Trans Polyunsaturated Fatty Acids Have More Adverse Effects than Saturated Fatty Acids on the Concentration and Composition of Lipoproteins Secreted by Human Hepatoma HepG2 Cells

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ABSTRACT The objective of this study was to assess the relative long-term effects of linoleic (cis, cis 18:2), linolelaidic (trans, trans 18:2), and palmitic (16:0) acids on hepatic lipoprotein production in HepG2 cells. All fatty acids increased the mass of triglycerides (TG) in the medium and the incorporation of [14C]-glycerol into secreted TG; the increase was more pronounced with linoleic acid than with linolelaidic and palmitic acids. The net accumulation in the medium of apolipoprotein (apo) A-I was not affected by the fatty acids tested and moderate changes in that of apoB resulted from apoB/apoA-I mass ratios of 1.05, 1.27 and 0.86 with linoleic, linolelaidic and palmitic acids, respectively. The incorporation of [14C]-acetate into cellular plus secreted total sterols was 9.1%, 33.6% and 17.4% of total [14C]-labeled lipids with linoleic, linolelaidic and palmitic acids, respectively. Relative to linoleic acid, palmitic acid, and to a greater extent (P < 0.05) linolelaidic acid, increased the secretion and cellular accumulation of [14C]-labeled free cholesterol (FC) and cholesteryl esters and decreased those of TG and phospholipids (PL). Compared with linoleic acid, linolelaidic acid increased LDL-cholesterol (C) and HDL-C by 154% (P < 0.001) and 50% (P = 0.016), respectively, whereas palmitic acid increased LDL-C by 17% (P > 0.1) and did not affect HDL-C. The LDL-C to HDL-C ratios were 0.70, 1.18 and 0.96 with linoleic, linolelaidic and palmitic acids, respectively. These differences were not due to altered LDL receptor activity. The PL to C ratios of HDL particles were 1.61, 0.40 and 0.77 with linoleic acid, linolelaidic acid and palmitic acid, respectively. These results suggest that relative to cis polyunsaturated and saturated fatty acids, trans PUFA more adversely affect the concentration and composition of apoA-I- and apoB-containing lipoproteins secreted by HepG2 cells. J. Nutr. 132: 2651-2659, 2002.

KEY WORDS: • cholesterol metabolism • triglyceride secretion • liver • LDL • HDL

Increased concentrations of plasma LDL-cholesterol (C) and apoB, the major apolipoprotein in LDL and reduced levels of HDL-C and apoA-I, the major apolipoprotein in HDL, contribute greatly to the risk of coronary artery disease (CAD) (1–3). The plasma concentrations of lipoproteins are markedly influenced by diet and numerous studies have led to the general consensus that dietary saturated fatty acids increase, whereas cis-isomers of (n-6) PUFA decrease, plasma concentrations of LDL-C (2). Trans PUFA have been reported to have adverse effects on the plasma lipid profile compared with their cis isomers (4–7) and in some studies, relative to saturated fatty acids (6,8,9). Trans-fatty acids are formed during hydrogenation of vegetable oils to transform them from liquids to semisolid states. Hydrogenation also results in saturation of a portion of double bonds, thus, increasing the saturated and monounsaturated fatty acids and decreasing the polyunsaturated content of vegetable oils. Based on availability or disappearance data, the estimated per capita dietary trans fatty acid intakes in the United States is between 8.1 and 12.8 g/d (5). Based on responses to questionnaires and surveys and analysis of self-selected diets, trans fatty acids in the U.S. diet account for 5–6% of total fat intake (5). The reported effects of trans fatty acids on human plasma lipoproteins include increased plasma concentrations of LDL-C and reduced levels of HDL-C (6,7,9–11) and possibly elevated concentrations of triglycerides (TG) (8). It has been shown that the ratio of LDL-C to HDL-C in plasma is higher with trans fatty acid intake than with saturated-fat acid intake (6,10) and replacement of dietary saturated fatty acids by trans fatty acids impairs endothelial function of brachial arteries (9). These studies suggest that trans fatty acids might be more atherogenic than saturated fatty acids and that consumption of large amounts of trans fatty acids may contribute to increased risk of CAD (12–14). The quantitative relationship between trans fatty acid intake and plasma total cholesterol, LDL-C and HDL-C 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

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in vivo studies is to put multiple changes in the diet when a hydrogenated vegetable oil replaces its nonhydrogenated form; therefore, it is difficult to fully attribute the observed effects to an increased trans fatty acid intake. The effect of individual fatty acids on lipoprotein metabolism in humans, specifically their production in the liver, has not been studied extensively.

Although the results of in vitro studies may not represent the exact situation in vivo, it is important to establish the direct and individual effects of dietary fatty acids on lipoprotein metabolism at the hepatic level and in the absence of other confounding variables that cannot be controlled for in the in vivo studies. The goal of this study was to test whether trans PUFA and saturated fatty acids have different effects on hepatic synthesis and secretion of lipoproteins and to assess their relative atherogenicity at this level. To this end, we determined the long-term direct effects of linoleic (cis, cis 18:2), linoleadiacid (trans, trans 18:2), and palmitic (16:0) acids on the concentration and composition of apoA-I- and apoB-containing lipoproteins. The human hepatoblastoma cell line HepG2 was selected as the experimental model representing human liver because they are the most suitable and accessible human-derived cells and retain many of the biochemical functions of human liver parenchymal cells (15,16), including the nutritional regulation of synthesis and secretion of plasma lipoproteins (17,18).

