Secretion of lipids, apolipoproteins, and lipoproteins by human hepatoma cell line, HepG2: effects of oleic acid and insulin

Nassrin Dashti[1] and Gertrud Wolfbauer[2]

Lipoprotein and Atherosclerosis Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

Abstract   The aim of this study was to determine the effect of oleic acid and insulin on the secretion of lipoproteins by HepG2 cells grown in minimum essential medium. Triglycerides were the major neutral lipid (57% of total) and apoB was the predominant apolipoprotein (56% of total) secreted by these cells. The addition of oleate resulted in a two-fold increase in the concentration of neutral lipids but only a slight to moderate increase in the apolipoprotein (A-I, A-II, B, and E) levels. The secretion of very low density lipoproteins (VLDL) was stimulated by 425%, low density lipoproteins (LDL) by 77%, and high density lipoproteins (HDL) by 68%. Whereas neutral lipid composition of LDL was unchanged, the VLDL particles contained a significantly higher percentage of triglyceride and lower percentages of cholesterol and cholesteryl esters compared with VLDL secreted in the absence of oleate. Oleate had no significant effect on the composition of apolipoproteins in VLDL, LDL and HDL. In basal medium, insulin caused a significant decrease in the secretion of neutral lipids and apolipoproteins, particularly triglycerides and apoB. In addition to a 60–68% reduction in the total concentration of VLDL and LDL, insulin altered their composition by producing particles that had a significantly lower content of triglycerides, contained less apoB, and were deficient in apoE. There were no major changes in the concentration or composition of HDL particles. Insulin had a similar but less pronounced effect on the concentration and composition of lipoproteins secreted in the presence of oleate. ▬ The increased accumulation of triglycerides in the HepG2 cells concomitant with their reduced levels in the medium suggests that insulin may affect the secretion rather than synthesis of triglyceride-rich lipoproteins. —**Dashti, N., and G. Wolfbauer.** Secretion of lipids, apolipoproteins, and lipoproteins by human hepatoma cell line, HepG2: effects of oleic acid and insulin. *J. Lipid Res.* 1987. **28:** 423–436.

Supplementary key words VLDL • LDL • HDL • fatty acids • hormones • triglyceride synthesis • liver

One of the important functions of the liver is to synthesize plasma lipoproteins (1–3). Freshly isolated hepatocytes (4–6), liver cells maintained in suspension (7), and hepatocytes in primary culture (8–10) have been shown to retain the ability to synthesize and secrete plasma lipoproteins. Several studies have also demonstrated that major apolipoproteins are synthesized by the intact rat liver (11–13) and cultured liver cells (8, 9, 14–16). The hepatic lipoprotein synthesis and secretion are influenced by many factors, including nutrients and hormones. A number of studies with the perfused liver (17–21), liver slices (22), and hepatocytes in primary culture (10, 15, 23) have shown that long-chain fatty acids have a stimulatory effect on the synthesis of triglyceride secreted in the form of very low density lipoproteins (VLDL). However, the enhanced secretory rates of lipids in response to fatty acid provision were not paralleled by similar changes in the apolipoprotein (apo) secretion (24–26). In contrast to the well-established effect of fatty acids, the role of insulin on the regulation of triglyceride secretion is controversial. In vivo studies have indicated that, in man, insulin increased hepatic triglyceride secretion (27–28). Direct stimulatory effect of insulin on fatty acid esterification and VLDL secretion by perfused rat liver (29–31) has also been demonstrated. Contrary to these results, acute in vivo inhibition of hepatic triglyceride secretion by insulin has been reported both in humans (32) and the rat (33). Furthermore, direct inhibitory effect of insulin on hepatic secretion of triglyceride-rich lipoproteins has been documented in two separate studies (34–35) using cultured rat hepatocytes. A recent report by Patsch, Gotto, and Patsch (36) has shown that insulin exerts its inhibitory effect through a receptor-mediated process which is subject to down-regulation by prior exposure of cells to this hormone.

