we suggest that these effects of fatty acid on hepatic cholesterol metabolism result from stimulation of secretion of triacylglycerol in the VLDL by fatty acids, and the absolute requirement of cholesterol as an important structural surface component of the VLDL necessary for transport of triacylglycerol from the liver.

INTRODUCTION

It has been reported previously by our laboratory [1–5] and others [6,7] that fatty acids stimulate the synthesis and secretion of the very-low-density lipoprotein (VLDL) lipids (triacylglycerol, phospholipids, cholesterol and cholesterol esters) by the isolated perfused rat liver. The increased secretion of cholesterol resulting from addition of fatty acid to the perfusion medium was accompanied by increased incorporation of $^3\text{H}_2\text{O}$ into cholesterol and stimulation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.34) in microsomal fractions isolated from these perfused livers [8,9]. Although HMG-CoA reductase is considered to be the rate-limiting enzyme in the biogenesis of cholesterol under most circumstances [10–13], it has been reported that, under some conditions, the overall rate of cholesterol synthesis may be limited by the rate of generation of HMG-CoA substrate [14–16]. The regulation of the enzymic steps that precede HMG-CoA reductase may therefore be of importance in determination of the rate of cholesterologenesis. Acetoacetyl-CoA (AcAc-CoA) thiolase (EC 2.3.1.9) and HMG-CoA synthase (EC 4.1.3.5), acting in sequence, generate

HMG-CoA in the cytoplasm for hepatic cholesterol biosynthesis [17,18]. AcAc-CoA may also be generated by the action of AcAc-CoA synthetase (EC 6.2.1.16). This enzyme enables the liver to use acetoacetate for lipogenesis [19], but can also, along with thiolase, contribute to the cytosolic pool of AcAc-CoA available for conversion into HMG-CoA [20–22]. The activities of these enzymes have been reported to be co-ordinately regulated under various physiological conditions. Cytoplasmic AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase are suppressed, along with HMG-CoA reductase, by dietary cholesterol or fasting [18,21], conditions that decrease the rate of hepatic cholesterologenesis. The activities of AcAc-CoA thiolase and HMG-CoA synthase are also depressed by exogenous sterol in cultured fibroblasts [23]. Conversely, these enzymes are activated, along with HMG-CoA reductase, by inclusion of cholestyramine and/or lovastatin in the diet, presumably by depletion of a metabolic pool of cholesterol in the liver [21,24]. Lovastatin has also been shown to induce AcAc-CoA thiolase and HMG-CoA synthase, as well as HMG-CoA reductase, in a fibroblast cell line [25].

We now report that oleic acid, in addition to

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; AcAc-CoA, acetoacetyl-CoA; TG, triacylglycerol; VLDL, very-low-density lipoprotein.

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stimulating HMG-CoA reductase activity in the perfused rat liver [8,9], augments the activity of AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase. We suggest that the action of fatty acids to stimulate the activities of these enzymes is a primary consequence of depletion of a hepatic pool of cholesterol required for the secretion of VLDL triacylglycerol [26]. Depletion of this pool of cholesterol may release the feedback inhibition of these enzymes. Preliminary results of this study have been reported [27,28].

EXPERIMENTAL

Animals

Male Sprague-Dawley rats (175–200 g), obtained from Harlan Industries, Indianapolis, IN, U.S.A., were housed with illumination from 05:00 to 17:00 h. Animals were allowed access to Purina Rodent Chow (23 % protein, 4.5 % fat, 6.0 % fibre, 8.0 % ash and 2.5 % added minerals; by wt.) and water *ad libitum* (fed group), or were deprived of food 24 h before the time of the perfusion experiments.