MATERIALS AND METHODS

Materials. Minimum essential medium (MEM), trypsin, sodium pyruvate, 1-glutamine, minimum essential vitamin solution and fetal bovine serum (FBS) were purchased from Grand Island Biological Company (Grand Island, NY). Fatty acids (purity >99% by capillary gas chromatography), Triton X-100, benzamidine, phenemethylsulfonyl fluoride, and leupeptin were from Sigma (St. Louis, MO). Fatty acid-free bovine serum albumin (BSA) was from Miles (Kankakee, IL). [3H]-glycerol and [14C]-acetate were obtained from Amersharm (Arlington Heights, IL).

Cell culture. The human hepatoblastoma HepG2 cell line was obtained from American Type Culture Collection (Rockville, MD). Whereas most of the reported in vivo dietary-induced changes in lipoprotein profile reflect the results of long-term studies, in the majority of in vitro studies the effects of fatty acids on hepatic lipoprotein production are determined after an acute treatment with fatty acids. Therefore, we examined potential differences in acute and long-term effects of fatty acids on lipoprotein production in HepG2 cells. To ensure that the concentrations of fatty acids used in this study were not cytotoxic, cells were incubated for 5–6 d in the presence of increasing concentrations of fatty acids bound to BSA at a constant fatty acid to BSA molar ratio of 1.8. The fatty acid concentrations tested were: 0.1 mmol/L bound to 3.75 g/L BSA, 0.2 mmol/L bound to 7.5 g/L BSA, 0.4 mmol/L bound to 15 g/L BSA, and 0.8 mmol/L bound to 30 g/L BSA. The fatty acids tested included linoleic acid (cis, cis 18:2), the predominant dietary PUFA; linoleadiacid (trans, trans 18:2); palmitic acid (16:0), the principal saturated fatty acid in the diet; and for comparison to linoleadiacid acid, elaidic acid (trans, trans 18:1), which is the major trans fatty acid in the diet, was also included. This pilot study showed that the prolonged incubation (5–6 d) of cells with ≥0.4 mmol/L of elaidic and linoleadiacid acids and ≥0.2 mmol/L of 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, a 15- to 24-h incubation of cells with 0.8 mmol/L of all fatty acids, and 0.4 mmol/L of linoleadiacid and palmitic acids was also cytotoxic. Therefore, in all long-term studies, cells were grown for 5–6 d in the presence of 0.1 mmol/L fatty acids and experiments were conducted in the presence of 0.2 mmol/L fatty acids bound to 7.5 g/L BSA. In acute studies, cells were grown in the absence of fatty acids and experiments were conducted in the presence of 0.2 mmol/L fatty acids bound to 7.5 g/L BSA.

To study the acute effect of fatty acids, cells were seeded onto tissue culture dishes in MEM containing 10% (v/v) FBS and to study the long-term effects of fatty acids, cells were seeded onto tissue culture dishes in MEM supplemented with 10% (v/v) FBS and either 1.8 g/L BSA (control) or 0.1 mmol/L fatty acids bound to 1.8 g/L BSA. All cells were incubated at 37°C in a 95% air, 5% CO2 atmosphere as previously described (17–19). Medium was changed 48 h later and daily thereafter. At the start of experiments, the maintenance medium was removed, monolayers were washed twice with PBS and serum-free MEM containing either 7.5 g/L BSA (control dishes) or 0.2 mmol/L fatty acid bound to 7.5 g/L BSA (experimental dishes) was added and cells were incubated for the indicated period. A longer incubation time, i.e., 15–24 h, was necessary to obtain a sufficient quantity of samples for accurate determinations of the mass of TG and apolipoproteins A-I and B in the medium due to the sensitivity limits of the assays used. In contrast, short-term incubation time, i.e., 2–6 h, is routinely used to assess potential effects in the early stages of synthesis and secretion of lipids and apolipoproteins. Therefore, the incorporation of [3H]-labeled glycerol and [14C]-labeled acetate into lipids was determined after 4–6 h incubation. After incubation, preservative cocktail at the final concentrations of 500,000 U/L penicillin-G, 50 mg/L streptomycin sulfate, 20 mg/L chloramphenicol, 1.3 g/L E-amino caproic acid, and 1 g/L EDTA was added to the conditioned medium to prevent oxidative and proteolysis damage (18,19). The medium was centrifuged at 250 × g for 30 min at 4°C to remove small amounts of broken cells and debris. For mass determinations, medium was concentrated ~10- to 15-fold as previously described (17–20). The monolayers were washed three times with PBS, scraped off the plate in PBS and sonicated.

Isolation of lipoprotein density classes, determination of fatty acid uptake and the net accumulation in the medium of TG and apolipoproteins. To determine the rate of fatty acid uptake, cells were grown in phenol red-free MEM (to prevent interference with the enzymatic colorimetric method used to measure the concentration of free fatty acids) containing 0.2 mmol/L of each fatty acid bound to 7.5 g/L BSA. After 4-, 17- and 24-h incubations, conditioned medium was collected and processed as above. The concentration of nonesterified fatty acids (NEFA) in the medium before and after incubation with the cells was determined using the Wako NEFA test kit (Wako Chemicals, Richmond, VA). LDL (d 1.006–1.033 kg/L) and HDL (d 1.063–1.21 kg/L) were isolated by sequential preparative ultracentrifugation (21) of conditioned medium. The isolated lipoproteins were concentrated by ultrafiltration and the preservatives described above. The mass of TG in the 10- to 15-fold concentrated conditioned medium was determined by gas chromatography (22) and the concentrations of apoA-I and apoB were measured by electroimmunoassays using monospecific polyclonal antibodies to these apolipoproteins as described in detail elsewhere (23,24). Cell protein was measured by the method of Lowry et al. (25).