Similar studies on the regulation of hepatic lipoprotein synthesis and secretion using the human liver system have been hampered by the lack of an adequate experimental model. The recently developed human hepatocellular carcinoma cell line HepG2 (37) has been shown to retain the normal biochemical functions of liver parenchymal cells (38–40). The ability of HepG2 cells to synthesize and secrete into the medium the human apolipoproteins A-I, A-II, B, C-II, C-III, and E (41, 42) has rendered these cells a suitable model for studying factors that regulate the human hepatic lipoprotein production. The purpose of the present study was to determine the secretion rates of neutral lipids, apolipoproteins, and major lipoprotein density classes of HepG2 cells, and to assess the effects of oleic acid and insulin on these processes. Preliminary results have been already reported in an abstract form (43).

MATERIALS AND METHODS

Materials

Modified minimum essential medium (MEM) and fetal calf serum (FCS) were purchased from Grand Island Biological Company (Grand Island, NY). Trypsin, sodium pyruvate, L-glutamine, and MEM vitamin solution were from KC Biological, Inc. (Lenexa, KS). Bovine insulin was obtained from Calbiochem-Behring (La Jolla, CA). Oleic acid was from Sigma Chemical Co. (St. Louis, MO). Fatty acid-free bovine serum albumin was purchased from Miles Laboratories (Elkhart, IN) and [1-^{14}C]oleic acid was from New England Nuclear (Boston, MA).

Cell cultures

Cells were seeded in 60-mm or 100-mm diameter dishes in 0.1–0.2 ml of supplemented MEM containing 10% FCS/cm^2 surface area as described previously (44). Five to 6 days after seeding, maintenance medium was removed, cells were washed three times with phosphate-buffered saline (PBS), pH 7.4, fresh medium without FCS was added, and cells were incubated for a certain time period as indicated for each experiment. In studies where the effect of fatty acid was to be assessed, medium was supplemented with 0.4 mM oleate bound to 1.5% bovine serum albumin. Insulin, prepared as previously described (15), was included when indicated. At the end of each incubation period, the medium was removed, adjusted to 0.1% EDTA, and centrifuged at 2000 rpm for 20 min at 4°C to remove small amounts of cells and debris. The supernatant fraction was concentrated approximately 10- to 15-fold with sucrose placed outside the dialysis bag (5000 mol wt cut–off).

Isolation of lipoprotein density classes

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 (45). The isolated lipoproteins were dialyzed against PBS containing 0.01% EDTA (pH 7.4).

Determination of neutral lipids and apolipoproteins

Triglycerides and cholesterol contents of concentrated culture medium, lipoprotein density classes, and HepG2 cells were determined as described by Kuksis et al. (46). The concentrations of apolipoproteins A-I, A-II, B, C-I, C-II, C-III, and E were measured by electroimmunoassays developed in this laboratory as previously described (47–51).

Determination of [1-^{14}C]oleic acid incorporation into various lipid fractions in culture medium and HepG2 cells

After 6 days in culture, maintenance medium was removed and cells were washed with PBS and incubated with MEM containing 0.4 mM [1-^{14}C]oleic acid–1.5% bovine serum albumin for 4 hr at 37°C in the presence or absence of insulin. At the end of incubation, medium was removed and cells were washed three times with PBS. The monolayers were scraped off the plates with a rubber policeman after addition of 2 × 1 ml of PBS and sonicated. sonicated. One-milliliter aliquots of medium and cell suspension were extracted with chloroform-methanol 2:1; extracts were washed and applied to thin-layer chromatography plates as described previously (52). Cell protein was measured by the method of Lowry et al. (53).