Liver perfusion

Animals were anaesthetized by intraperitoneal injection of pentobarbitol (60 mg/kg) between 09:00 and 11:00 h. Livers were removed surgically and perfused in a recycling system [29] with a medium and conditions as described previously [30], except that bovine serum albumin was 6 g/dl in our original buffer containing the infusate fatty acid complex. The initial volume was 80 ml, and the haematocrit was 30 %. A complex of oleic acid was prepared with lipid-free bovine serum albumin, purified as described previously [4]. After a 20 min period of equilibration, a fatty acid-albumin complex (6 g of albumin/dl, 1410 μ mol of oleic acid/dl) was infused at a rate of 166 μ mol/h (11.7 ml/h), which sustained an average concentration in the erythrocyte-free perfusate of 0.61 ± 0.05 mM ($n = 13$). In control experiments, the same concentration of albumin without fatty acid was infused. At the end of a 4 h perfusion period, samples of liver were removed for analysis of lipids and enzyme activity. VLDL lipoprotein was isolated from the perfusate by ultracentrifugation [4], and secretion of VLDL triacylglycerol and cholesterol was determined as reported previously [31,32].

Enzyme assays

For determination of activities of cytosolic AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase, livers were homogenized with 4 vol of ice-cold 0.2 M-Tris/HCl buffer, pH 8.2, containing 0.1 M-KCl and 0.5 mM-dithiothreitol. The homogenate was centrifuged at 48000 g for 10 min, and the pellet discarded, after which the supernatant was centrifuged at 144000 g for 1 h. The post-microsomal supernatant was used for determination of AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase. The fractions were maintained at -70°C until assayed. For determination of HMG-CoA reductase activity, liver samples were homogenized with 3 vol. of 10 mM-EDTA, pH 7.4, containing 0.3 mM-sucrose, 1 mM-dithiothreitol and 50 mM-NaF. The hepatic microsomal fraction (144000 g pellet) was used for determination of HMG-CoA reductase activity.

The activity of AcAc-CoA synthetase was determined

spectrophotometrically by measurement of the disappearance of NADH (at 340 nm) in the presence of acetoacetate, CoA, ATP and L-(+)-hydroxyacyl-CoA dehydrogenase from pig heart [33]. AcAc-CoA thiolase activity measured by monitoring degradation of AcAc-CoA (measured by the absorption of the enol form at 303 nm) caused by the addition of CoA [34]. Thiolase was also assayed in the reverse direction towards formation of AcAc-CoA, by monitoring the NADH (340 nm)-dependent reduction of AcAc-CoA to 3-hydroxybutyryl-CoA in the presence of 3-hydroxybutyryl-CoA dehydrogenase [18]. HMG-CoA synthase activity was assayed by measurement of the incorporation of [$1-^{14}\text{C}$]acetyl-CoA into HMG-CoA in the presence of 20 μ M-AcAc-CoA [35]. The cytosolic fractions were dialysed for 24 h against the same buffer before assay. HMG-CoA reductase activity was determined radiochemically in microsomal fractions of livers homogenized with or without NaF (50 mM) by reduction of DL-[3- ^{14}C]-HMG-CoA to mevalonate in the presence of D-[5- ^3H]-mevalonate as an internal standard [36]. Cytoplasmic and microsomal protein was determined by the method of Lowry *et al.* [37].

AcAc-CoA and acetyl-CoA were prepared as previously described [38]. Citrate synthase was assayed in both crude liver homogenate and cytosol (144000 g supernatant) by the method of Coore *et al.* [39].

Reagents

DL-[3- ^{14}C]HMG-CoA, D-[5- ^3H]mevalonate and [$1-^{14}\text{C}$]acetyl-CoA were purchased from New England Nuclear. Dithiothreitol, CoA, acetic anhydride, diketene, acetoacetate, oxaloacetate, dithionitrobenzoate, ATP, NADH, HMG-CoA, NADPH, glucose 6-phosphate, glucose-6-phosphate dehydrogenase and mevalonic acid lactone were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Thin-layer plates of silica gel G were obtained from Quantum Inc., Fairfield, NJ, U.S.A. Oleate was obtained from Nu-Chek Prep., Elysian, MN, U.S.A. All other chemicals were of analytical grade.

Statistics

Values in all Tables are reported as means $\pm$ S.E.M. Statistical comparisons were made by using Student's one-tailed t test for unpaired data. Statistical significance was taken as $P < 0.05$.

RESULTS

Perfusion experiments: effects of oleic acid on the activity of cytosolic AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase

Infusion of oleic acid, as expected [2,40], increased secretion of VLDL cholesterol and triacylglycerol (Table 1). Over 90 % of the fatty acid supplied was taken up by the livers, regardless of the nutritional state of the animals (Table 1). After perfusion of livers from fed rats with oleic acid, a 2-fold increase was observed in the activity of AcAc-CoA thiolase, assayed by formation of acetyl-CoA (Table 2). When AcAc-CoA thiolase was assayed in the reverse direction towards AcAc-CoA formation (acetyl-CoA condensation), the basal enzyme activity was about 4 % that of AcAc-CoA cleavage rate in livers from fed animals, and infusion of oleic acid increased thiolase activity by similar proportion as for the cleavage reaction (11 ± 2 versus 29 ± 1.5 nmol/min per mg of

Table 1. Effects of oleic acid on the net output of VLDL cholesterol and triacylglycerol by the perfused liver

Livers were perfused for 4 h. Where indicated, 166 μmol of oleic acid/h was infused. VLDL were then isolated by ultracentrifugation, washed, delipidated and separated by SDS/polyacrylamide-gel electrophoresis, as described in the text. VLDL lipid classes were separated by t.l.c. as described in the text. The hepatic uptake of non-esterified fatty acids is calculated by disappearance from the medium. All values ($\mu\text{mol/h}$ per g of liver) are means $\pm$ S.E.M. Numbers of observations are given in parentheses; *different from corresponding value in the absence of oleate ($P < 0.05$).

Group	VLDL cholesterol	VLDL triacylglycerol	Uptake of non-esterified fatty acid
(A) Fed			
1. No oleate	0.027 ± 0.003 (17)	0.203 ± 0.025 (17)	-0.48 ± 0.01 (10)
2. With oleate	$0.077 \pm 0.006^*$ (11)	$0.775 \pm 0.090^*$ (11)	$12.58 \pm 0.50^*$ (8)
(B) Fasted			
1. No oleate	0.027 ± 0.009 (9)	0.145 ± 0.08 (10)	-0.56 ± 0.11 (5)
2. With oleate	$0.064 \pm 0.016^*$ (9)	$0.309 \pm 0.070^*$ (9)	$10.09 \pm 1.1^*$ (6)

Table 2. Effects of prior perfusion of the liver with oleic acid on the activities of HMG-CoA synthase, AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA reductase

Livers were perfused for 4 h. Where indicated, 166 μmol of oleic acid/h was infused. The hepatic cytosol or microsomal fractions were then isolated and enzyme activities were assayed as described in the text. AcAc-CoA thiolase activity was measured in the direction of cleavage of AcAc-CoA to generate acetyl-CoA. HMG-CoA reductase was assayed in the presence of NaF (50 mM). All values are means $\pm$ S.E.M. Numbers of observations are given in parentheses; *different from corresponding value in the absence of oleic acid ($P < 0.05$); †different from corresponding value in livers from fed animals ($P < 0.05$). ND, not determined.

Group	HMG-CoA synthase (nmol/min per mg of protein)	AcAc-CoA thiolase (nmol/min per mg of protein)	AcAc-CoA synthetase (nmol/min per mg of protein)	HMG-CoA reductase (pmol/min per mg of protein)
(A) Fed				
1. No oleate	5.32 ± 0.82 (9)	223 ± 39 (11)	20.2 ± 6.8 (9)	12.8 ± 4.9 (5)
2. With oleate	$7.30 \pm 0.78^*$ (11)	$483 \pm 77^*$ (7)	$44.0 \pm 10.5^*$ (9)	$33.6 \pm 6.9^*$ (5)
(B) Fasted				
1. No oleate	$2.46 \pm 0.24^\dagger$ (5)	141 ± 20 (4)	10.8 ± 3.1 (6)	ND
2. With oleate	$4.96 \pm 0.55^\dagger^*$ (5)	$380 \pm 38^*$ (4)	21.1 ± 6.0 (6)	ND

protein for control and oleic acid respectively). This increase was also observed in livers from fasted animals (4.4 ± 1 versus 12 ± 1 nmol/min per mg). AcAc-CoA synthetase activity was also increased about 2-fold, and a smaller increase in the activity of HMG-CoA synthase was detected in the hepatic cytosol. In agreement with our previous observations [8,9], HMG-CoA reductase activity determined after homogenization and assay in the presence of NaF was also stimulated by prior perfusion with oleic acid (Table 2). In separate experiments, livers perfused under similar conditions were homogenized in the absence of NaF (as described by Salam *et al.* [41]), and HMG-CoA reductase activity was determined to be 72.9 ± 6.8 ($n = 5$) and 153 ± 30 ($n = 5$) pmol/min per mg of protein for the control and oleic acid infusions respectively ($P < 0.02$). As expected, these values were higher than those in presence of NaF (Table 2).

Perfusion of livers from fasted rats with oleic acid resulted in increases in the activities of HMG-CoA synthase and AcAc-CoA thiolase similar to those

observed in the fed state. AcAc-CoA synthetase was not significantly activated in these livers. The activities observed in the presence of oleic acid tended to be lower in livers from fasted than in those from fed animals, although the differences between fasted and fed groups achieved statistical significance only for HMG-CoA synthase ($P < 0.04$).

Effects of prior perfusion of the liver with oleic acid on kinetic constants of cytosolic AcAc-CoA thiolase, AcAc-CoA synthetase and HMG-CoA synthase

Perfusion of livers from fed rats with oleic acid increased the $V_{\max}$ of cytoplasmic AcA-CoA thiolase without changing the apparent K_m of the enzyme for AcAc-CoA (Fig. 1a, Table 3). The K_m for AcAc-CoA under these conditions was unchanged: $20.9 \pm 2.2 \mu\text{M}$ for the control ($n = 3$) and $16.5 \pm 3.8 \mu\text{M}$ for the experimental ($n = 3$) groups. In contrast (Fig. 1b, Table 3), the apparent K_m of HMG-CoA synthase for acetyl-CoA was diminished by perfusion of the liver with oleic acid, but the $V_{\max}$ of the enzyme was unaltered. Perfusion with

Fig. 1. Effects of oleic acid on the kinetic constants of AcAc-CoA thiolase (a), HMG-CoA synthase (b) and AcAc-CoA synthetase (c)

Activities of enzymes under study were assayed, as described in the text, in cytosols isolated from livers of fed rats, after perfusion for 4 h with (■) or without (□) oleic acid (0.6 mM), with different concentrations of AcAc-CoA, acetyl-CoA or acetoacetate as substrates for AcAc-CoA thiolase, HMG-CoA synthase or AcAc-CoA synthetase respectively. The $V_{\max}$ and K_m values at a saturating concentration of CoA (50 μM) for AcAc-CoA thiolase, of AcAc-CoA (20 μM) for HMG-CoA synthase, and of NADH (50 μM), CoA (100 μM) and ATP (200 μM) for AcAc-CoA synthetase, were calculated from the intercept of the reciprocal reaction velocity and intercept of reciprocal substrate concentrations respectively. Each point on the graph represents the mean $\pm$ S.E.M. of three observations. The best linear least-squares fit to the data points is indicated.

oleic acid increased the $V_{\max}$ of AcAc-CoA synthetase (Fig 1c, Table 3) and tended to increase the K_m of this enzyme for acetoacetate ($P = 0.055$).

Addition of an oleic acid-albumin complex to the

assay mixture *in vitro* (final fatty acid concn. 1.2 mM) failed to 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 microsomal fractions (Table 4). Moreover, when oleoyl-CoA (100 μM) was added to the cytosolic preparations of the enzymes *in vitro* before assay, no stimulatory effects on the activities of AcAc-CoA thiolase, AcAc-CoA synthetase or HMG-CoA synthase were observed (results not shown).

To assess possible contamination of the 144000 g supernatant with the mitochondrial forms of AcAc-CoA thiolase or HMG-CoA synthase [24,35] we measured the activity of a mitochondrial marker enzyme, citrate synthase, in livers perfused with or without oleic acid. In both groups, citrate synthase in the cytosol was about 5% of the total activity in the crude homogenate (5.6 ± 1.4 and $5.2 \pm 1.2\%$ for oleate and control groups respectively; $n = 3$ in each group). On the basis of these measurements of the activity of citrate synthase, it does not appear that activities of cytosolic AcAc-CoA thiolase or HMG-CoA synthase were disturbed significantly by leakage of the mitochondrial forms of these enzymes. However, some investigators have reported that citrate synthase can associate with the mitochondrial inner membrane [42].