Determination of [3H]-glycerol and [14C]-acetate incorporation into various lipid fractions. HepG2 cells were plated and grown in MEM containing 10% FBS and 0.1 mmol/L fatty acids bound to 1.8 g/L BSA. After 5–6 d in culture, maintenance medium was removed, cells were washed twice with PBS and serum-free MEM containing 7.5 g/L BSA or 0.2 mmol/L fatty acid bound to 7.5 g/L BSA and [3H]-glycerol or [14C]-acetate was 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 described above. Aliquots of cell suspension, medium, and lipoprotein fractions (1 mL) were extracted with chloroform:methanol (2:1); extracts were washed as previously described (26) and adjusted to 25 mL with chloroform:methanol (2:1). Duplicate 1-mL aliquots of the final washed extracts were placed in counting vials, evaporated under nitrogen and counted; this number was multiplied by 25 to obtain the radioactivity in total lipids (phospholipids and neutral lipids) in 1 mL of medium, cell suspension, or lipoprotein fractions. Other 15-mL aliquots of extracts were evaporated at a small amount in a small amount of petroleum ether, applied to TLC plates, and various lipids were separated using a hexanediethyl ether:acetic acid (80:20:1) solvent system as previously described (26). The bands corresponding to phospholipids, free cholesterol, monoglycerides + diglycerides, TG, and cholesterol es-
Acute cellular uptakes of fatty acids (A) and the acute effects of fatty acids on the secretion rate of total sterols (B) in HepG2 cells. (A) Cells were grown for 5 d in phenol red-free minimum essential medium (MEM) containing 10% FBS. The rate of fatty acids (0.2 mmol/L bound to 7.5 g/L BSA) was determined after 4 h incubation. Values are means ± SE, n = 3. (B) Cells were grown for 5 d in MEM containing 10% FBS as described above. The incorporation of $[{}^{14}C]$-acetate (111 MBq/L of medium) into digitonin-precipitable total sterols secreted into the medium after a 3-, 6-, and 24-h incubation in the presence and absence of 0.2 mmol/L fatty acid bound to 7.5 g/L BSA was determined. Values are means ± SE, n = 3; those without a common letter differ, $P < 0.05$.

FIGURE 1 Acute cellular uptakes of fatty acids (A) and the acute effects of fatty acids on the secretion rate of total sterols (B) in HepG2 cells. (A) Cells were grown for 5 d in phenol red-free minimum essential medium (MEM) containing 10% FBS. The rate of fatty acids (0.2 mmol/L bound to 7.5 g/L BSA) was determined after 4 h incubation. Values are means ± SE, n = 3. (B) Cells were grown for 5 d in MEM containing 10% FBS as described above. The incorporation of $[{}^{14}C]$-acetate (111 MBq/L of medium) into digitonin-precipitable total sterols secreted into the medium after a 3-, 6-, and 24-h incubation in the presence and absence of 0.2 mmol/L fatty acid bound to 7.5 g/L BSA was determined. Values are means ± SE, n = 3; those without a common letter differ, $P < 0.05$.

TABLE 1

<table>
<thead>
<tr>
<th>Additions</th>
<th>TG mass $^3$ (nmol/g cell protein · h)</th>
<th>$[^{14}C]$-Labeled TG $^4$ (dpm/mg cell protein · h)</th>
<th>ApoA-I mass $^3$ ($\mu$g/g cell protein · h)</th>
<th>ApoB mass $^3$ ($\mu$g/g cell protein · h)</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>151.17 ± 4.50$^a$</td>
<td>5,600 ± 376$^d$</td>
<td>120.00 ± 9.48</td>
<td>94.46 ± 5.05</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>209.28 ± 13.38$^b$</td>
<td>14,637 ± 874$^a$</td>
<td>111.12 ± 10.12</td>
<td>105.56 ± 9.87</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>Linolelaidic acid</td>
<td>195.30 ± 26.00$^b$</td>
<td>12,625 ± 476$^b$</td>
<td>115.47 ± 3.87</td>
<td>112.33 ± 10.97</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>246.59 ± 7.29$^a$</td>
<td>8,775 ± 451$^c$</td>
<td>106.18 ± 5.42</td>
<td>100.67 ± 7.62</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>162.35 ± 6.87$^c$</td>
<td>8,225 ± 225$^d$</td>
<td>109.00 ± 3.08</td>
<td>106.59 ± 3.17</td>
<td>0.98 ± 0.05</td>
</tr>
</tbody>
</table>

1 Values are means ± SE, n = 3; those in a column with superscripts without a common letter differ, $P < 0.05$.
2 HepG2 cells were incubated in serum-free MEM containing either 7.5 g/L BSA (control) or 0.2 mmol/L of the indicated fatty acid bound to 7.5 g/L BSA as described in Materials and Methods.
3 The mass of TG and apolipoproteins A-I and B in the medium was measured after an 18-h incubation.
4 The incorporation of $[^{3}H]$glycerol into secreted newly synthesized TG was determined after a 4-h incubation.
by all fatty acids tested and this increase was more pronounced with linoleic acid (161%, \( P < 0.001 \)) than with linolelaidic acid (125%, \( P < 0.001 \)), elaidic acid (57%, \( P < 0.001 \)), and palmitic acid (47%, \( P = 0.003 \); Table 1). Long-term (5–6 d) incubation of HepG2 cells with linoleic acid increased \( (P < 0.001) \) the mass of TG in the medium by 175% (Table 2). All fatty acids tested stimulated the secretion of \( [3H] \)-labeled TG; the increase due to linoleic acid (272%, \( P < 0.001 \)) was more pronounced than with linolelaidic (125%, \( P < 0.001 \)), elaidic (175% \( P < 0.001 \)), and palmitic (135%, \( P < 0.001 \)) acids (Table 2).

**Net accumulation in the medium of apolipoproteins A-I and B.** Acute incubation of cells with fatty acids tested did not affect the net accumulation in the medium of apolipoproteins A-I and B or the ratio of apoB to apoA-I (Table 1). Although the apoA-I and apoB masses were not affected significantly by long-term incubation with fatty acids, the apoB to apoA-I ratio was greater (48%, \( P = 0.017 \)) with linolelaidic and elaidic acids than with palmitic acid (Table 2).

**De novo synthesis and secretion of total sterols and fatty acids.** Under acute experimental conditions, the incorporation of \( [14C] \)-acetate into total sterols (in cells plus medium) was not affected by linoleic and palmitic acids but was increased with linolelaidic acid (71%, \( P < 0.001 \)) and elaidic acid (40%, \( P = 0.001 \)) compared with the BSA control (Fig. 2). Compared with palmitic acid, total \( [14C] \)-labeled sterols were stimulated with linolelaidic acid (53%, \( P < 0.001 \)) and elaidic acid (25%, \( P = 0.016 \)) (Fig. 2). The long-term effect of fatty acids on the synthesis and secretion of total sterols was somewhat different from the acute effect. Under long-term experimental conditions, the synthesis and secretion of sterols were decreased by linoleic acid (58%, \( P < 0.001 \)), stimulated by linolelaidic acid (36%, \( P < 0.001 \)) and elaidic acid (96%, \( P < 0.001 \)) and were not affected by palmitic acid relative to the BSA control (Fig. 2). Compared with linoleic acid, the synthesis and secretion of total sterols were increased by linolelaidic acid (223%, \( P < 0.001 \)), elaidic acid (364%, \( P < 0.001 \)), and palmitic acids (150% \( P < 0.001 \); Fig. 2). Compared with palmitic acid, total sterol production was increased by linolelaidic acid (29%, \( P = 0.001 \)) and elaidic acids (85%, \( P < 0.001 \); Fig. 2). The percentages of total \( [14C] \)-acetate converted to total lipids (total fatty acids + sterols) in the cells and secreted into the medium that was recovered as digitonin-precipitable sterols were 9.08, 33.62, 29.84, and 17.37 for linoleic, linolelaidic, elaidic, and palmitic acids, respectively (Table 3). The percentages of total \( [14C] \)-acetate converted to total lipids in the cells and secreted into the medium that was recovered as total fatty acids (free fatty acids plus the fatty acid moiety of phospholipids, diglycerides, TG, and cholesteryl esters) were 90.97, 66.38, 70.19, and 82.63, respectively (data not shown).

Results shown in Figure 1, A and B, Figure 2, and Tables 1 and 2 demonstrate that within acute and long-term treatment groups, there were differences in the effects of fatty acids on lipoprotein production in HepG2 cells. Therefore, because most of the in vivo studies reflect prolonged dietary-induced changes in lipoprotein profile, in all subsequent studies only the long-term effects of fatty acids on lipoprotein synthesis and secretion in HepG2 cells were assessed.

**De novo synthesis and secretion of phospholipids and neutral lipids.** All fatty acids tested decreased the incorporation of \( [14C] \)-acetate into secreted TG (Table 4) and cellular

**TABLE 2**

*Long-term effects of fatty acids on the net accumulation in the medium of triglycerides (TG) and apolipoproteins A-I, B, and \( [3H] \)TG secretion in HepG2 cells*.

<table>
<thead>
<tr>
<th>Additions</th>
<th>TG mass</th>
<th>( [3H] )-labeled TG</th>
<th>ApoA-I mass</th>
<th>ApoB mass</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>( 206.06 \pm 20.14 )</td>
<td>( 5,767 \pm 544 )</td>
<td>( 114.05 \pm 10.20 )</td>
<td>( 119.20 \pm 13.58 )</td>
<td>( 1.11 \pm 0.11 )</td>
</tr>
<tr>
<td>Linoleic</td>
<td>( 567.29 \pm 72.67 )</td>
<td>( 21,474 \pm 631 )</td>
<td>( 123.72 \pm 11.39 )</td>
<td>( 131.23 \pm 17.52 )</td>
<td>( 1.05 \pm 0.08 )</td>
</tr>
<tr>
<td>Linolelaidic acid</td>
<td>( 256.80 \pm 1.13 )</td>
<td>( 12,962 \pm 1,083 )</td>
<td>( 107.26 \pm 9.43 )</td>
<td>( 135.38 \pm 12.00 )</td>
<td>( 1.27 \pm 0.07 )</td>
</tr>
<tr>
<td>Elaidic</td>
<td>( 262.95 \pm 3.56 )</td>
<td>( 15,773 \pm 647 )</td>
<td>( 106.62 \pm 6.76 )</td>
<td>( 134.56 \pm 10.58 )</td>
<td>( 1.27 \pm 0.10 )</td>
</tr>
<tr>
<td>Palmitic</td>
<td>( 261.64 \pm 8.58 )</td>
<td>( 13,533 \pm 1,773 )</td>
<td>( 111.23 \pm 11.45 )</td>
<td>( 96.41 \pm 6.67 )</td>
<td>( 0.86 \pm 0.07 )</td>
</tr>
</tbody>
</table>

1 Values are means \( \pm SD, n = 3 \) for TG mass, \( n = 6 \) for secreted \( [3H] \)-labeled triglycerides, \( n = 8 \) for apoA-I and apoB mass; those in a column with superscripts without a common letter differ, \( P < 0.05 \).
2 HepG2 cells were incubated in MEM containing either 7.5 g/L BSA or 0.2 mmol/L of fatty acid bound to 7.5 g/L BSA as described in Materials and Methods.
3 The mass of TG and apolipoproteins A-I and B in the medium was measured after an overnight (18–22 h) incubation.
4 The incorporation of \( [3H] \)-glycerol into secreted TG was determined after a 4-h incubation.

Figure 2. Acute and chronic effects of fatty acids on the synthesis and secretion of digitonin-precipitable total sterols in HepG2 cells. Cells were grown for 5 d under conditions described in Materials and Methods. The incorporation of \( [14C] \)-acetate (111 MBq/L of medium) into cellular and secreted digitonin-precipitable total sterols in the presence and absence of 0.2 mmol/L of fatty acids bound to 7.5 g/L BSA was determined after 6 h incubation. Values are means \( \pm SE, n = 3 \) in acute experiments and \( n = 6 \) in chronic experiments. Values within a panel without a common letter differ, \( P < 0.05 \).
and Methods and the incorporation of hence, their incorporation into secreted and cellular phospho-
esters was more effectively inhibited by linoleic acid (74%, $P < 0.001$) than by linolelaidic (24%, $P < 0.001$), elaidic (55%, $P < 0.001$), and palmitic acids (61–64%, $P < 0.001$; Table 4). In contrast, the secretion of cholesteryl esters was more effectively inhibited by linoleic acid (74%, $P < 0.001$) than by linolelaidic (52%, $P < 0.001$), elaidic (24%, $P < 0.001$) and palmitic (55%, $P < 0.001$) acids (Table 4). The secretion of free cholesterol was decreased by linoleic acid (48%, $P = 0.001$), stimulated by elaidic acid (64%, $P < 0.001$) and was not affected by linolelaidic and palmitic acids (Table 4). Similar results were obtained on the cellular accumulation of lipids (Table 5).

Compared with linoleic acid, linolelaidic acid: 1) decreased the secretion (54%, $P = 0.001$) and cellular content (63%, $P < 0.001$) of phospholipids; 2) stimulated the secretion (82%, $P < 0.001$) and cellular content (125%, $P < 0.001$) of free cholesterol; 3) reduced the secretion (70%, $P = 0.002$) and cellular content (55%, $P < 0.001$) of TG, and 4) enhanced the secretion (86%, $P = 0.002$) and cellular content (147%, $P = 0.003$) of cholesteryl esters. Relative to linoleic acid, elaidic acid: 1) tended to decrease the secretion (28%, $P = 0.15$) and decreased the cellular content (49%, $P < 0.001$) of phospho-
lipids; 2) stimulated the secretion (220%, $P < 0.001$) and cellular content (197%, $P < 0.001$) of free cholesterol, 3) reduced the secretion (38%, $P = 0.005$) and cellular content (21%, $P = 0.002$) of TG, and 4) enhanced the secretion (192%, $P < 0.001$) and cellular content (208%, $P = 0.001$) of cholesteryl esters. Compared with linoleic acid, palmitic acid: 1) decreased the secretion (32%, $P = 0.095$) and cellular content (48%, $P < 0.001$) of phospholipids; 2) increased the secretion (54%, $P = 0.008$) and cellular content (99%, $P < 0.001$) of free cholesterol; 3) reduced the secretion (40%, $P = 0.002$) and cellular content (32%, $P = 0.008$) of TG, and 4) stimulated the secretion (77%, $P = 0.009$) and cellular content (60%, $P = 0.008$) of cholesteryl esters.

### Cholesterol concentration of secreted lipoprotein density classes.
Linoleic and palmitic acids did not affect LDL-C or HDL-C concentrations (Fig. 3). Compared with the BSA control, elaidic acid increased LDL-C by 136% ($P < 0.001$) and HDL-C by 90% ($P = 0.003$), and linolelaidic acid stimulated LDL-C by 130% ($P < 0.001$) and HDL-C by 74% ($P < 0.001$; Fig. 3). Compared with linoleic acid, linolelaidic acid increased LDL-C and HDL-C by 154% ($P < 0.001$) and 50% ($P = 0.016$, respectively; elaidic acid increased LDL-C and HDL-C by 161% ($P < 0.001$) and 65% ($P = 0.003$, respectively; and palmitic acid did not affect LDL-C and HDL-C (Fig. 3). Furthermore, compared with palmitic acid, both linolelaidic and elaidic acids significantly ($P < 0.001$) increased LDL-C (117–123%) and to a lesser extent (70–88%, $P

### TABLE 3

<table>
<thead>
<tr>
<th>Additions</th>
<th>14C-labeled total sterols$^3$</th>
<th>LDL-C/HDL-C ratio</th>
<th>Phospholipid/cholesterol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of total labeled lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL</td>
<td>HDL</td>
</tr>
<tr>
<td>BSA</td>
<td>8.89 ± 0.15$^b$</td>
<td>0.89 ± 0.06$^{bc}$</td>
<td>2.26 ± 0.04$^a$</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>9.08 ± 1.71$^b$</td>
<td>0.69 ± 0.01$^c$</td>
<td>0.78 ± 0.03$^b$</td>
</tr>
<tr>
<td>Linolelaidic acid</td>
<td>33.62 ± 2.21$^a$</td>
<td>1.18 ± 0.02$^a$</td>
<td>0.23 ± 0.02$^d$</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>29.84 ± 2.52$^a$</td>
<td>1.09 ± 0.04$^{ab}$</td>
<td>0.25 ± 0.01$^d$</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>17.37 ± 1.17$^c$</td>
<td>0.96 ± 0.10$^{ab}$</td>
<td>0.39 ± 0.01$^c$</td>
</tr>
</tbody>
</table>

1 Values are means ± SE, $n = 6$ for 14C-labeled total sterols, $n = 3$ for LDL-C/HDL-C and phospholipid/cholesterol ratios of LDL and HDL; those in a column with superscripts without a common letter differ, $P < 0.05$.
2 Cells were grown as described in Materials and Methods and the incorporation of [14C]acetate (185 MBq/L of medium) into total sterols and the cholesterol and phospholipid moieties of LDL and HDL was determined after 15 h of incubation.
3 Values are expressed as the percentage of total [14C]acetate converted to lipids (total fatty acids + sterols) in the cells and secreted into the medium that was recovered as digitonin-precipitable sterols.

### TABLE 4

<table>
<thead>
<tr>
<th>Additions</th>
<th>Phospholipids</th>
<th>Free cholesterol</th>
<th>Triglycerides</th>
<th>Cholesterol esters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpmmg cell protein h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>1860 ± 132$^a$</td>
<td>677 ± 33$^b$</td>
<td>2598 ± 42$^a$</td>
<td>143 ± 6$^a$</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1056 ± 112$^b$</td>
<td>347 ± 5$^c$</td>
<td>1555 ± 83$^b$</td>
<td>37 ± 4$^d$</td>
</tr>
<tr>
<td>Linolelaidic acid</td>
<td>483 ± 36$^a$</td>
<td>632 ± 16$^b$</td>
<td>483 ± 5$^d$</td>
<td>68 ± 4$^c$</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>759 ± 16$^{bc}$</td>
<td>1109 ± 77$^a$</td>
<td>959 ± 71$^c$</td>
<td>108 ± 2$^b$</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>721 ± 49$^{bc}$</td>
<td>535 ± 19$^b$</td>
<td>929 ± 24$^c$</td>
<td>65 ± 3$^c$</td>
</tr>
</tbody>
</table>

1 Values are means ± SE, $n = 3$; those in a column with superscripts without a common letter differ, $P < 0.05$.
2 HepG2 cells were incubated in serum-free MEM containing 0.2 mmol/L of the indicated fatty acid bound to 7.5 g/L BSA as described in Materials and Methods and the incorporation of [14C]acetate (111 MBq/L of medium) into secreted lipids was determined after 6 h.
The major lipid constituents of LDL (Table 3). In the presence of linoleic acid, TG and phospholipids were among the fatty acids tested (data not shown). However, there were no differences in LDL receptor activity LDL binding to HepG2 cells compared with BSA alone.

LDL receptor activity. To determine whether the changes in LDL-C (Fig. 3) were due to differential effects of fatty acids on LDL receptor activity, the binding of [125I]-labeled plasma LDL at 5, 12.5, 25, and 50 mg protein/L, to HepG2 cells was determined. The binding of LDL at 5 mg protein/L of culture medium was not affected by any of the fatty acids tested. At concentrations > 12.5 mg/L of medium, all fatty acids had moderate but significant (P < 0.05) inhibitory effects on the LDL binding to HepG2 cells compared with BSA alone. However, there were no differences in LDL receptor activity among the fatty acids tested (data not shown).

Lipid composition of secreted lipoprotein density classes. In the presence of linoleic acid, TG and phospholipids were the major lipid constituents of LDL (Table 6) and HDL (Table 7), respectively. In contrast, cholesterol was the major lipid component of both LDL and HDL in cells incubated with linoleoleic, linoleidic, elaidic, and palmitic acids, respectively (Table 3). Thus, compared with linoleic acid, the PL:C ratio of both LDL and HDL were lower (P < 0.001) with trans and saturated fatty acids. Compared with palmitic acid, the PL:C ratio of LDL was lower in cells incubated with linoleidic (P = 0.007) and elaidic (P = 0.017) acids and that of HDL were significantly (P < 0.001) lower with both trans fatty acids (Table 3).

DISCUSSION

We have previously reported the relative long-term effects of cis and trans monounsaturated and saturated fatty acids on the synthesis and secretion of apoA-I and apoB-containing lipoproteins in HepG2 cells (29). The purpose of this study was to test whether trans PUFA and saturated fatty acids have different effects on the concentration and composition of lipoproteins secreted by HepG2 cells. The higher TG secretion and concentration with linoleic acid than with linoleoleic, elaidic, and palmitic acids might be due to a lower selectivity for the incorporation of saturated and trans unsaturated fatty acids into TG compared with cis unsaturated fatty acids (5). Our results are consistent with studies in perfused rat liver demonstrating that trans, trans-18:2 decreased the secretion of TG compared with cis, cis-18:2 (30), and with results obtained in human-derived intestinal Caco2 cells showing that TG secretion was higher with linoleic acid than with palmitic acid (31,32). Studies in humans, however, have indicated that saturated and trans unsaturated fatty acids increase the serum concentration of TG compared with cis unsaturated fatty acids (6,8). Although the results of in vitro studies cannot be directly compared with those obtained in vivo, these differences may be due to variations in the catabolism and/or differential modification or remodeling in circulation of hepatic TG-rich lipoproteins produced in the presence of different fatty acids. Consistent with this possibility, studies by Chung et al. (33) demonstrated that polyunsaturated TG in VLDL and/or chylomicrons are more susceptible to lipolysis than saturated TG.

Studies in both humans (34,35) and nonhuman primates

![FIGURE 3](https://academic.oup.com/jn/article-abstract/132/9/2651/4687935)

**FIGURE 3** Long-term effects of fatty acids on the LDL-cholesterol (C) and HDL-C secreted by HepG2 cells. Cells were grown for 5 d under conditions described in Materials and Methods. The incorporation of [14C]-acetate (185 MBq/L of medium) into LDL-C and HDL-C in the presence and absence of 0.2 mmol/L of fatty acids bound to 7.5 g/L BSA was determined after 15 h incubation. Values are means ± SE, n = 3; those within a panel without a common letter differ, P < 0.05.
human primates (36) indicate that enhanced catabolism, rather than decreased secretion, contribute to the reduction of HDL-apoA-I concentration when unsaturated fat is substituted for saturated fat. The concentrations of TG and apoB in the medium did not change in parallel with fatty acids tested, an observation consistent with that in rat liver (39) and rat hepatocytes (40). As in nonhuman primates (38), there were no major differences between the effects of saturated and PUFA on the mass of apoB in the medium. The higher apoB/apoA-I ratios in cells incubated with linolealaidic and elaidic acids than in those with linoleic and palmitic acids are consistent with studies in humans (6,8,10,11,42,43) and non-human primates (44) and suggest that trans unsaturated fatty acids have a more adverse effect than saturated fatty acid on the mass ratio of apoB to apoA-I, considered to be a good indicator of risk for CAD (45,46).

The observed decrease in de novo synthesis and secretion of total fatty acids in HepG2 cells incubated with elaidic and linolealaidic acids compared with linoleic and palmitic acids may be due to increased formation of ketone bodies with trans fatty acids as demonstrated in the rat liver (30). The higher synthesis and secretion of total sterols in cells incubated with elaidic and linolealaidic acids than with linoleic and palmitic acids are compatible with studies in humans (4–7). Taken together, the above results suggest that a greater proportion of acetyl-CoA was channeled to cholesterol synthesis in the presence of trans fatty acids compared with cis and saturated fatty acids. As discussed in a recent review (47), cis PUFA apparently exert their beneficial effect by activating the transcription factor peroxisome proliferator activator receptor α and by suppressing the expression of sterol regulatory element binding protein-1 resulting in fuel repartitioning. These effects seem to be reversed by the hydrogenation of PUFA and production of trans fatty acids.

Consistent with in vivo studies in humans (9–11), the LDL-C/HDL-C ratios were higher in cells incubated with trans fatty acids than in cells incubated with cis and saturated fatty acids. However, the unchanged LDL-C in cells incubated with palmitic acid differs from in vivo studies, demonstrating a hypercholesteroleic effect of this fatty acid (2,48). The increase in LDL-C associated with a saturated-fat diet may be due to a decrease in LDL receptor activity in the presence of dietary cholesterol (2,49), a component not included in our experiments. This may also explain the lack of effects of the fatty acids on LDL receptor activity in this study.

Our data demonstrate that the LDL particles secreted by HepG2 cells incubated with linoleic acid contained less free cholesterol and cholesteryl esters than cells incubated with elaidic, linolealaidic, and palmitic acids, an observation consistent with the reported cholesterol-lowering property of linoleic acid. An interesting finding of this study was that both elaidic and linolealaidic acids markedly increased the secretion of cholesterol-rich, apoB-containing lipoproteins compared with palmitic acid, implying that trans fatty acids may be more atherogenic than saturated fatty acids. The ratio of phospholipid to cholesterol (PL:C) in HDL particles was lower in cells incubated with elaidic and linolealaidic acids than with linoleic and palmitic acids (Table 3). Several studies have demonstrated a positive correlation between the PL:C ratio of HDL

### Table 6

**Long-term effects of fatty acids on the lipid composition of LDL secreted by HepG2 cells**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
<th>Free fatty acids</th>
<th>Triglycerides</th>
<th>Cholesteryl esters</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>23.93 ± 0.90a</td>
<td>10.76 ± 0.01b</td>
<td>1.22 ± 0.07c</td>
<td>60.28 ± 1.32a</td>
<td>3.83 ± 0.49c</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>17.87 ± 0.20b</td>
<td>25.61 ± 1.71d</td>
<td>4.82 ± 0.06d</td>
<td>49.75 ± 1.95b</td>
<td>1.95 ± 0.50d</td>
</tr>
<tr>
<td>Linolealaidic</td>
<td>11.27 ± 1.68b</td>
<td>46.56 ± 0.47a</td>
<td>3.23 ± 0.05c</td>
<td>32.81 ± 0.40d</td>
<td>6.14 ± 0.30c</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>10.43 ± 0.38b</td>
<td>39.16 ± 0.42b</td>
<td>1.74 ± 0.11c</td>
<td>39.83 ± 0.66c</td>
<td>8.86 ± 0.24a</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>12.17 ± 0.44b</td>
<td>30.85 ± 0.47c</td>
<td>3.41 ± 0.28b</td>
<td>49.45 ± 0.01b</td>
<td>4.13 ± 0.30c</td>
</tr>
</tbody>
</table>

1 Values are means ± se, n = 3; those in a column with superscripts without a common letter differ, P < 0.05.

### Table 7

**Long-term effects of fatty acids on the lipid composition of HDL secreted by HepG2 cells**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
<th>Free fatty acids</th>
<th>Triglycerides</th>
<th>Cholesteryl esters</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>59.91 ± 1.16a</td>
<td>17.37 ± 1.54d</td>
<td>2.94 ± 0.28c</td>
<td>16.68 ± 0.03b</td>
<td>3.13 ± 0.23b</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>51.43 ± 2.55b</td>
<td>30.02 ± 1.57c</td>
<td>6.00 ± 0.12a</td>
<td>11.47 ± 0.61c</td>
<td>1.08 ± 0.26c</td>
</tr>
<tr>
<td>Linolealaidic</td>
<td>24.35 ± 0.98bc</td>
<td>62.27 ± 0.45a</td>
<td>3.92 ± 0.39bc</td>
<td>6.29 ± 0.40d</td>
<td>3.19 ± 0.11b</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>23.01 ± 0.35c</td>
<td>59.13 ± 0.44a</td>
<td>2.88 ± 0.14c</td>
<td>9.87 ± 0.04c</td>
<td>5.12 ± 0.01a</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>29.20 ± 0.83c</td>
<td>39.20 ± 1.36b</td>
<td>5.17 ± 0.35ab</td>
<td>22.88 ± 0.97a</td>
<td>3.56 ± 0.11b</td>
</tr>
</tbody>
</table>

1 Values are means ± se, n = 3; those in a column with superscripts without a common letter differ, P < 0.05.

2 Cells were grown as described in Materials and Methods and the incorporation of [14C]acetate (185 MBq/L of medium) into secreted HDL was measured after 15 h of incubation.
and the capacity of serum to accept cellular cholesterol (50–52). It has been suggested that a low PL:C ratio in HDL is associated with increased risk for ischemic vascular disease (53) and CAD (54). Our data, therefore, suggest that compared with linoleic and palmitic acids, the HDL particles secreted in the presence of trans unsaturated fatty acids may be less efficient in promoting cholesterol efflux from the cells.

Linoleic acid increased the association of apoB with larger, TG-rich lipoproteins, i.e., these particles had a higher mass ratio of TG to apoB (3.37) than their counterparts produced in the presence of elaidic and linolelaidic acids (1.67), or palmitic acid (2.4). These results suggest that compared with the BSA control, linoleic acid had no major effect on the number of apoB-containing lipoproteins but produced larger, TG-rich particles. In contrast, apoB-containing lipoprotein particles produced in the presence of palmitic acid, and to a greater extent, trans fatty acids, contained less TG and more free cholesterol and cholesteryl ester and, therefore, may be less susceptible to lipolysis than their counterparts secreted in the presence of linoleic acid (33).

We have demonstrated that long-term incubation of HepG2 cells with linoleic acid decreased the synthesis and secretion of cholesterol-rich apoB-containing lipoproteins compared with linolelaidic, elaidic, and palmitic acids. The LDL:C-HDL-C ratio was lower in cells incubated with linoleic acid than with linolelaidic, elaidic, and palmitic acids. The LDL and HDL particles secreted in the presence of linoleic acid contained less free cholesterol and cholesteryl ester and more phospholipid and TG than those secreted in the presence of linolelaidic, elaidic, and palmitic acids. An important finding of this study is that compared with palmitic acid, linolelaidic and elaidic acids stimulated the synthesis and secretion of cholesteryl and apoB, increased the ratios of LDL:C-HDL-C and apoB:ApoA-I, and produced LDL and HDL particles that contained more cholesterol and less phospholipid and TG, all of which are directly correlated with increased risk for CAD. Based on these results, we suggest that relative to cis polyunsaturated and saturated fatty acids, trans PUFA more adversely affect the concentration and composition of apoA-I and apoB-containing lipoproteins and, therefore, may be more atherogenic.

ACKNOWLEDGMENTS

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LITERATURE CITED

29. Dashi, N., Feng, Q. & Franklin, F. A. (2000) 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 and, therefore, may be more atherogenic.

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