RESULTS

Rates of neutral lipid and apolipoprotein accumulation in the culture medium

After 5–6 days in culture medium containing 10% FCS, the maintenance medium was removed and cells were washed with PBS. Fresh medium without FCS (basal medium) was added and the levels of triglycerides, cholesterol, and apolipoproteins were measured in the culture medium during the 24-hr incubation period. As shown in **Fig. 1,** the rates of triglycerides and total cholesterol accumulation in the culture medium were constant for 24 hr. In a series of six experiments, the maximum rates of triglycerides and cholesterol accumulation in basal culture medium containing 5.5 mM glucose were 505 and 233 μg/hr per g of cell protein, respectively. Thus, the HepG2 cells synthesized and secreted similar molar quantities of triglycerides and cholesterol. However, in
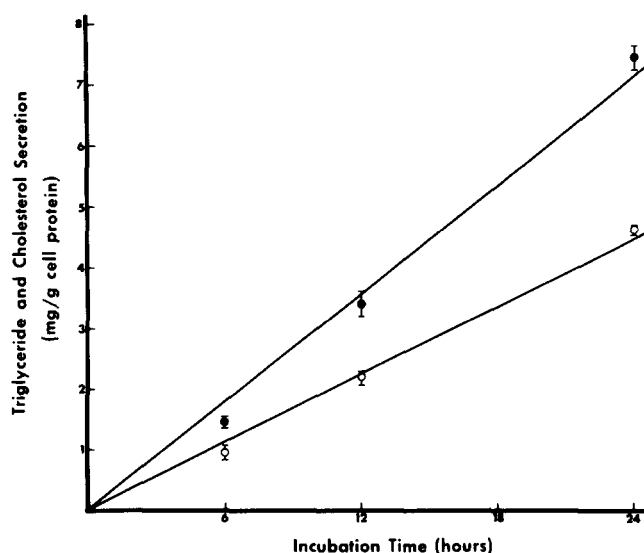
Fig. 1. Triglyceride and cholesterol secretion by Hep G2 cells. Cells were cultured in minimum essential medium containing 10% fetal calf serum for 5–6 days. The maintenance medium was removed, and cells were washed three times with PBS. Fresh medium without fetal calf serum was added and cells were incubated for 24 hr. At each time point the medium was removed and assayed for triglycerides (●) and total cholesterol (O). Each value is the mean ± SEM of triplicate plates.

weight percentages, triglycerides accounted for 57%, while unesterified cholesterol accounted for 31%, and cholesteryl esters for 12% of the total neutral lipid content (**Table 1**). Approximately 80% of cholesterol secreted by HepG2 cells was in the unesterified form and the remaining 20% was esterified.

As shown in **Table 2**, apoB was the major apolipoprotein secreted by HepG2 cells (56% of the total) followed by apoA-I (22% of the total), apoE (13% of the total), and apoA-II (9% of the total). Although apolipoproteins C-I, C-II, and C-III were detected in the culture medium, due to their low concentrations, their levels, particularly those of apoC-I and apoC-II, could not be accurately measured by the available immunoassays. In a separate study, the

concentrations of lipoprotein B and lipoprotein B:E particles in the medium were measured over a 24-hr incubation period. The results demonstrated that the rates of accumulation of these particles in the medium were constant and that lipoprotein B particles were secreted independently and at a higher rate than lipoprotein B:E particles (Dashti, N., and G. Wolfbauer, unpublished data). A constant rate of accumulation of neutral lipids (Fig. 1) and apolipoproteins B and E (data not shown) in the culture medium for several hours is indicative of a steady state and reflects constant rates of both synthesis and secretion. Thus, the term production, used herein, refers to the combined processes of synthesis and secretion of products by the cells.

Effects of insulin on the concentrations of neutral lipids and apolipoproteins in the basal culture medium

Incubation of cells in the presence of 8–1,000 ng/ml (200–25,000 μU/ml) of insulin resulted in decreased accumulation of unesterified cholesterol, cholesteryl esters, and triglycerides in the medium (**Fig. 2**). This reduction in the concentration of neutral lipids was dose-dependent and was observed when cells were incubated with either basal medium (Fig. 2A) or basal medium supplemented with oleate (Fig. 2B). The maximum effect of insulin, in both systems, was observed at 1 μg/ml of medium. Based on these results, all subsequent experiments were performed in the presence or absence of pharmacological concentrations of 1 μg insulin/ml of medium.

Addition of insulin produced a significant decrease in the concentrations of the secreted triglycerides, cholesteryl esters, and total cholesterol (Table 1). Unesterified cholesterol accounted for 84% of the total cholesterol in the insulin-supplemented medium (Table 1). The concentrations of apolipoproteins A-I, A-II, B, and E were also decreased in response to insulin administration (Table 2). The decrease in apoB secretion was more pronounced than that observed in other apolipoproteins (Table 2).

TABLE 1.   Effect of insulin on the secretion of triglycerides and cholesterol by
Hep G2 cells in minimum essential medium

	Triglycerides	Unesterified Cholesterol	Cholesteryl Esters	Total Cholesterol
		μg/g cell protein per hr		
Medium	318.11 ± 70.25	171.63 ± 22.52	66.21 ± 8.82	211.37 ± 25.99
Medium + insulin	123.82 ± 42.94	104.57 ± 21.01	30.33 ± 7.72	124.79 ± 27.13
% decrease	61.1	39.1	54.2	41.0
P^a	< 0.05	NS[b]	< 0.02	< 0.05

After 24 hr incubation in serum-free MEM in the presence and absence of insulin (1 μg/ml), the concentrations of triglycerides and cholesterol in the medium were determined by gas–liquid chromatography. Values are means ± SEM of six experiments.
[a]Determined by Student's t-test.
[b]NS, not significant ($P > 0.05$).