DISCUSSION

To the best of our knowledge, fatty acids are the only physiological substrates known to stimulate hepatic synthesis and secretion of cholesterol [1,7]. Although the mechanism by which this stimulation is affected is not known, it has been suggested by the work of our 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 triacylglycerol in the VLDL [43,44]. If cholesterol depletion is the immediate cause of increased rates of cholesterol biogenesis and of increased HMG-CoA reductase activity, it might be expected that the increased activity of this enzyme would also be accompanied by increased activity of cytosolic AcAc-CoA synthetase, AcAc-CoA thiolase and HMG-CoA synthase, since these enzymes are all activated by treatment with cholestyramine and lovastatin [21,24,45], drugs presumed to act by depletion of hepatic cholesterol.

Other laboratories have shown that short-term perfusion of liver with fatty acids [46] or incubation for 1 h of hepatocytes with oleic acid [22] decreases rather than increases cholesterol synthesis. These data with hepatocytes have recently been confirmed in our laboratory (L. M. Cagen, M. B. Elam, W. H. Salam & M. Heimberg, unpublished work). In the study by Pullinger & Gibbons [22], cholesterol synthesis and HMG-CoA reductase activity were both increased after 4 h of exposure to the fatty acid. In the work reported here, livers were perfused for 4 h with or without 0.6 mM-oleic acid. This concentration is within the normal range for plasma non-esterified fatty acids [4]. Oleate was reported in a previous study to be more effective than either palmitate or linoleate in stimulating hepatic HMG-CoA reductase activity [2,4], although the molar ratio of hepatic output of cholesterol/triacylglycerol was in the order palmitate > oleate > linoleate.

As shown in Table 2, the activities of AcAc-CoA

Table 3. Effects of prior perfusion of the liver with oleic acid on kinetic constants of cytosolic HMG-CoA synthase, AcAc-CoA thiolase and AcAc-CoA synthetase

Livers were perfused for 4 h. Where indicated, 166 μmol of oleic acid/h was infused. Cytosolic fractions of the liver were isolated after perfusion for determination of enzyme activities, as described in the text. For each enzyme, the initial velocity was determined as a function of concentration of one substrate (acetyl-CoA for HMG-CoA synthase, AcAc-CoA for thiolase and acetoacetate for AcAc-CoA synthetase) in the presence of saturating concentrations of alternative substrates. Values of K_m were determined as described in Fig. 1. All values are means $\pm$ S.E.M. for three observations; *different from corresponding value in the absence of oleate ($P < 0.05$).

Enzyme	No oleate		With oleate	
	K_m (μM)	V_{max} (nmol/min per mg)	K_m (μM)	V_{max} (nmol/min per mg)
HMG-CoA synthase	47.6 ± 10.5	9.1 ± 1.6	$8.2 \pm 0.05^*$	8.4 ± 0.05
AcAc-CoA thiolase	20.9 ± 2.2	257 ± 18	16.5 ± 3.8	$468 \pm 69^*$
AcAc-CoA synthetase	7.5 ± 0.33	28 ± 1.1	10.90 ± 1.20	$82 \pm 7^*$

Table 4. Effects of oleic acid and bovine serum albumin on activities of cytosolic AcAc-CoA thiolase and HMG-CoA synthase, and on microsomal HMG-CoA reductase

Livers from fed rats were perfused for 4 h with infusion of bovine serum albumin alone, to serve as controls. The hepatic cytosol or microsomal fractions were then isolated, and enzyme activities were assayed to establish baseline activities (A). HMG-CoA reductase was assayed in the presence of NaF (50 mM). After prior incubation of the cytosol or microsomal fractions with oleic acid (1.2 mM) as a complex with bovine albumin (6%, w/v), or with albumin alone (6%), for 30 or 60 min, enzyme activities were again measured. All values are means $\pm$ S.E.M. for three observations; *different from corresponding value in group A ($P < 0.05$).

Addition to assay system	HMG-CoA synthase (nmol/min per mg of protein)	AcAc-CoA thiolase (nmol/min per mg of protein)	HMG-CoA reductase (pmol/min per mg of protein)
(A) None	4.36 ± 0.63	286 ± 15.6	18.20 ± 1.67
(B) Plus oleate-albumin			
1.30 min	2.96 ± 0.33	249 ± 6.98	$11.90 \pm 0.85^*$
2.60 min	2.84 ± 0.21	222 ± 36.2	$13.00 \pm 1.30^*$
(C) Plus albumin alone			
1.30 min	3.97 ± 0.66	283 ± 29.1	$13.10 \pm 1.63^*$
2.60 min	3.96 ± 0.69	294 ± 22.3	16.30 ± 2.20

synthetase, AcAc-CoA thiolase and HMG-CoA synthase were all elevated after 4 h of perfusion with oleic acid. The present model therefore suggests the presence of a common regulatory factor that stimulates the activities of these enzymes along with HMG-CoA reductase. The lack of any stimulatory effect in the assay system of added oleic acid or oleoyl-CoA on the activities of these enzymes makes it unlikely that the fatty acid or its primary metabolite could be responsible for stimulation of activity by a direct interaction with the enzyme. Moreover, Lehrer *et al.* [47] observed a direct inhibition *in vitro* of microsomal HMG-CoA reductase by oleic acid. Inhibition of cholesterologenesis from acetate in rat liver homogenates by high concentrations of oleic acid has been previously reported [48].

The effects of oleic acid on the kinetic constants of HMG-CoA synthase, AcAc-CoA thiolase and AcAc-CoA synthetase are presented in Table 3 and Fig. 1. The V_{max} of AcAc-CoA thiolase or AcAc-CoA synthetase was increased by oleic acid, whereas the K_m values of these enzymes for the indicated substrates were unchanged. The K_m of rat liver cytoplasmic thiolase for

AcAc-CoA had been reported previously to be 33 μM under normal conditions [21]. This might indicate that perfusion with oleic acid augments the activity of AcAc-CoA thiolase or AcAc-CoA synthetase by increasing the amounts of enzyme protein in the hepatic cytosol. The increase in V_{max} is much larger than the apparent effect on K_m for acetoacetate; uptake of fatty acid would also increase the cytoplasmic concentration of acetoacetate. The net effect of oleic acid should then be in the direction of increased rate of formation of AcAc-CoA by this pathway. In contrast, the V_{max} of HMG-CoA synthase was not altered by oleic acid, whereas the K_m for acetyl-CoA was diminished (Table 3; Fig. 1b). The value of K_m in controls is close to that reported previously by others (50 μM) [31]. These results suggest that augmentation of HMG-CoA synthase activity by oleic acid might be due to modification of pre-existing enzyme protein. Increased HMG-CoA synthase activity in rat liver brought about by treatment with cholestyramine and lovastatin has recently been shown to be accompanied by increases in mRNA for the enzyme in the cytoplasm [45]. This increase is accompanied by an

increase in HMG-CoA synthase protein. It may be that the mechanism of augmentation of HMG-CoA synthase activity brought about by chronic cholestyramine and lovastatin treatment differs from that elicited by acute exposure to oleic acid. The actions of cholestyramine and lovastatin on HMG-CoA reductase and on HMG-CoA synthase are thought to be an indirect consequence of depletion of a metabolic pool of cholesterol in the liver. It is certainly possible that augmentation of cholesterol synthesis and of individual enzymes of the cholesterol-biosynthetic pathway by oleic acid is not due to depletion of an intrahepatic pool of cholesterol, but is a response to some other signal generated by the fatty acid.

The mechanism(s) by which oleic acid (and perhaps other fatty acids) affects the activities of these enzymes of cholesterol biosynthesis require investigation. It is our conviction that cholesterol is required for the secretion of the VLDL, a process stimulated by fatty acids [26]. The requirement for cholesterol in the surface coat of the VLDL may be the physiological basis for the stimulation by fatty acids of secretion and synthesis of cholesterol. Removal of free cholesterol from a putative hepatic metabolic pool in the process of formation and secretion of the VLDL may decrease the inhibitory signal on cholesterol biosynthesis.

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