Long-term effects of *cis* and *trans* monounsaturated (18:1) and saturated (16:0) fatty acids on the synthesis and secretion of apolipoprotein A-I- and apolipoprotein B-containing lipoproteins in HepG2 cells

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Abstract **The objective of this study was to compare the long-term effects of oleic (*cis* 18:1), elaidic (*trans* 18:1), and palmitic (16:0) acids on hepatic lipoprotein production, using HepG2 cells as an experimental model. The net accumulation in the medium of apolipoprotein A-I (apoA-I) was not significantly altered by fatty acids, whereas that of apoB was increased with oleic and elaidic acids. Oleic acid, and to a lesser extent elaidic and palmitic acids, increased the mass of triglycerides in the medium and the incorporation of [^{3}H]glycerol into secreted triglycerides. The incorporation of [^{14}C]acetate into cellular and secreted total cholesterol was stimulated by 96% and 83%, respectively, with elaidic acid but was not significantly modified by oleic or palmitic acid. Relative to oleic acid, the secretion of ^{14}C-labeled phospholipids and triglycerides was decreased 28% to 31% with elaidic and palmitic acids whereas that of free cholesterol and cholesteryl esters was enhanced 93% and 73%, respectively, with elaidic acid but remained unchanged with palmitic acid. Compared with oleic acid, elaidic acid stimulated the secretion of very low density lipoprotein cholesterol (VLDL-Chol), low density lipoprotein cholesterol (LDL-Chol), and high density lipoprotein cholesterol (HDL-Chol) by 43%, 70%, and 34%, respectively, whereas palmitic acid decreased VLDL-Chol but had no significant effect on LDL-Chol and HDL-Chol. The ratios of total cholesterol to HDL-Chol were 3.17, 3.60, and 3.25 with oleic, elaidic, and palmitic acids, respectively; the corresponding ratios of LDL-Chol to HDL-Chol were 0.87, 1.10, and 0.93, respectively. Compared with oleic and palmitic acids, the LDL and HDL particles secreted in the presence of elaidic acid contained higher levels of free cholesterol and cholesteryl esters and a lower content of phospholipids. The phospholipid-to-total cholesterol ratios of HDL were 1.05, 0.40, and 0.76 with oleic, elaidic, and palmitic acids, respectively. Our results indicate that in comparison with *cis* monounsaturated and saturated fatty acids, *trans* fatty acids have more adverse effects on the concentration and composition of lipoproteins secreted by HepG2 cells.**—Dashti, N., Q. Feng, and F. A. Franklin. **Long-term effects of *cis* and *trans* monounsaturated (18:1) and saturated (16:0) fatty acids on the synthesis and secretion of apolipoprotein A-I- and apolipoprotein B-containing lipoproteins in HepG2 cells.** ***J. Lipid Res.*** **2000.** 41: **1980–1990.**

Supplementary key words cholesterol metabolism • triglyceride secretion • liver • low density lipoproteins • high density lipoproteins • regulation of lipoprotein production

Epidemiologic studies have established that the risk of coronary artery disease (CAD) is positively related to the concentrations of total cholesterol and low density lipoprotein cholesterol (LDL-Chol) and their major apolipoprotein (apo), apoB, but negatively related to that of high density lipoprotein cholesterol (HDL-Chol) and their main apolipoprotein, apoA-I (1–3). The plasma concentrations of lipoproteins are markedly influenced by diet and numerous studies have been conducted to establish the relative effects of major dietary fatty acids on the concentration and composition of plasma lipoproteins. Collectively, these studies have led to the general agreement that dietary saturated fatty acids increase while *cis* isomers of ω-6 polyunsaturated fatty acids lower the plasma concentrations of LDL-Chol (2). The *cis* isomer of monounsaturated fatty acids are thought either to have no effect (2) or to decrease (4) LDL-Chol concentration whereas their *trans* isomers have been reported to have adverse effects on plasma lipid levels (5–8). *Trans* fatty acids are naturally present in small amounts in meat and dairy products and are also formed during hydrogenation of vegetable oils in order to transform them from liquid form to a semisolid state. Hydrogenation also results in saturation of a portion of double bonds, thus increasing the saturated

Abbreviations: apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; BSA, bovine serum albumin; CAD, coronary artery disease; Chol, cholesterol; FBS, fetal bovine serum; HDL, high density lipoproteins; LDL, low density lipoproteins; MEM, minimum essential medium; PBS, phosphate-buffered saline; VLDL, very low density lipoproteins.

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and monounsaturated fatty acids and decreasing the polyunsaturated content of vegetable oils. Currently, *trans* fatty acids contribute approximately 8% to the total dietary fat intake in the United States (5).

The reported effects of *trans* fatty acids on human plasma lipoproteins include increased plasma concentration of LDL-Chol and reduced level of HDL-Chol (6, 7, 9, 10) and possibly elevated concentration of triglycerides (11). Therefore, it has been suggested that consumption of high amounts of *trans* fatty acids putatively contributes to increased risk of CAD (12–14). Studies by Mensink and Katan (6) demonstrated that replacement of diet high in oleic acid (*cis*-18:1) with a diet high in elaidic acid (*trans*-18:1) increased total cholesterol and LDL-Chol and decreased HDL-Chol (6, 15). In contrast, replacement of oleic acid with saturated fatty acids caused an increase in LDL-Chol level similar to that with *trans* fatty acids without affecting that of HDL-Chol, resulting in a higher ratio of LDL-Chol to HDL-Chol with *trans* fatty acid than with saturated fatty acids (6). This unfavorable effect of *trans* fatty acids relative to both *cis* unsaturated fatty acids and saturated fatty acids was demonstrated in several subsequent studies (9). In addition, it has been shown that relative to saturated and *cis* unsaturated fatty acids, *trans* fatty acids decrease apoA-I and increase apoB levels in plasma (6, 15, 16). The quantitative relation between *trans* fatty acid intake and plasma total cholesterol, LDL-Chol, and HDL-Chol and their contribution to the risk of CAD relative to saturated fatty acids, nonetheless, remain a matter of some dispute (5). The inconsistency in the results of in vivo studies is partly due to multiple changes in the diet when a hydrogenated vegetable oil replaces its nonhydrogenated form and, therefore, it is difficult to fully attribute the observed effects to increased *trans* fatty acid intake.

Few studies have examined the effect of individual fatty acids on lipoprotein metabolism in humans, specifically at the hepatic level. In the present study we investigated the in vitro direct and independent effects of dietary *cis* and *trans* 18:1 and saturated 16:0 fatty acids on the hepatic synthesis and secretion of apoA-I- and apoB-containing lipoproteins. The HepG2 cell line was selected as the experimental model because these cells retain many of the biochemical functions of human liver parenchymal cells (17, 18) including the regulation of synthesis and secretion of plasma lipoproteins and apolipoproteins by dietary fatty acids and cholesterol (19–22). Part of this work has been reported in an abstract form (23).

EXPERIMENTAL PROCEDURES

Materials

Minimum essential medium (MEM), trypsin, sodium pyruvate, and L-glutamine were purchased from GIBCO (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Whittaker Bioproducts (Walkersville, MD). Triton X-100, leupeptin, fatty acids, and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). The purity of fatty acids was greater than 99% by capillary gas chromatography. [^{3}H]glycerol and [^{14}C]acetate were obtained from Amersham (Arlington Heights, IL).

Cell culture

The human hepatoblastoma cell line HepG2 was obtained from the American Type Culture Collection (Rockville, MD). Cells were seeded into tissue culture dishes in MEM supplemented with 10% FBS and either 0.18% BSA (control) or 0.1 mM fatty acids bound to 0.18% BSA and were incubated at 37°C in a 95% air, 5% CO_2 atmosphere as previously described (19–22). At the start of experiments the maintenance medium was removed, monolayers were washed twice with phosphate-buffered saline (PBS), and serum-free MEM containing either 0.75% BSA (control dishes) or 0.2 mM fatty acid bound to 0.75% BSA (experimental dishes) was added and cells were incubated for the indicated time period. The conditioned medium was removed and a preservative cocktail including final concentrations of penicillin G (500 units/ml), streptomycin sulfate (50 μg/ml), chloramphenicol (20 μg/ml), ε-amino caproic acid (1.3 mg/ml), and ethylenediaminetetraacetic acid (1 mg/ml) was added. The medium was centrifuged at 2,000 rpm for 30 min to remove cells and debris. The monolayers were washed three times with PBS, scraped off the plate after addition of PBS, and analyzed for cellular protein.

Isolation of lipoprotein density classes and determination of the mass of triglycerides and apolipoproteins in the medium

Very low density lipoproteins (VLDL) (d < 1.006 g/ml), LDL (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) were isolated by sequential preparative ultracentrifugation (24). The isolated lipoproteins were dialyzed against PBS containing the preservatives described above. The mass of triglycerides in the 10- to 15-fold concentrated medium was determined by gas-liquid chromatography (25) and the concentrations of apoA-I and apoB were measured by electroimmunoassays using monospecific polyclonal antibodies to these apolipoproteins as described in detail elsewhere (26, 27). These antibodies have been used in our laboratory for determination of the corresponding apolipoprotein concentration and for immunoprecipitation and immunoblotting analyses (19–22). Cell protein was measured by the method of Lowry et al. (28).

Determination of [^{3}H]glycerol and [^{14}C]acetate incorporation into various lipid fractions

HepG2 cells were plated and grown in MEM containing 10% FBS and 0.18% BSA (control) or 0.1 mM fatty acids bound to 0.18% BSA. After 5–6 days in culture, maintenance medium was removed, cells were washed twice with PBS, and serum-free MEM containing either 0.75% BSA or 0.2 mM fatty acid bound to 0.75% BSA and [^{3}H]glycerol or [^{14}C]acetate were added. At the end of incubation, medium was removed and cells were washed three times with PBS and scraped off the plates as described above. Lipoprotein density fractions were isolated as previously described (24). One-milliliter aliquots of cell suspension, medium, and lipoprotein fractions were extracted with chloroform–methanol 2:1, and extracts were washed and applied to thin-layer chromatography plates to separate phospholipids, unesterified cholesterol, free fatty acids, triglycerides, and cholesteryl esters as previously described (29). The incorporation of [^{14}C]acetate into cellular and secreted digitonin-precipitable sterols and total fatty acids (free fatty acids plus fatty acid moiety of phospholipids, diglycerides, triglycerides, and cholesteryl esters) was determined as previously described (29).

Determination of LDL receptor activity

The effect of fatty acids on the LDL receptor activity was assessed by the rate of ^{125}I-labeled LDL binding to HepG2 cells. LDL was isolated from the plasma of a normolipemic subject and was iodinated as previously described (30). Cells were plated

and grown in MEM containing 10% FBS and 0.1 mM fatty acids bound to 0.18% BSA. After 5 days in culture, maintenance medium was removed, cells were washed twice with PBS, and serum-free MEM containing either 0.75% BSA or 0.2 mM fatty acid bound to 0.75% BSA was added. After a 15-h incubation, medium was removed, cells were washed three times with cold PBS, and serum-free MEM supplemented with 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (pH 7.4) was added. The binding of ^{125}I-labeled LDL at 4°C during a 3-h incubation was determined as previously described (30).

Statistical analysis

Statistical analysis was performed by a Student's *t*-test.

RESULTS

Many of the reported changes in the lipoprotein profile by diet represent the results of long-term studies. Therefore, in the present study, the effects of fatty acids on hepatic lipoprotein production were determined after a 5- to 6-day incubation of HepG2 cells with fatty acids unless otherwise stated. To assure that under the experimental conditions used in this study, the concentration of fatty acids was not cytotoxic, cells were incubated for 5–6 days in the presence of 0.1, 0.2, 0.4, and 0.8 mM fatty acids bound to BSA at a fatty acid-to-BSA ratio of 1.8. The fatty acids tested included the following: oleic acid (*cis* 18:1), the predominant dietary monounsaturated fatty acid; elaidic acid (*trans* 18:1), the major *trans* fatty acid in the diet; and palmitic acid (16:0), the major saturated fatty acid in the diet. Results of this pilot study showed that prolonged incubation (5–6 days) of cells with ⩾0.4 mM elaidic acid and ⩾0.2 mM palmitic acid had cytotoxic effects and caused cell detachment from the dishes as determined by light microscopy and cell protein concentration. In a separate series of experiments, we observed that a 15- to 24-h incubation of cells with an 0.8 mM concentration of all fatty acids, and 0.4 mM palmitic acid, was also cytotoxic. Therefore, cells were incubated for 5–6 days with 0.1 mM fatty acids and experiments were conducted in the presence of 0.2 mM fatty acids bound to 0.75% BSA unless otherwise stated.

Effect of fatty acids on the production of apoA-I and apoB

The net accumulation of apoA-I in the medium was not significantly altered by any of the fatty acids tested whereas that of apoB was increased by 34% and 26% with oleic and elaidic acids, respectively, but remained unchanged with palmitic acid (**Table 1**). The ratio of apoB to apoA-I was 1.1 with both oleic and elaidic acids and 0.97 with palmitic acid.

Effects of fatty acids on the secretion and the mass of triglycerides in the medium

The effects of fatty acids on the secretion of newly synthesized triglycerides from ^{3}H-labeled glycerol precursor are shown in **Table 2**. All fatty acids stimulated the secretion of ^{3}H-labeled triglycerides; the increase with oleic acid (+126%) was more pronounced than that with elaidic (+56%) or palmitic (+47%) acids. Similarly, the increase in cellular ^{3}H-labeled triglycerides was greater with oleic acid (+144%) than with elaidic (+118%) or palmitic (+124%) acids (data not shown). In addition, a higher percentage of cellular ^{3}H-labeled triglycerides was secreted into the medium with oleic acid (2.44%) than with elaidic or palmitic acids (1.73–1.93%). In comparison with oleic acid, there was a significant decrease in the secretion of ^{3}H-labeled triglycerides with elaidic (−31%, $P = 0.014$) and palmitic (−35%, $P = 0.003$) acids. The mass of triglycerides in the medium was increased by 105%, 23%, and 27% with oleic, elaidic, and palmitic acids, respectively (Table 2), corroborating the results of labeling studies. As with ^{3}H-labeled triglycerides, their mass in the medium was significantly lower with elaidic (−40%, $P = 0.007$) and palmitic (−38%, $P = 0.01$) acids when compared with oleic acid (Table 2).

TABLE 1. Effects of fatty acids on net accumulation of apolipoproteins A-I and B in culture medium of HepG2 cells

Addition	Net Accumulation in the Medium	
	ApoA-I	ApoB
	μg/g cell protein/h	
BSA	105.9 ± 5.9	97.0 ± 8.9
Oleic acid	118.2 ± 6.1	129.5 ± 10.4[a]
Elaidic acid	109.3 ± 2.9	122.1 ± 3.5[b]
Palmitic acid	94.39 ± 2.8	91.24 ± 4.1

Cells were plated and grown in MEM containing 10% FBS and either 0.18% BSA (control) or 0.1 mM fatty acid bound to 0.18% BSA. After 5 days in culture, the maintenance medium was removed, cells were washed with PBS, and serum-free MEM containing either 0.75% BSA (control) or 0.2 mM fatty acids bound to 0.75% BSA was added. The net accumulation of apolipoproteins A-I and B in the medium after a 16-h incubation was determined by electroimmunoassays. Values are means ± SEM of six dishes from two separate experiments.

The difference between BSA control and fatty acids was significant at [a] $P = 0.04$ and [b] $P = 0.026$.

Effect of fatty acids on de novo synthesis and secretion of total fatty acids and cholesterol

The secretion rate of de novo synthesized cholesterol was assessed by pulse labeling the cells with [^{14}C]acetate

TABLE 2. Effects of fatty acids on net accumulation in medium and incorporation of [^{3}H]glycerol into secreted triglycerides

Addition	Triglyceride Mass	^{3}H-Labeled Triglyceride
	μg/g cell protein/h	*dpm/mg cell protein/h*
BSA	160.48 ± 22.20	5,597 ± 538
Oleic acid	328.68 ± 15.20[a]	12,647 ± 98[b]
Elaidic acid	197.00 ± 3.90	8,772 ± 635[c]
Palmitic acid	203.55 ± 9.12	8,210 ± 323[c]

HepG2 cells were grown under the experimental conditions described in the legend to Table 1. The mass of triglycerides in the medium after a 24-h incubation with 0.2 mM fatty acids bound to 0.75% BSA was determined by gas-liquid chromatography. The incorporation of [^{3}H]glycerol (5 μCi/ml of medium) into secreted triglycerides was determined after a 4-h incubation with 0.2 mM fatty acids bound to 0.75% BSA. Values are means ± SD of duplicate dishes.

The difference between the BSA control and fatty acids was significant at [a] $P = 0.01$, [b] $P = 0.003$, and [c] $P = 0.03$.

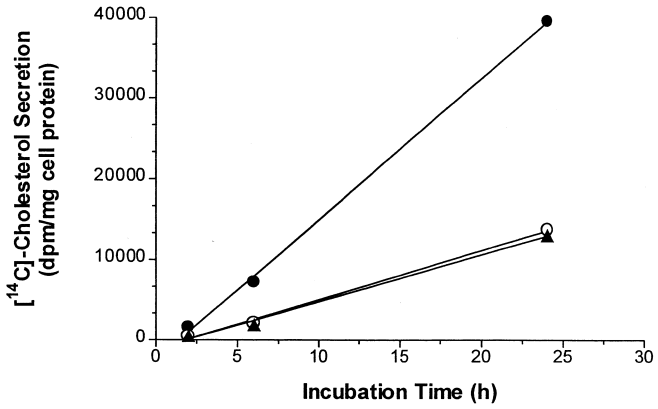

Fig. 1. Effects of fatty acids on the rate of cholesterol secretion. HepG2 cells were plated and grown in MEM containing 10% FBS and either 0.18% BSA (control) or 0.1 mM fatty acid bound to 0.18% BSA. After 5 days in culture, medium was removed, cells were washed with PBS, and serum-free MEM containing either 0.75% BSA (control) or 0.2 mM fatty acid bound to 0.75% BSA was added. Cells were pulsed with [^{14}C]acetate (3 μCi/ml of medium) and chased for 2, 6, and 24 h. The secretion of digitonin-precipitable ^{14}C-labeled sterols in the presence of BSA (solid triangles), oleic acid (open circles), and elaidic acid (solid circles) was determined as described in Experimental Procedures. Values are means ± SD of duplicate dishes. The difference between oleic and elaidic acids after a 2-h chase was significant at $P = 0.03$. The difference between oleic and elaidic acids after a 6-h chase was significant at $P = 0.0005$. The difference between oleic and elaidic acids after a 24-h chase was significant at $P = 0.0002$.

precursor (3 μCi/ml of medium) followed by a 2-, 6-, and 24-h chase. As shown in **Fig. 1**, the rate of [^{14}C]acetate incorporation into secreted cholesterol was linear up to 24 h of chase in both the control and fatty acid-treated cells. In comparison with BSA control, oleic acid had no significant effect on the secretion of [^{14}C]cholesterol at 2, 6, or 24 h of incubation (Fig. 1). In contrast, elaidic acid significantly increased the secretion of [^{14}C]cholesterol at 2 h (+190%, $P = 0.03$), 6 h (+276%, $P = 0.0006$), and 24 h (+202%, $P = 0.0001$). Thus, the effect of elaidic acid was rapid and prolonged. On the basis of this study, the effects of exogenous fatty acids on the incorporation of [^{14}C]acetate into de novo synthesized and secreted lipids and lipoprotein density classes were determined after a 6- and 15-h incubation, respectively.

Results in **Table 3** show the incorporation of [^{14}C]-acetate into cellular and secreted total fatty acids (i.e., free fatty acids plus fatty acid moiety of phospholipids, diglycerides, triglycerides, and cholesteryl esters) and total digitonin-precipitable sterols. As expected, all exogenous fatty acids inhibited de novo synthesis and secretion of fatty acids from [^{14}C]acetate precursor (Table 3). In comparison with the BSA control, the incorporation of [^{14}C]acetate into total fatty acids in the medium was decreased with oleic (−43%; $P = 0.002$) and elaidic (−46%; $P = 0.002$) acids (Table 3). The incorporation of [^{14}C]acetate into cellular total fatty acids was also decreased with oleic (−46%; $P = 0.002$), elaidic (−53%; $P = 0.0006$), and palmitic (−50%, $P = 0.001$) acids (Table 3). However, the percentage of cellular ^{14}C-labeled total fatty acids that were secreted into the medium was not affected with oleic and elaidic acids (11%) but was increased to 20% with palmitic acid when compared with BSA alone. Thus, in comparison with BSA alone, the incorporation of [^{14}C]acetate into cellular plus secreted total fatty acids was decreased similarly (−46%, $P = 0.002$) with oleic and palmitic acids and to a greater extent (−52%, $P = 0.0007$) with elaidic acid, that is, less ^{14}C-labeled fatty acid was found within the cells and medium after incubation with elaidic acid than with oleic or palmitic acids.

Oleic and palmitic acids had no significant effect on the incorporation of [^{14}C]acetate into either the secreted or cellular cholesterol (Table 3). In contrast, elaidic acid significantly increased the incorporation of [^{14}C]acetate into both the secreted (+83%, $P = 0.0001$) and cellular (+96%, $P < 0.0001$) cholesterol (Table 3). This effect of elaidic acid was observed after 2 h and was sustained for up to 24 h of incubation (Fig. 1). In comparison with oleic acid, elaidic acid significantly ($P = 0.0001$) increased [^{14}C]cholesterol in both the medium (+85%) and the cells (+106%) whereas palmitic acid had a modest (+13%) stimulatory effect only on the cellular [^{14}C]-cholesterol (Table 3). The percentage of cellular cholesterol that was secreted into the medium (3% to 3.3%) was not significantly different among the fatty acids. Data in

TABLE 3. Effects of fatty acids on incorporation of [^{14}C]acetate into total fatty acids and sterols in HepG2 cells

	[^{14}C]Acetate Incorporation			
	Total Fatty Acids		Total Sterols	
Addition	Medium	Cells	Medium	Cells
	dpm/mg cell protein × 10^{-3}			
BSA	83.97 ± 5.25	780.57 ±42.82	2.62 ± 0.14	80.57 ± 4.04
Oleic acid	47.52 ± 0.73[a]	423.74 ± 24.62[a]	2.59 ± 0.13	76.62 ± 2.15
Elaidic acid	45.23 ± 1.14[a]	365.27 ± 3.70[b]	4.79 ± 0.01[c]	158.00 ± 2.23[c]
Palmitic acid	83.15 ± 2.77	387.43 ± 17.35[d]	2.34 ± 0.25	86.27 ± 0.41

Cells were plated and grown under the experimental conditions described in the legend to Table 1. The incorporation of [^{14}C]acetate (3 μCi/ml of medium) into secreted and cellular total fatty acids and sterols was determined after a 6-h incubation. Values are means ± SEM of triplicate dishes.

The difference between the BSA control and fatty acids was significant at [a] $P = 0.002$, [b] $P = 0.0006$, [c] $P = 0.0001$, and [d] $P = 0.001$.

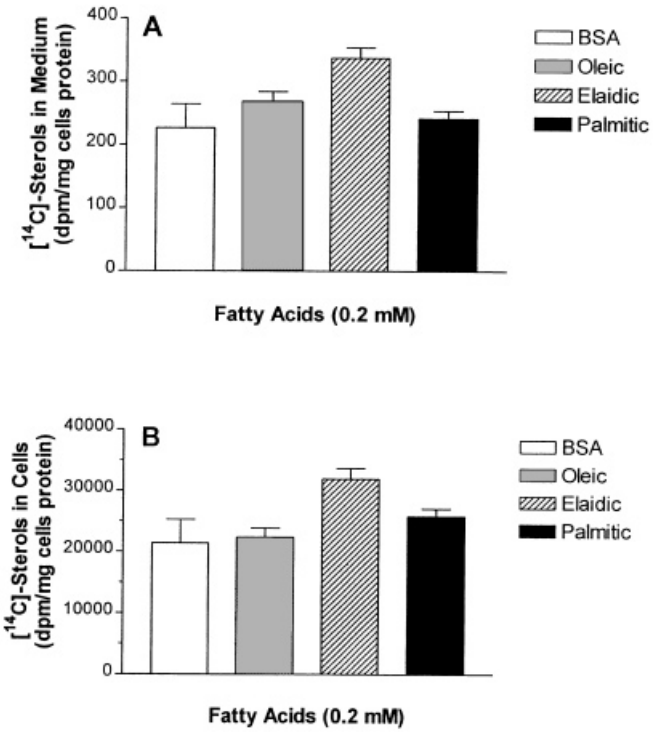

Fig. 2. Acute effects of fatty acids on the secretion of newly synthesized sterols. HepG2 cells were plated and grown in MEM containing 10% FBS. After 5 days in culture, the maintenance medium was removed, cells were washed with PBS, and serum-free MEM containing either 0.75% BSA (control) or 0.2 mM fatty acid bound to 0.75% BSA was added. The incorporation of [^{14}C]acetate into cellular and secreted digitonin-precipitable sterols was determined after a 4-h incubation. Values are means ± SEM of triplicate dishes.

Table 3 demonstrate that the total [^{14}C]acetate incorporation into fatty acids and cholesterol in medium plus cells, expressed as disintegrations per minute per milligram of cell protein, was similar with oleic (550,470 ± 26,737), elaidic (576,299 ± 1,087), and palmitic (558,415 ± 11,498) acids. However, the percentage of total [^{14}C]acetate incorporation into fatty acids (medium plus cells) was 86%, 71%, and 84% with oleic, elaidic, and palmitic acids, respectively, and that converted into cholesterol was 14%, 29%, and 16%, respectively. Because acetate pool size may be different among various fatty acids after 4–5 days of incubation, the acute effect of fatty acids on the incorporation of [^{14}C]acetate into secreted and cellular cholesterol was assessed. Cells were grown in MEM containing 10% FBS without any fatty acids for 4–5 days. The maintenance medium was removed, cells were washed with PBS, and serum-free MEM containing either 0.75% BSA or 0.2 mM fatty acid bound to 0.75% BSA was added. The incorporation of [^{14}C]acetate (2 μCi/ml of medium) into secreted and cellular cholesterol after a 4-h incubation was determined. Results showed that elaidic acid significantly increased the incorporation of [^{14}C]acetate into secreted (**Fig. 2A**) and cellular (Fig. 2B) cholesterol, when compared with BSA alone, or with oleic or palmitic acid. This experiment, together with that shown in Fig. 1, validated the results obtained after a 4- to 5-day incubation with 0.1 mM fatty acids followed by a 6- to 15-h incubation with 0.2 mM fatty acids, using [^{14}C]acetate. However, the unequivocal stimulatory effect of elaidic acid on the synthesis and secretion of cholesterol remains to be established using $^{3}H_2O$ precursor.

Effect of fatty acids on de novo synthesis and secretion of phospholipids and neutral lipids

Consistent with the results of total fatty acids shown in Table 3, all exogenous fatty acids caused a significant decrease in the incorporation of [^{14}C]acetate into secreted (**Table 4**) and cellular (**Table 5**) phospholipids and triglycerides, and this decrease was similar for elaidic and palmitic acids. In contrast, the incorporation of [^{14}C]acetate into both the secreted and cellular unesterified cholesterol and cholesteryl esters was significantly higher with elaidic acid than with oleic and palmitic acids, corroborating the results shown in Table 3. In comparison with oleic acid, elaidic acid *1*) decreased the secretion (−28%, P = 0.05) and cellular content (−30%; P = 0.0001) of ^{14}C-labeled phospholipids, *2*) reduced the secretion (−27%; P = 0.02) and cellular content (−12%; P = 0.003) of ^{14}C-labeled triglycerides, *3*) stimulated the secretion (+93%, P = 0.003) and cellular content (+89%, P = 0.0004) of ^{14}C-labeled unesterified cholesterol, and *4*) enhanced the secretion (+73%; P = 0.0007) and cellular content (+109%; P = 0.0001) of ^{14}C-labeled cholesteryl esters. Relative to oleic acid, palmitic acid *1*) decreased the secretion (−31%; P = 0.05) and cellular content (−29%; P = 0.0001) of ^{14}C-labeled phospholipids, *2*) reduced the se-

TABLE 4. Effects of fatty acids on incorporation of [^{14}C]acetate into secreted lipids in HepG2 cells

	[^{14}C]Acetate Incorporation				
Addition	Phospholipids	Unesterified Cholesterol	Free Fatty Acids	Triglycerides	Cholesteryl Esters
	dpm/mg cell protein × 10^{-3}				
BSA	11.16 ± 0.79	4.06 ± 0.20	43.24 ± 0.59	15.59 ± 0.25	0.86 ± 0.03
Oleicacid	6.30 ± 0.63 (100%)[a]	3.44 ± 0.14 (100%)	23.07 ± 1.41 (100%)[b]	7.89 ± 0.40 (100%)[c]	0.37 ± 0.03 (100%)[d]
Elaidic acid	4.55 ± 0.10 (72%)[e]	6.66 ± 0.46 (193%)[f]	17.17 ± 1.65 (74%)[g]	5.75 ± 0.43 (73%)[c]	0.65 ± 0.01 (173%)[h]
Palmitic acid	4.33 ± 0.29 (69%)[e]	3.21 ± 0.12 (93%)[i]	49.06 ± 2.2 (213%)	5.58 ± 0.15 (71%)[c]	0.39 ± 0.02 (105%)[d]

Cells were plated and grown under the experimental conditions described in the legend to Table 1. The incorporation of [^{14}C]acetate (3 μCi/ml of medium) into secreted lipids was measured after a 6-h incubation. Values are means ± SEM of triplicate dishes. Numbers in parentheses represent relative changes from oleic acid set at 100%.

The difference between the BSA control and fatty acids was significant at [a] P = 0.009, [b] P = 0.0002, [c] P < 0.0001, [d] P = 0.0003, [e] P = 0.001, [f] P = 0.007, [g] P = 0.0001, [h] P = 0.004, and [i] P = 0.02.

TABLE 5. Effects of fatty acids on incorporation of [^{14}C]acetate into cellular lipids in HepG2 cells

Addition	[^{14}C]Acetate Incorporation				
	Phospholipids	Unesterified Cholesterol	Free Fatty Acids	Triglycerides	Cholesteryl Esters
	dpm/mg cell protein $\times 10^{-3}$				
BSA	519.50 ± 9.78	120.20 ± 6.38	32.34 ± 2.73	411.08 ± 17.36	37.97 ±1.96
Oleic acid	191.66 ± 2.46 (100%)[a]	89.98 ± 6.86 (100%)[b]	11.68 ± 0.79 (100%)[c]	344.20 ± 5.74 (100%)[d]	19.95 ± 0.48 (100%)[e]
Elaidic acid	134.49 ± 3.19 (70%)[a]	170.42 ± 2.68 (189%)[c]	13.27 ± 0.54 (114%)[c]	304.34 ± 2.70 (88%)[f]	41.69 ± 0.93 (209%)
Palmitic acid	136.68 ± 2.30 (71%)[a]	114.23 ± 1.16 (127%)	24.61 ± 1.24 (211%)	261.70 ± 12.33 (76%)[c]	21.64 ± 0.68 (108%)[g]

Cells were plated and grown under the experimental conditions described in the legend to Table 1. The incorporation of [^{14}C]acetate (3 μCi/ml of medium) into cellular lipids was measured after a 6-h incubation. Values are means ± SEM of triplicate dishes. Numbers in parentheses represent relative changes from oleic acid set at 100%.

The difference between the BSA control and fatty acids was significant at [a] $P < 0.0001$, [b] $P = 0.032$, [c] $P = 0.002$, [d] $P = 0.02$, [e] $P = 0.0009$, [f] $P = 0.004$, and [g] $P = 0.0014$.

cretion (−29%; $P = 0.007$) and cellular content (−24%; $P = 0.003$) of ^{14}C-labeled triglycerides, *3*) had no significant effect on the secretion of ^{14}C-labeled unesterified cholesterol but increased (+27%, $P = 0.03$) its cellular content, and *4*) had no significant effect on the secretion or cellular accumulation of ^{14}C-labeled cholesteryl esters. The lipid composition of the medium and the cells in the presence of oleic acid was similar to that of BSA alone. A higher content of unesterified cholesterol and cholesteryl esters and lower level of phospholipids and triglycerides, relative to oleic acid, characterized the lipid composition of medium in the presence of elaidic acid. The lipid composition of medium in the presence of palmitic acid was intermediate between that of oleic and elaidic acids.

Effect of fatty acids on the cholesterol concentration of secreted lipoprotein density classes

After a 15-h incubation, the incorporation of [^{14}C]acetate into cholesterol secreted into the medium was increased by 20% and 82% with oleic and elaidic acids, respectively, but remained unchanged with palmitic acid (**Fig. 3A**). Oleic acid increased the VLDL-Chol (Fig. 3B), LDL-Chol (Fig. 3C), and HDL-Chol (Fig. 3D) by 107%, 40%, and 42%, respectively. Elaidic acid had a significantly

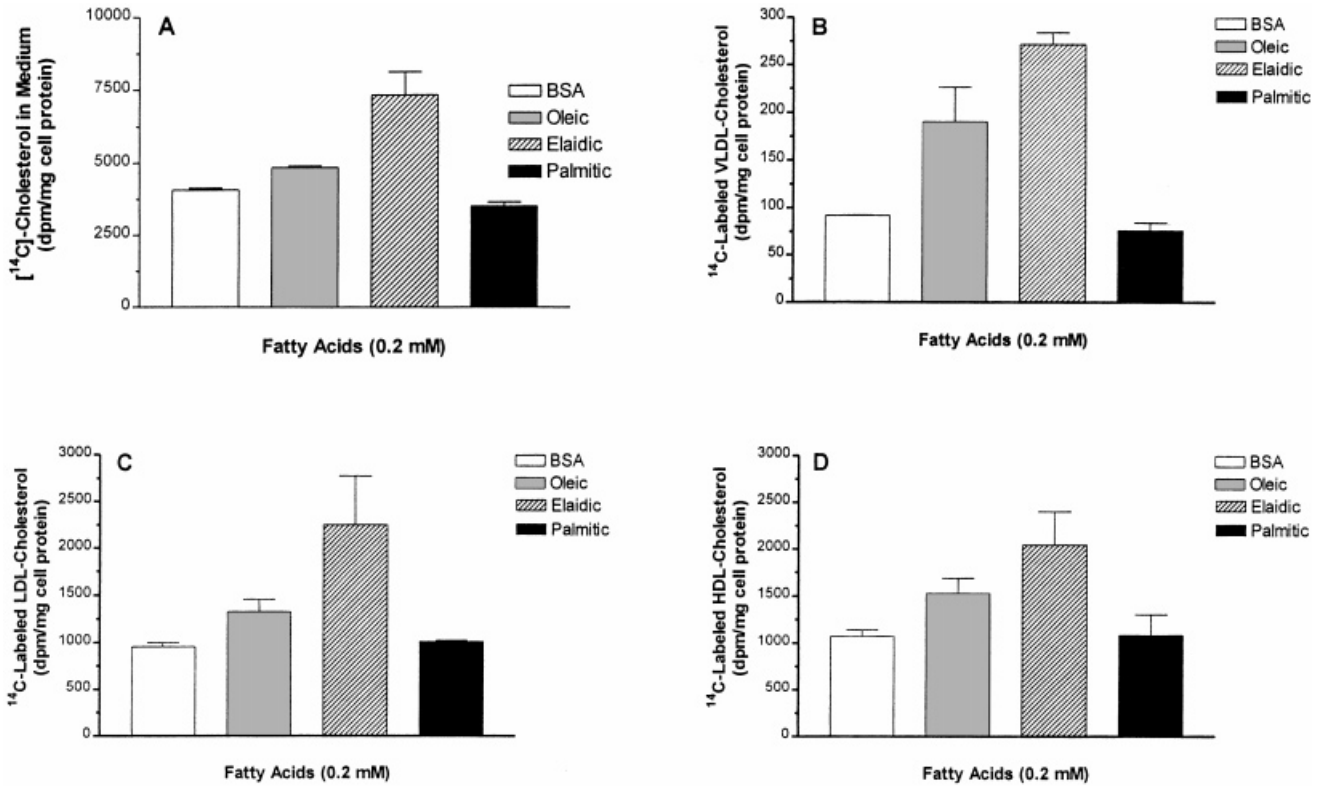

Fig. 3. Effects of fatty acids on lipoprotein cholesterol concentration. HepG2 cells were grown for 5 days in MEM containing 10% FCS and 0.1 mM fatty acids. The medium was replaced with serum-free MEM containing 0.2 mM fatty acid bound to 0.75% BSA and the incorporation of [^{14}C]acetate (5 μCi/ml) into cholesterol in the medium (A), VLDL-Chol (B), LDL-Chol (C), and HDL-Chol (D) was determined as described in Experimental Procedures. Values are means ± SD of duplicate dishes. The difference in the [^{14}C]cholesterol in the medium between BSA and both oleic and elaidic acids was significant at $P = 0.02$. The difference in the [^{14}C]cholesterol in VLDL between BSA and elaidic acid was significant at $P = 0.005$.

TABLE 6. Effects of fatty acids on lipid composition of low sensity lipoproteins secreted by HepG2 cells

Addition	Composition of Secreted Low Density Lipoproteins				
	Phospholipids	Cholesterol	Free Fatty Acids	Triglycerides	Cholesteryl Esters
			% of total lipids		
BSA	23.93 ± 0.90	10.76 ± 0.01	1.22 ± 0.07	60.28 ± 1.32	3.83 ± 0.49
Oleic acid	23.19 ± 0.84	27.74 ± 0.83[a]	2.48 ± 0.21[b]	44.26 ± 3.52[c]	4.43 ± 0.35
Elaidic acid	10.43 ± 0.53[d]	39.16 ± 0.59[e]	1.74 ± 0.15[f]	39.83 ± 0.93[d]	8.86 ± 0.34[g]
Palmitic acid	12.17 ± 0.44[d]	30.85 ± 0.47[e]	3.41 ± 0.28[h]	49.45 ± 0.01[g]	4.13 ± 0.30

Cells were plated and grown under the experimental conditions described in the legend to Table 1. The incorporation of [^{14}C]acetate (5 μCi/ml of medium) into secreted phospholipids and neutral lipids of low density lipoproteins was determined after a 15-h incubation. Values are means ± SD of duplicate dishes.

The difference between the BSA control and fatty acids was significant at [a] $P = 0.001$, [b] $P = 0.02$, [c] $P = 0.03$, [d] $P = 0.003$, [e] $P = 0.0003$, [f] $P = 0.05$, [g] $P = 0.007$, and [h] $P = 0.009$.

higher stimulatory effect on the secretion of VLDL-Chol (+195%), LDL-Chol (+138%), and HDL-Chol (+90%) than oleic acid (Fig. 3B–D). These changes appeared to be due, primarily, to absolute increases in lipoprotein cholesterol level. Palmitic acid caused a small decrease in VLDL-Chol (Fig. 3B) but did not affect LDL-Chol (Fig. 3C) or HDL-Chol (Fig. 3D). Thus, in comparison with oleic acid, elaidic acid increased the secretion of VLDL-Chol, LDL-Chol, and HDL-Chol by 43%, 70%, and 34%, respectively, whereas palmitic acid decreased VLDL-Chol but had no major effect on LDL-Chol and HDL-Chol. These changes resulted in ratios of total cholesterol to HDL-Chol of 3.17, 3.60, and 3.25, with oleic, elaidic, and palmitic acids, respectively; the corresponding ratios of LDL-Chol to HDL-Chol were 0.87, 1.10, and 0.93, respectively.

Effect of dietary fatty acids on the lipid composition of secreted lipoprotein density classes

Because of low concentrations of VLDL, their composition could not accurately be determined. The differential effects of fatty acids on the composition of secreted LDL and HDL particles are shown in **Tables 6** and **7**. In the presence of oleic acid, triglycerides and phospholipids were the major lipid constituents of LDL (Table 6) and HDL (Table 7), respectively. Relative to oleic acid, a significantly higher percent content of unesterified cholesterol and cholesteryl esters and lower level of phospholipids characterized the LDL and HDL particles secreted in the presence of elaidic acid. The compositions of LDL and HDL particles secreted in the presence of palmitic acid were intermediate between those of oleic and elaidic acids, that is, they had lower contents of unesterified cholesterol and cholesteryl esters and higher levels of phospholipids and triglycerides when compared with elaidic acid (Tables 6 and 7). The phospholipid-to-cholesterol ratios of LDL particles in the presence of oleic, elaidic, and palmitic acids were 0.83, 0.26, and 0.39, respectively, and those of HDL were 1.04, 0.39, and 0.74, respectively.

Effects of fatty acids on LDL receptor activity

Changes in LDL receptor activity under the present experimental conditions may contribute to the observed effects of fatty acids on the concentration and composition of apoB-containing lipoproteins. As shown in **Fig. 4**, the binding at 4°C of ^{125}I-labeled human LDL to HepG2 cells grown in the presence of different fatty acids was a curvilinear function of substrate concentration and did not reach a plateau up to 50 μg of LDL protein per ml of medium. The binding of ^{125}I-labeled LDL at 5 μg of protein per ml of culture medium was not significantly altered by any of the fatty acids tested (Fig. 4). However, at concentrations ⩾12.5 μg/ml, all fatty acids had a modest inhibitory effect on ^{125}I-labeled LDL binding to HepG2 cells when compared with BSA alone. Relative to oleic acid, elaidic and palmitic acids caused a 13–20% increase in the binding of ^{125}I-labeled LDL to HepG2 cells (Fig. 4).

TABLE 7. Effects of fatty acids on lipid composition of high density lipoproteins secreted by HepG2 cells

Addition	Composition of Secreted High Density Lipoproteins				
	Phospholipids	Cholesterol	Free Fatty Acids	Triglycerides	Cholesteryl Esters
			% of total lipids		
BSA	59.91 ± 1.16	17.37 ± 1.54	2.94 ± 0.28	16.68 ± 0.03	3.13 ± 0.23
Oleic acid	42.77 ± 0.15[a]	41.28 ± 0.04[b]	3.67 ± 0.07[c]	9.77 ± 0.01[d]	2.52 ± 0.18
Elaidic acid	23.01 ± 0.50[e]	59.13 ± 0.62[f]	2.88 ± 0.19	9.87 ± 0.05[d]	5.12 ± 0.01[g]
Palmitic acid	29.20 ± 0.83[b]	39.20 ± 1.36[a]	5.17 ± 0.35[c]	22.88 ± 0.97[h]	3.56 ± 0.11

Cells were plated and grown under the experimental conditions described in the legend to Table 1. The incorporation of [^{14}C]acetate (5 μCi/ml of medium) into secreted phospholipids and neutral lipids of high density lipoproteins was determined after a 15-h incubation. Values are means ± SD of duplicate dishes.

The difference between the BSA control and fatty acids was significant at [a] $P = 0.004$, [b] $P = 0.002$, [c] $P = 0.02$, [d] $P < 0.0001$, [e] $P = 0.001$, [f] $P = 0.0008$, [g] $P = 0.007$, and [h] $P = 0.01$.

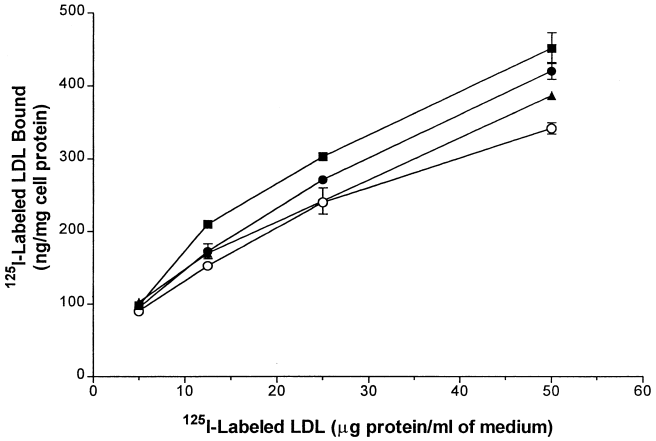

Fig. 4. Effects of fatty acids on LDL receptor activity. HepG2 cells were grown for 5 days in MEM containing 10% FCS and 0.1 mM fatty acids. The medium was replaced with serum-free MEM containing a 0.2 mM concentration of the indicated fatty acid bound to 0.75% BSA and the binding of ^{125}I-labeled plasma LDL at 4°C during a 3-h incubation in the presence of BSA (solid squares), oleic acid (open circles), elaidic acid (solid circles), and palmitic acid (solid triangles) was determined. Values are means ± SEM of triplicate dishes. The difference in the binding of LDL at 12.5 μg/ml of medium between BSA and oleic, elaidic, and palmitic acids was significant at $P = 0.0008$, $P = 0.03$, and $P = 0.001$, respectively. The difference in the binding of LDL at 25 μg/ml of medium between BSA and oleic, elaidic, and palmitic acids was significant at $P = 0.0001$, $P = 0.004$, and $P = 0.03$, respectively. The difference in the binding of LDL at 50 μg/ml of medium between BSA and oleic and palmitic acids was significant at $P = 0.008$ and $P = 0.04$, respectively.

DISCUSSION

In the present study, we compared the effects of oleic (*cis* 18:1), elaidic (*trans* 18:1), and palmitic (16:0) acids on the concentration and composition of apoA-I- and apoB-containing lipoproteins in HepG2 cells. The net accumulation of apoA-I in the medium was not significantly affected by any of the fatty acids tested whereas that of apoB was increased with both oleic and elaidic acids, resulting in a higher mass ratio of apoB to apoA-I with elaidic acid than with palmitic acid. These results agree with studies of human subjects (6, 10, 11, 15, 16) and nonhuman primates (31, 32) and suggest that *trans* fatty acids may have a more adverse effect on the ratio of apoB to apoA-I, a good indicator of risk of CAD (33), than do saturated fatty acids. Studies of human subjects demonstrated that, in comparison with oleic acid, saturated fatty acids increased while *trans* fatty acids decreased plasma concentration of apoA-I (6, 11), and that *trans* fatty acids raised plasma apoB level either similarly to (11) or to a greater extent than (6) that of saturated fatty acid. In nonhuman primates, it was shown that saturated fatty acids increased plasma concentrations of both apoA-I and apoB when compared with monounsaturated fatty acids (31, 32). Our results suggest that as in nonhuman primates (31), the decrease in apoA-I level with monounsaturated fat relative to saturated fat is most likely due to enhanced catabolism rather than decreased production rate of apoA-I-containing lipoproteins.

The difference in relative effects of oleic and palmitic acids on the net accumulation of apoB in this study and those reported in human subjects (6, 10, 11) and nonhuman primates (31, 32) may be due to the presence of dietary cholesterol in the above-described in vivo studies. This is supported by studies of Caco-2 cells, demonstrating that in the absence of cholesterol apoB production was higher with oleic acid than with palmitic acid (34, 35). Experimental evidence from several studies (17, 18) suggests that the regulation of apoB production is posttranscriptional and that intracellular degradation of newly synthesized apoB is a major regulatory mechanism (36). Studies of nonhuman primates have shown that monounsaturated and saturated fatty acids have similar effects on the fractional catabolic rate of LDL-apoB (32) and support our observation indicating no major differences in the effect of fatty acids on LDL receptor activity. However, because we did not examine the catabolism of nascent lipoproteins, which has been reported to be insignificant under normal culture conditions (37), the possibility of variation in the reuptake of nascent apoB-containing lipoproteins secreted in the presence of different fatty acids cannot be ruled out. The effects of fatty acids on the secretion and intracellular degradation of apoB and potential modification of secreted lipoproteins remain to be established.

Addition of all fatty acids stimulated the incorporation of [^{3}H]glycerol into cellular and secreted triglycerides and this increase was more pronounced with oleic acid than with elaidic and palmitic acids. Furthermore, the proportion of cellular ^{3}H-labeled triglycerides that was secreted into the medium was greater with oleic acid (2.44%) than with elaidic and palmitic acids (1.7% to 1.9%). Determination of the mass of triglycerides in the medium corroborated the results of labeling studies. The observed relative effects of fatty acids on triglyceride synthesis and secretion may, in part, be explained by a lower selectivity value for incorporation of saturated and *trans* unsaturated fatty acids into triglycerides compared with *cis* unsaturated fatty acids (5). Our results agree with studies of rat liver (38) showing enhanced triglyceride secretion with *cis* 18:1 compared with *trans* 18:1, and of liver of cynomolgus monkey (31), human colonic adenocarcinoma Caco-2 cells (34), and human subjects (39) demonstrating increased triglyceride concentration with monounsaturated fatty acids relative to saturated fatty acids. Few studies of human subjects (6, 11), however, have indicated that compared with oleic acid, *trans* and saturated fatty acids increase serum triglyceride levels. While the in vitro observations of HepG2 cells cannot be directly compared with those obtained in vivo, these differences might be due to modification of hepatic lipoproteins in plasma and/or variable catabolic rates of triglycerides produced in the presence of different fatty acids. Alternatively, as demonstrated in Caco-2 cells (35), the stimulatory effect of elaidic acid on triglyceride secretion compared with oleic and palmitic acids may occur at the intestinal rather than the hepatic level.

Consistent with the reported inhibitory effect of exogenous fatty acids on de novo synthesis of fatty acids, the net incorporation of [^{14}C]acetate into cellular plus secreted to-

tal fatty acids was decreased by oleic, palmitic, and to a greater extent elaidic acids. As in humans and several animal species (2, 40), oleic acid did not alter the net incorporation of [^{14}C]acetate into cellular plus secreted cholesterol and under the experimental conditions used, the effect of palmitic acid was similar to that of oleic acid. In contrast, elaidic acid significantly increased the incorporation of [^{14}C]acetate into cellular and secreted cholesterol both after an acute and long-term incubation. Pulse-chase studies demonstrated that the effect of elaidic acid on cholesterol secretion was rapid and prolonged, that is, it was evident after 2 h and was sustained for up to 24 h of chase. The incorporation of [^{14}C]acetate into total lipids, that is, total fatty acids and cholesterol in cells plus medium, was similar for all three fatty acids, suggesting that a greater proportion of acetyl-CoA was channeled to cholesterol synthesis in the presence of elaidic acid compared with oleic and palmitic acids. This was further supported by a higher incorporation of [^{14}C]acetate into unesterified cholesterol and cholesteryl esters with elaidic acid than with oleic and palmitic acids. The unequivocal stimulatory effect of elaidic acid on cholesterol synthesis and secretion from $^{3}H_2O$ remains to be established. Our results on the relative effects of oleic and elaidic acids on the synthesis and secretion of cholesterol are compatible with studies of human subjects (5–8), but those observed with palmitic acid differ from in vivo studies showing a hypercholesterolemic effect of this saturated fatty acid (2, 40). As shown in nonhuman primates (41) and discussed in two review articles (2, 42), the increase in plasma LDL-Chol with a saturated fat diet may be explained by a decrease in LDL receptor activity in the presence of dietary cholesterol, which was not included in the present study.

In the absence of cholesterol, oleic and palmitic acids had a similar effect on LDL-Chol concentration, which is in agreement with studies of nonhuman primates (43, 44) and guinea pigs (45–48). The decrease in VLDL-Chol with palmitic acid relative to oleic acid observed in this study may be attributed to the inability of palmitic acid to stimulate triglyceride secretion and hence VLDL production (2). Relative to oleic and pamitic acids, elaidic acid stimulated the secretion of cholesterol in all lipoprotein density fractions; however, the increase in VLDL-Chol and LDL-Chol was greater than that in HDL-Chol. These changes resulted in higher ratios of total cholesterol to HDL-Chol, LDL-Chol to HDL-Chol, and VLDL-Chol plus LDL-Chol to HDL-Chol with elaidic acid than with oleic and palmitic acids and are consistent with several in vivo studies of human subjects (6, 7, 10, 15, 16). Our results demonstrated that elaidic acid increased the LDL receptor activity by 13% to 20% and LDL-Chol by 70% when compared with oleic acid and suggest that enhanced secretion rather than decreased catabolism of apoB-containing lipoproteins was the most likely regulatory mechanism involved in elaidic acid action.

Consistent with studies of human subjects (49) and nonhuman primates (32, 43, 44, 50), the lipid composition of LDL particles was similar with oleic and palmitic acids. In contrast, the LDL particles secreted in the presence of elaidic acid were enriched with unesterified cholesterol and cholesteryl esters and had a lower content of phospholipids when compared with oleic acid; observations that are in agreement with results in human subjects (6, 7). The lipid composition of HDL secreted in the absence of fatty acid was similar to that reported by Thrift et al. (51), that is, they contained phospholipids as their major lipids, had a high content of unesterified cholesterol, and were deficient in cholesteryl esters when compared with their plasma counterparts (52), presumably because of low activity of lecithin:cholesterol acyltransferase in the culture medium (12, 51). The HDL particles secreted in the presence of elaidic acid contained a lower content of phospholipids and higher amounts of unesterified cholesterol and cholesteryl esters and hence a lower phospholipid-to-cholesterol ratio when compared with oleic and palmitic acids. Several studies (53–55) have suggested that the low phospholipid-to-cholesterol ratio of HDL is associated with increased risk for ischemic vascular disease (56) and CAD (57) because of their lower capacity to accept cellular cholesterol. Our data suggest that compared with oleic and palmitic acids, the HDL particles secreted in the presence of elaidic acid might be less efficient in promoting cholesterol efflux from the cells.

We have demonstrated that in HepG2 cells the incorporation of [^{14}C]acetate into cellular and secreted cholesterol was stimulated markedly by elaidic acid when compared with oleic and palmitic acids. The ratios of LDL-Chol to HDL-Chol, and of apoB to apoA-I, considered to be good indicators of risk of CAD (33, 58), were higher with elaidic acid than with oleic and palmitic acids. In comparison with oleic and palmitic acids, the LDL and HDL particles secreted in the presence of elaidic acid contained a higher content of unesterified cholesterol and cholesteryl esters and the HDL particles had a lower phospholipid-to-cholesterol ratio, rendering them less efficient in accepting cellular cholesterol. Our results suggest that in HepG2 cells, *trans* monounsaturated fatty acids have more adverse effects on the concentration and composition of lipoproteins than both *cis* monounsaturated and saturated fatty acids, that is, they increase the synthesis and secretion of potentially more atherogenic cholesterol-rich and phospholipid-poor LDL and HDL particles.

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