TABLE 2. Effect of insulin on the secretion of apolipoproteins by Hep G2 cells in minimum essential medium

	Apolipoproteins			
	A-I	A-II	B	E
	μg/g cell protein per hr			
Medium	120.84 ± 11.18	47.52 ± 6.70	312.36 ± 15.06	74.54 ± 12.82
Medium + insulin	91.34 ± 6.78	38.00 ± 5.74	143.23 ± 15.08	57.13 ± 13.73
% decrease	24.4	20	54.1	23.4
P^a	< 0.05	NS[b]	< 0.02	NS[c]

Experimental conditions are described in Table 1. Values are means ± SEM of six experiments.
[a]Determined by Student's *t*-test.
[b]NS, not significant ($P > 0.05$).

Rates of accumulation of neutral lipids and apolipoproteins in the medium in response to the addition of oleate

The concentrations of triglycerides and cholesterol in the culture medium were elevated significantly ($P < 0.05$) upon addition of oleate (**Table 3**) as compared to basal medium alone (Table 1). The increase in triglycerides secretion was predominantly due to the elevation of a triglyceride consisting mainly of three fatty acids of 18-carbon atoms (designated TG-57) whose absolute concentration in oleate-supplemented medium was 300% higher than that in basal medium. Major neutral lipids secreted in the presence of oleate (Table 3) were triglycerides which accounted for 58% of the total lipid; they were followed by unesterified cholesterol (32%) and cholesteryl esters (8.8%). The major apolipoprotein synthesized and secreted by HepG2 cells incubated in oleate-supplemented medium was apoB (**Table 4**). The percent composition of apolipoproteins A-I, A-II, B, and E in the presence of oleate (Table 4) was similar to that observed in the basal medium (Table 2). Oleate produced small to moderate stimulation in the secretion of apolipoproteins A-I (10%), A-II (19%), B (28%), and E (8%).

Insulin-related changes in the concentrations of neutral lipids and apolipoproteins in the oleate-supplemented medium

The addition of insulin to HepG2 cells incubated in the presence of oleate caused a marked reduction in the concentrations of triglycerides and cholesteryl esters in the medium (Table 3). There was a concomitant decrease in the secretion of apolipoproteins B and E upon insulin administration (Table 4). On the other hand, the concentrations of apolipoproteins A-I and A-II in the medium were unchanged by this hormone (Table 4). Whereas insulin had no major effect on the cellular cholesterol, it caused a 65% increase ($P < 0.05$) in the accumulation of triglycerides in the cells.

Effects of oleate and insulin on the concentrations of lipoprotein density classes

In the basal culture medium VLDL, LDL, and HDL accounted for 14%, 60%, and 26%, respectively, of the total lipoproteins (**Fig. 3**). Due to the low concentration of lipoproteins with d < 1.006 g/ml in the culture medium, the contribution of LDL₁ (d 1.006–1.03) to the total lipoproteins was further examined. In two separate experiments, it was determined that the contributions of VLDL + LDL₁(d < 1.03 g/ml), LDL₂ (d 1.03–1.063 g/ml), and HDL + VHDL (d > 1.063 g/ml) to the total lipoproteins were 40.33 ± 7.11%, 41.29 ± 5.70%, and 18.38 ± 1.40% (mean ± SEM), respectively. These data suggest that the high concentration of LDL in basal culture medium is partially due to the presence of lipoproteins with d 1.006–1.03 g/ml. The addition of oleic acid to the culture medium stimulated the secretion of VLDL by 425% ($P < 0.05$) and LDL by 77% ($P < 0.01$). The observed 68% elevation in the HDL concentration was not statistically significant (Fig. 3). Thus, in the presence of oleate, VLDL, LDL, and HDL accounted for 33%, 47%, and 20%, respectively, of the total lipoproteins. The amount of VLDL secreted by HepG2 cells in both basal and oleate-supplemented media was reduced by 60% ($P < 0.05$) in response to insulin administration (Fig. 3). