Fatty acids as determinants of triglyceride and cholesteryl ester synthesis by isolated hepatocytes: kinetics as a function of various fatty acids

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Abstract Triglyceride synthesis by hepatocytes is currently thought to be the rate-limiting step for lipoprotein formation. In order to determine whether triglyceride and cholesteryl ester syntheses in hepatocytes are sensitive to physiological fluctuations of serum fatty acids, the fatty acid dependence of these pathways was examined. Uptake of various fatty acids and incorporation into triglyceride and cholesteryl esters were studied in isolated rat hepatocytes. Rates were determined under conditions of linear incorporation and related to the concentrations of total and unbound fatty acid in a mixture of fatty acids and albumin. The findings were: pathways for triglyceride and cholesteryl ester synthesis saturated at unbound fatty acid concentrations (or fatty acid albumin ratios) within the range of serum values and thus would be acutely modulated by fluctuations in serum fatty acids. Addition of cholesterol to the medium increased cellular cholesterol, but did not alter rates of cholesterol esterification, suggesting that endogenous cholesterol synthesis provided the needed substrate. Oleate, palmitate, and linoleate were comparable in their saturation kinetics and ability to support triglyceride and cholesteryl ester synthesis. Consequently, their binding affinity for serum albumin would determine their lipid-incorporation rates. On this basis, in humans, oleate would yield the lowest rates as it has the lowest unbound fatty acid at each fatty acid–albumin ratio. Stearate, in contrast to the other fatty acids, was poorly esterified into neutral lipids by hepatocytes. Poor hepatic metabolism of stearate most likely explains previous findings of a hypocholesteremic effect of diets high in stearate as compared to other saturated fatty acids. The findings are generally consistent with an important role of serum fatty acid–albumin ratios in acutely modulating lipid and consequently lipoprotein synthesis by hepatocytes.—**Kvilekval, K., J. Lin, W. Cheng, and N. Abumrad.** Fatty acids as determinants of triglyceride and cholesteryl ester synthesis by isolated hepatocytes: kinetics as a function of various fatty acids. *J. Lipid Res.* 1994. **35:** 1786–1794.

Supplementary key words unbound fatty acid • saturation kinetics

Levels of serum lipoproteins have been positively correlated with the risk for atherosclerosis (1–3). Two major contituents of lipoproteins, triglycerides (TG) and cholesteryl esters (ChE), utilize long-chain fatty acids (FA) as essential precursors. TG synthesis was recently reported to be the rate-limiting step in hepatic lipoprotein production (4), suggesting a potential regulatory role for serum FA (5, 6). However, this would only apply if hepatic synthesis of TG and ChE were sensitive to fluctuations in serum FA. Although earlier reports established the relationship of serum FA to hepatic lipids (6), more recent studies failed to show such a correlation (7, 8). Two main reasons could explain this discrepancy. First, determinations were conducted in vivo or in the perfused liver and were thus complicated by factors such as diffusion in the tissue and/or the very rapid turnover of serum FA. Second, FA in serum is bound to albumin, with the concentration of free or unbound FA (ubFA) not exceeding 0.3% of the total (9). UbFA, a function of the FA–albumin molar ratio, does not change in proportion with total FA (10, 11) and determines cellular FA uptake (12–15). However, ubFA has not been considered in metabolic studies.

In this report, we have explored the dependence of pathways for TG and ChE synthesis by isolated hepatocytes on FA and ubFA concentrations. Hepatocyte suspensions minimize problems of substrate diffusion and allow short time measurements, so kinetic characteristics of both pathways could be determined. Oleate, palmitate, linoleate, and stearate, the predominant long-chain FA in serum, were compared for their ability to support production of these lipids. The information obtained should help relate fluctuations in serum FA to hepatic lipid metabolism and to lipoprotein production.

Abbreviations: TG, triglyceride; ChE, cholesteryl ester; FA, fatty acid; ubFA, unbound fatty acid; KRH, Krebs-Henseleit-HEPES buffer; KHB, Krebs-Henseleit bicarbonate buffer; PCV, packed cell volume.

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METHODS

Materials

[^{14}C(U)]glucose, [9,10-^{3}H]oleic acid, [9,10-^{3}H]palmitic acid, [1-^{14}C]stearic acid, and [1,2-^{3}H(N)]cholesterol were obtained from New England Nuclear (Boston, MA). [1-^{14}C]linoleic acid and aqueous scintillation fluid were obtained from Amersham (Arlington Heights, IL). Collagenase (Type III) was from Worthington (Freehold, NJ). Fatty acids, diolein, triolein, cholesterol, cholesteryl esters, albumin (bovine serum albumin, BSA, fraction V, essentially fatty acid-free), phosphatidylcholine, and low density lipoproteins were from Sigma (St. Louis, MO). Silica G thin-layer plates were from Analtech (Newark, DE).

Cell preparation

Hepatocytes were obtained from Sprague-Dawley rats weighing 200–250 g. Rats were anesthetized with sodium pentobarbital injected into the peritoneum. The livers were then perfused and the hepatocytes were isolated essentially as described by Claus, Pilkis, and Park (16). Briefly, livers were initially perfused with Ca^{2+}-free Krebs-Henseleit buffered with HEPES (KRH) for about 10 min. The perfusate was then switched to KRH containing 1 mM Ca^{2+} and Type III collagenase (30 mg%) for 30–40 min. At the end of this perfusion, the liver was harvested, cut into small pieces, and shaken for 20 min at 37°C in collagenase-containing medium with continuous gassing by a 95% O_2/5% CO_2 mixture. The cell suspension was then filtered through nylon mesh and washed once with Krebs-Henseleit bicarbonate buffer (KHB), containing 0.4% albumin, 0.1% glutamate, and 0.08% pyruvate, and then twice with KHB without albumin. The washed cells were suspended in 2–4 ml of KHB (without albumin) to give a cell density of 20–30% (v/v) using the packed cell volume, determined by centrifugation of a 7-μl aliquot in microcapillary tubes. Cell viability was tested by Trypan blue exclusion and was greater than 90%. An aliquot of cell suspension corresponding to 10–50 μl packed cell volume (PCV) was used for each time point measurement. A 25-μl PCV consisted of about 3.9×10^6 cells or of 4.8 mg protein.

Isotope preparation

The isotope solution consisted of KHB containing 3 mM glucose, unlabeled and labeled FA (^{3}H at 3000 cpm/μl, ^{14}C at 1000 cpm/μl), complexed to albumin at different molar ratios (13, 17). The ratios were obtained both by keeping the concentration of albumin constant and varying the concentration of FA or by varying the albumin at constant total FA. The labeled and unlabeled FA were premixed and dissolved in a small volume of ethanol, added slowly with stirring to warm KHB containing albumin, and allowed to equilibrate for 1 h. The final concentration of ethanol did not exceed 0.1%. Alternatively, labeled and unlabeled FA were mixed and evaporated under N_2 in a glass tube. Warm KHB with albumin was added and the mixture was left stirring until most of the radioactivity added was in solution. For stearic acid, an additional procedure (18) involved recovering the FA off the side of the glass tube in 2 mM NaOH at 70°C. Warm KHB with albumin (1.5 to 2 × concentrated) was then added. All isotopic solutions were subjected to filtration (Milipore 0.4 μm) and the radioactivity recovered (about 90%) after filtration was considered to reflect the complexed FA.

Uptake and incorporation of FA

Before the assay the isotopic solution was prewarmed to 37°C and gassed with an O_2–CO_2 95:5 mixture. The volume of isotopic solution was kept greater than 20 times that of the cell suspension and the concentration of FA (60–440 μM) used always ensured that FA in the medium did not fall below 70% of the starting concentration. At the desired time, an aliquot of cell incubation corresponding to 10–25 μl packed cells was removed and cells and media were separated by centrifugation. The cell pellet was washed twice with 1 ml ice-cold KRB, resuspended in 100 μl of buffer and processed for lipid extraction. Similar protocols were used for all FA. Comparisons between the different FA were always from experiments where each FA was used in parallel with oleate with the same cell preparation.

FA incorporation rates were derived from the linear portions of complete time courses and were related to the concentration of ubFA in the incubation medium. This was calculated by a computer routine as previously described (11, 13) using dissociation constants determined by Spector, John, and Fletcher (14).

Extraction and separation of lipids

Total lipids from washed cell pellets and media were extracted by the method of Bligh and Dyer (19), and were separated by thin-layer chromatography (TLC) on silica gel G with hexane–diethyl ether–acetic acid 80:20:1 (v/v). The major lipid fractions were identified by comparison with standards that were run simultaneously on one side of the plate and visualized by Rhodamine. For counting, the silica was added directly to the counting fluid (ACS) and the vials were shaken for 20 min before counting. To quantitate radioactivity in a lipid fraction, the percent (of total silica counts) recovered in that fraction was multiplied by the total cellular uptake (20, 21). Nonspecific background radioactivity, determined from zero-time incubations, was very low for all FA (less than 3% of uptake values) except for the case of stearate.

Preparation of cholesterol dispersions

In some experiments, hepatocytes were preincubated in medium containing cholesterol-phospholipid dispersions (22). Briefly, egg phosphatidylcholine (48 mg) was dissolved in hexane and dried under N_2 in a glass tube, and free cholesterol (65 mg) in benzene was added. After mixing, the solvent was evaporated under N_2 in a vacuum. Twelve ml of 0.15 M NaCl was added and the mixture was sonicated (Bransonic 12) under N_2 for 1 h with the temperature kept under 50°C. The mixture was centrifuged for 30 min at 26,000 rpm to sediment unincorporated or crystalline cholesterol. About 50–60% of the cholesterol originally added was sedimented as determined in separate experiments using [^{3}H]cholesterol. Thus the final cholesterol to phospholipid ratio was about 1.2–1.4. Aliquots of the supernatant, which contained the cholesterol dispersions, were then added to the cell suspension (approximately 150 μg cholesterol/ml).

Measurement of cholesterol, cholesteryl esters, and of free FA

Cellular cholesterol and cholesteryl esters were measured in total lipid extracts enzymatically according to established procedures (23). Levels of unesterified FA were measured using an enzymatic colorimetric assay from Wako Pure Chemical Industries (Biochemical Diagnostics, Edgewood, NY).

RESULTS

Time course for oleate uptake and incorporation into lipids

Time courses for uptake (free and esterified) of [^{3}H]oleate, expressed as nmol/50 μl PCV, at various FA–albumin ratios, are shown in **Fig. 1** (top panel). In all experiments, the cells took up less than 25% of medium oleate at the end of 30 min, as tested from determination of both cell-associated radioactivity and of radioactivity remaining in the medium. Cell viability, tested at the end of the 30-min incubations, was higher than 85%. Thus, time courses that extended from 1- to 30 min were used to generate rates for oleate uptake or incorporation into lipids using linear regression.

The time course for oleate incorporation into TG (Fig. 1, middle panel) closely paralleled that of total cellular uptake (Fig. 1, top panel). This reflected the fact that a large fraction of intracellular radioactivity (about 60%) was recovered in TG. Significant incorporation was also measured into diglycerides (about 15%) and into cholesteryl esters (6–10%). Recovery of radioactivity in cholesterol was minimal and undetectable in most experiments (<1%). The remaining radioactivity was in phospholipids, FA, and monoglycerides which together accounted for about 15% of cellular counts.

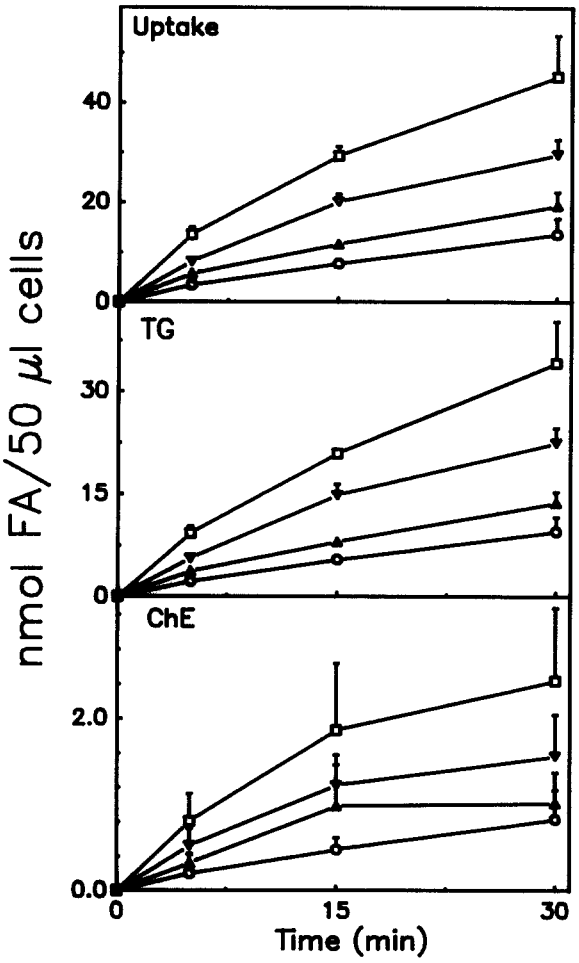

Fig. 1. Time course for [^{3}H]oleate uptake and incorporation into triglyceride (TG) and cholesteryl esters (ChE). Hepatocytes were incubated (about 15% v/v) with [^{3}H]oleate for the times indicated on the abscissa. The FA–albumin molar ratios were 0.4 (○), 0.8 (△), 1.5 (▽) and 3.0 (□). The ubFA concentrations corresponding to these ratios were, respectively: 0.032, 0.084, 0.24, and 1.3 μM. Ratios were obtained by changing the albumin concentration at constant oleate (300 μM) or by varying the FA, keeping albumin constant as described under Results. Extraction and separation of the lipid fractions were as described under Experimental Procedures. Data presented are averages from five experiments and are shown with their standard errors (in some cases, standard error bars are hidden by the symbols). Top panel shows uptake of oleate; middle and bottom panels show oleate incorporation into TG and ChE, respectively.

Validation of rate measurements

The rates determined for oleate uptake and incorporation were adequate estimates of true rates based on the following. *1)* They were derived from the linear portions of time courses. Under such conditions, supply of substrate was steady and changes in the FA albumin ratio and/or in availability of ubFA were not significant enough to alter linearity of the time course. At the highest FA–albumin ratios used, however, the curves showed a tendency to plateau earlier than at the lowest ratios so shorter portions of the time course were used for rate determinations.

2) The FA in the medium, measured chemically at the end of the 30-min incubations, changed slightly and in parallel with medium counts, indicating that the specific activity of FA in the medium remained constant. *3)* Free intracellular [^{3}H]oleate, determined from chromatography of cell lipid, stabilized by the first minute of incubation at a very low value (about 1% of oleate uptake) reflecting rapid equilibration of a small intracellular free FA pool with [^{3}H]ubFA in the medium. *4)* In separate experiments, [^{14}C]glucose was used simultaneously with [^{3}H]oleate, as previously described for adipocytes and fibroblasts (20, 21, 24) to assess intracellular dilution of [^{3}H]oleate. The ratios of [^{3}H]FA to [^{14}C]glycerol incorporated into TG exceeded 4 at 15 min and were down to about 3 at 30 min. These values, when compared to the chemically expected ratio in TG (3:1), indicated that dilution of [^{3}H]oleate by endogenous FA, on its way to esterification, was minimal (data not shown). All the above observations indicated that the use of medium specific activity for estimation of oleate incorporation was adequate. *5)* Finally, in all experiments, the medium glucose concentration used, 3 mM, was shown to support optimal FA esterification. Increasing medium glucose to 20 mM was not associated with detectable changes in oleate incorporation into either TG or ChE (data not shown).

Dependence of oleate incorporation on the concentration of ubFA

Rates of oleate incorporation, determined from time courses up to 30 min, were related to the concentration of FA in the medium. First, it was established that incorporation was dependent on the molar ratio of FA to albumin and not on total FA concentration. This was demonstrated under conditions where the concentration of the FA–albumin complex was not limiting (the volume of isotopic solution relative to that of cell suspension was kept at greater than 20:1 to provide enough dissociating FA–albumin complexes) and as follows. Duplicate experiments were performed at the same range of molar ratios of FA–albumin (0.2 to 3.0), achieved using two different experimental approaches. In the first, total oleate was kept at 300 μM and the concentration of BSA was varied from 1.5 to 0.1 mM. In the second condition, the concentration of BSA was kept constant at 147 μM and the concentration of oleate was varied from 60- to 440 μM. Oleate incorporation values were found to vary as a function of the FA–BSA ratio, and were independent of total FA. Under both experimental conditions, lipid incorporation of oleate was similar at each molar ratio of FA–albumin and the saturation kinetics exhibited (described below) were similar, so results from both sets of experiments were pooled.

Incorporation rates of oleate (nmol FA/min/50 μl cells) into TG and ChE (determined from the linear portion of the curve) were saturable when related to the concentration of ubFA (**Fig. 2A and B,** top panels). On the other hand, when incorporation rates were plotted against total FA concentration, the process appeared to be less saturable (inset Fig. 2A). This reflected the fact that the concentration of ubFA does not change in direct proportion with that of total FA (11, 14). As FA are supplied to hepatocytes bound to serum albumin and ubFA is the FA fraction that should be considered (12), the data indicate that pathways for synthesis of both TG and ChE are highly saturable with respect to the FA substrate.

Fig. 2 (bottom panels) shows the data analyzed by Hanes plot (S/v versus S) to yield the kinetic parameters for TG and ChE synthesis. Incorporation of oleate into TG had a K_m of 0.14 μM and a V_{max} of 1.57 nmol/min/50 μl. Incorporation into ChE, had a K_m of 0.08 μM and a V_{max} of 0.09 nmol/min/50 μl PCV. The half-saturation values for TG and ChE syntheses by hepatocytes were within the range of unbound FA concentrations (0.01–0.3 μM) in serum.

The oleate K_m and the values cited above for serum ubFA are based on the association constants for FA–BSA reported by Spector et al. (14) as these are the most complete determinations available. However, these studies might have overestimated the concentration of unbound FA for the various FA–albumin ratios. Recent studies (18, 25) using alternative methodology to that used by Goodman (9) and Spector et al. (14), reported higher association constants for FA–albumin binding and consequently lower ubFA concentrations. These higher constants may reflect, in part, a lower probability for FA dimerization (26, 27) in the new assays. Based on the new constants, circulating ubFA would be in the range of 10–300 nM and our experiments would yield a K_m of 3 nM for oleate esterification by hepatocytes as opposed to 0.14 μM or 140 nM (Fig. 2).

Effect of exogenous cholesterol on ChE synthesis by hepatocytes

In all the above experiments the cholesterol needed for ChE synthesis was derived from endogenous sources. As hepatocytes have a high capacity for cholesterol synthesis, its supply would not be expected to be limiting. To ensure that this was the case, the effect of including cholesterol-rich lipid dispersions in the medium on rates of cholesterol esterification was determined. Addition of cholesterol dispersions to hepatocyte suspensions almost doubled cellular cholesterol from 116 to 216 μg/50 μl PCV. However, cellular ChE remained unchanged at 90 μg/50 μl PCV. Consistent with this, [^{3}H]oleate incorporation into TG or ChE was not altered by the addition of liposomes containing cholesterol. Cholesterol dispersions have been shown to be effective in providing cholesterol to the cells for esterification into cholesteryl esters (22). Consistent with this, we could measure incorporation of medium cholesterol into cholesteryl esters when [^{3}H]cho-

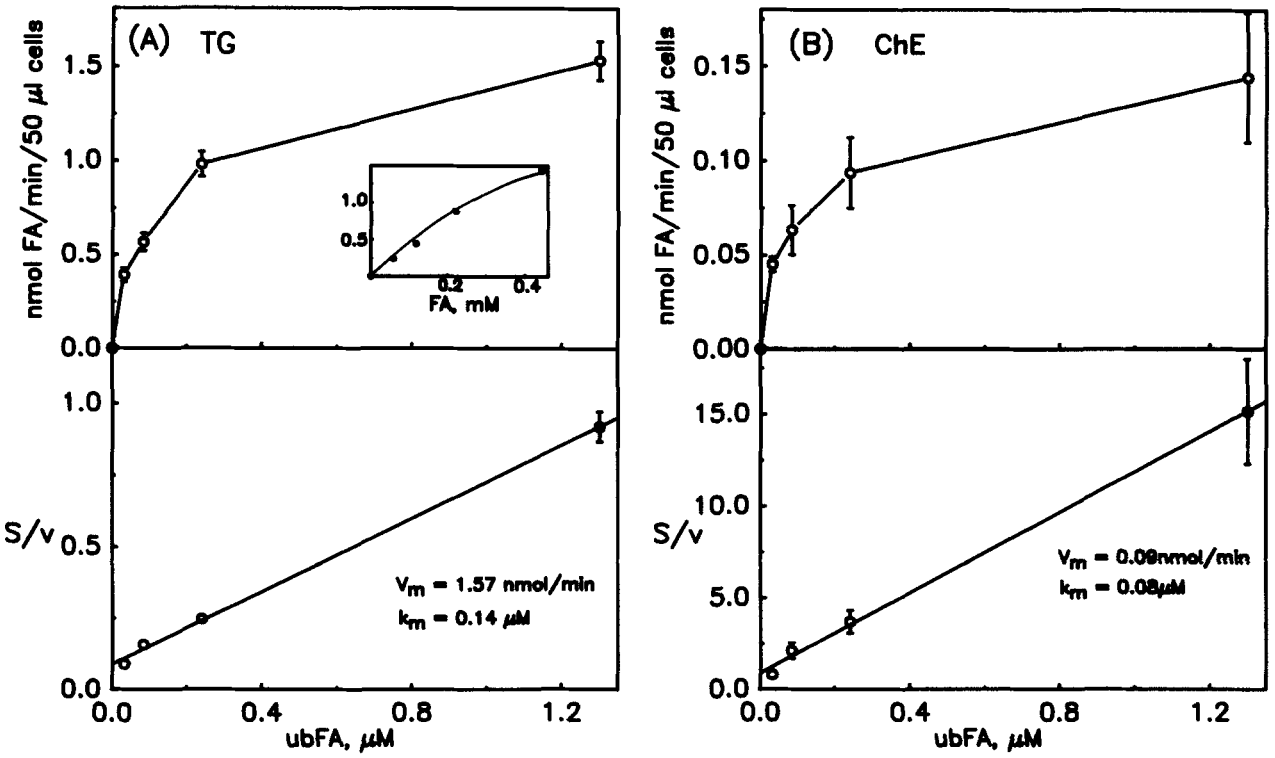

Fig. 2. Incorporation of oleate into TG and ChE as a function of ubFA concentration. Rates were derived from the linear portion of uptake time courses illustrated in Fig. 1. Top two panels show uptake rates plotted as a function of ubFA concentration calculated for the various FA–albumin ratios. Inset shows uptake incorporation in TG as a function of total FA in the medium. The bottom two panels show Hanes-Wolf plots (S/v versus S) of the data to derive the kinetic coefficients, where v = nmol/min/50 μl cells and S = μM ubFA calculated (12) based on the association constants determined by Spector et al. (14). The x-intercept = $-K_m$ and the slope = $1/V_{max}$.

lesterol-phospholipid dispersions were used in the incubations (data not shown). However, in order to rule out the possibility that the lack of effect of cholesterol dispersions was due to a nonphysiological cholesterol delivery system, the effect of including lipoproteins in the medium was examined. As shown in **Table 1**, addition of lipoproteins, to yield final concentrations of 100 and 160 μg/ml cholesterol, failed to alter rates of fatty acid incorporation into cholesteryl esters, similar to the results with cholesterol-containing lipid dispersions.

TABLE 1. Effect of incubating cells with cholesterol-containing liposome dispersions or with lipoproteins on fatty acid incorporation into triglycerides (TG) and cholesteryl esters (ChE)

	TG	ChE
	μg/50 μl PCV	
No additions	50 ± 2	3 ± 0.2
+ Lipoproteins	53 ± 3	3 ± 0.2
No additions	73 ± 3	6 ± 0.4
+ Liposomes	74 ± 3	6 ± 0.4

Lipoproteins were added to yield 100 and 160 μg cholesterol/ml with similar results. Liposomes were added to a final cholesterol concentration of 150 μg/ml. Incubations were conducted for 0–60 min. Data shown for lipoproteins are averages of palmitate incorporations at 15 and 30 min for duplicate cell incubations from two separate experiments. Incorporation (oleate) data for liposomes were obtained as described for lipoproteins and were pooled from four experiments.

Incorporation rates for palmitate, linoleate, and stearate

Uptake and lipid incorporation rates for palmitate, linoleate, and stearate were examined and compared to those for oleate. Assays for each FA were conducted in parallel with oleate using the same cell suspension to avoid artifacts introduced by variability between different hepatocyte preparations. Molar ratios of FA-BSA that would yield similar concentrations of ubFA for the respective FA were used in each experiment. Finally, as for oleate, rates were derived from complete time courses and were related to the concentration of ubFA in the medium.

Total uptake of palmitate, linoleate, and oleate exhibited similar time courses. Incorporation into TG and ChE showed similar trends. All three FA were preferentially channelled into TG but both palmitate and linoleate appeared to support less ChE synthesis than oleate (**Table 2**). Incorporation of palmitate and linoleate, similar to that of oleate, showed saturation at low levels of ubFA (**Fig. 3**). The K_ms for TG synthesis ranged from

TABLE 2. Kinetics of incorporation into triglycerides (TG) and cholesteryl esters (ChE) for oleate, palmitate, and linoleate

FA	TG		ChE	
	K_m	V_{max}	K_m	V_{max}
	μM	*nmol/h*	μM	*nmol/h*
Oleate	0.2 ± 0.02	90 ± 7	0.13 ± 0.02	9 ± 0.8
Linoleate	0.2 ± 0.02	60 ± 4	0.03 ± 0.01	3.6 ± 0.2
Palmitate	0.1 ± 0.02	72 ± 4	0.07 ± 0.01	4.2 ± 0.2
Stearate	0.05 ± 0.01	7 ± 1	0.05 ± 0.01	0.08 ± 0.009

Hepatocytes were incubated with the different FA as described under Experimental Procedures. Rates (nmol/h 50 μl cells) were derived from the linear portions of time courses from four experiments and were related to the concentration of ubFA.

0.08 μM (palmitate) to 0.18 μM (linoleate) using the association constants determined by Spector et al. (14) and about 1/20 of these values if constants reported by Richieri et al. (18) and by Bojesen and Bojesen (25) are used.

Stearate, in contrast to the other FA tested, was metabolized poorly by hepatocytes (Table 2). Most of the stearate recovered in lipid extracts of hepatocytes incubated with the FA remained in the unesterified form (60–80%), as compared to about 1% in the case of oleate.

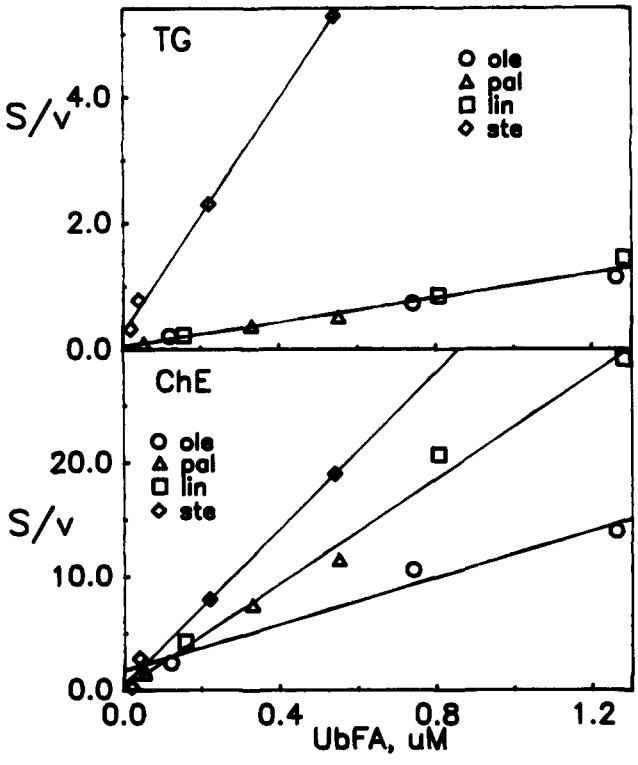

Fig. 3. Saturation kinetics for palmitate, linoleate, and stearate incorporation into TG and ChE. Each FA was assayed in parallel with oleate using the same hepatocyte preparation. Rates were derived as described in the text and in the legends to Figs. 1 and 2. Data shown are from four experiments using complete time courses.

A large fraction of this unesterified stearate reflected FA that adsorbed to the cell surface and to the assay tubes and that was not removed by the washing buffer as a result of the poor solubility of stearate. This was determined from background radioactivity, which, in the case of stearate, amounted to about 50% of uptake values at 30 min. Incorporation of stearate into TG and ChE was about one-tenth that of oleate and about one-fifth that of palmitate and linoleate at comparable ubFA concentrations. The lower metabolism of stearate did not reflect a deleterious effect of the FA on hepatocytes. Cell viability, using trypan blue exclusion, and protein content of the pelleted and washed cells were determined for hepatocytes incubated with various ratios of stearate–albumin. At the end of the 30-min incubation, cell viability was 85–87% (versus 90–95% at time zero) which was identical to that in parallel incubations with palmitate. Protein content of washed cell pellets from aliquots taken at the end of 30-min incubations with palmiate or stearate were also identical. In addition, although incorporation of stearate into neutral lipids was limited, that into polar lipids was similar to that determined for palmitate in the same experiments. Finally, incorporation of stearate proceeded linearly with time, as was the case with other FA, arguing against effects on esterification enzymes.

DISCUSSION

The present study examined regulation of hepatic TG and ChE formation by the supply of long-chain FA. Formation of both lipids, from a range of natural FA, was estimated as a function of the physiological substrate, ubFA, and saturation kinetics were determined. Our findings indicated that syntheses of TG and ChE by hepatocytes exhibit half-saturation values within the range of circulating ubFA concentrations. This indicated that fluctuations of serum FA–albumin ratios, such as those associated with fasting, diabetes, or high fat meals, would acutely modulate formation of TG and ChE by hepatocytes. Further-

more, our findings support the interpretation that supply of long-chain FA is the main determinant for synthesis of both TG and ChE by hepatocytes under physiological conditions. This is discussed in more detail below.

Saturation kinetics of TG and ChE synthesis as a function of ubFA concentration

In hepatocytes, relating oleate lipid-incorporation rates to medium ubFA concentration indicated that pathways for synthesis of TG and ChE were highly saturable, contrary to previous reports which related synthesis to total FA concentration (28). TG synthesis by hepatocytes saturated at FA–albumin ratios that are in the range of physiological values (9, 10). If lipid synthesis in rat hepatocytes is representative of human cells, the data imply that increases in serum FA would acutely increase TG synthesis. Maximal rates of TG formation would be generated at high physiological FA–albumin ratios (about 2:1) such as those that occur with hormonal action, fasting, the ingestion of fatty food, diabetes, etc. As TG synthesis and cellular TG levels regulate hepatic lipoprotein production (5, 29), increases in serum FA will be directly reflected in increases in lipoprotein secretion. Our data also indicated that increasing cholesterol supply to hepatocytes and doubling cellular cholesterol did not acutely increase lipid synthesis. A similar result was obtained when medium glucose was increased. Thus, although chronic effects of cholesterol and glucose on hepatic synthesis of TG and ChE are likely, and are not ruled out by the data, acute fluctuations in serum FA appear to be the most significant modulator. However, it is worth noting that under physiological conditions, the hepatocyte can also derive FA from lipoproteins and chylomicrons, after their intravascular lipolysis. The relative contribution of these various sources to the intracellular FA pool remains to be established.

Cholesteryl ester formation saturated with a K_m that was within the range of physiological ubFA concentrations (irrespective of which FA–albumin association constants are used for estimation of ubFA). As serum FA–albumin ratios increase, ubFA concentration increases resulting in a rise in intracellular FA. Availability of FA would then promote esterification and thus trapping of cholesterol within the cell.

Finally, exogenous FA might contribute to regulating hepatocyte lipid by an additional secondary mechanism. As cellular TG and ChE are in a continuous state of turnover (30), high FA–albumin ratios would be expected to reduce net hydrolysis of these lipids by limiting cellular FA release and promoting FA re-esterification with glycerol or cholesterol. The data generally emphasize the primary role of exogenous FA–albumin ratios in regulating triglyceride and cholesteryl ester levels within hepatocytes.

Comparison of lipid-incorporation rates for various long-chain FA

Several differences were noted in the ability of four native long-chain FA to support TG and ChE synthesis. Oleate, palmitate, and linoleate exhibited similar incorporation kinetics. The K_m and V_{max} for TG and ChE syntheses for all three FA were essentially similar. This indicated that, under physiological conditions, their incorporation rates will be determined by their affinity for serum albumin and by their FA–albumin molar ratios. Based on this, in humans, oleate, as compared with palmitate and linoleate, would generate lower levels of TG and ChE by hepatocytes, as at each FA–albumin ratio, unbound oleate is about one-fifth unbound palmitate or linoleate (9, 10). Serum unbound oleate is likely to remain within a range of concentrations (0.002–0.05 μM) that are lower than those saturating TG and ChE synthesis. Thus, oleate would be unlikely to yield maximal rates for hepatic synthesis of these lipids. These properties of oleate would be expected to promote lower rates of lipoprotein formation. Finally, it is uncertain whether oleate's ability to promote the highest ChE/TG incorporation ratios will be reflected in the type of lipoprotein formed by hepatocytes.

Stearate metabolism by hepatocytes

In contrast to the other long-chain FA tested, stearate exhibited very low lipid-incorporation rates at both low and high stearate to albumin ratios. A significant increase in cellular nonesterified FA was observed in hepatocytes incubated with stearate even at a ratio of 0.2. The free intracellular fatty acid was about 70% of the stearate taken up. The parallel decreases in TG and ChE formation would indicate that conversion of stearate to stearoyl-CoA might be the rate-limiting step, possibly reflecting poor metabolism of this FA by the palmitoyl-CoA synthase (ref. 31 and Abumrad, N. A., unpublished observations). An increase in intracellular unesterified stearate was previously reported in studies using Erlich ascites tumor cells (8). In human serum, stearate averages about 13% of the total concentration of unesterified FA (32) and its affinity for human albumin is relatively high (9, 10). Thus, in healthy humans under normal dietary conditions, with serum FA varying between 0.2 and 2.0 mM, stearate concentration would range between 0.02 and 0.2 mM. These values would correspond to molar ratios of stearate to human albumin that are between 0.05 and 0.5. These conditions might not lead to a significant accumulation of free unesterified stearate inside the cell. However, under conditions where stearate levels increase in the serum as a result of diets high in this FA, one would predict that levels of TG and ChE produced by hepatocytes would decrease as a result of the increased proportion of a poorly metabolized FA. Our results would explain previous ob-

servations related to the effects of diets high in stearate on plasma lipids. For example, it was noted previously that when the proportion of stearate in the diet is increased from 4 to 11%, there was a lowering of total plasma cholesterol as compared with a diet containing the same proportion of other saturated FA (33, 34). In addition, plasma cholesterol remained similar in subjects receiving 80% more saturated FA when the predominant FA given was stearate (34). Thus, high levels of dietary stearate would lead to a decreased synthesis of cholesteryl esters and triglycerides by hepatocytes and consequently to a decreased production of lipoproteins by these cells. Although effects of stearate on the LDL receptor or on apolipoprotein synthesis have been hypothesized (34) and remain a possibility, our results would indicate that the main mechanism mediating the hypocholerestemic action of diets high in stearate is likely to be poor stearate incorporation into neutral lipid by liver cells. In this context, limited incorporation of stearate into TG by rat adipocytes and low levels of the FA in adipocyte TG have been reported (35) suggesting similar esterification profiles for the FA in both tissues.

In general, the data presented emphasize the primary role of serum FA and more specifically of serum FA–albumin ratios in modulating hepatic lipid synthesis and, consequently, levels of plasma lipoproteins. By extrapolation, a potentially important role for serum albumin levels can be inferred in certain conditions. Although large fluctuations in serum albumin are not generally observed, they do occur in certain diseases such as in the nephrotic syndrome (36). In individuals with this syndrome, albumin loss in the urine leads to a marked drop in circulating albumin. The ensuing increase in circulating FA–albumin ratios may be a significant factor behind the high serum lipids and the increased risk of vascular disease observed in these patients (36).

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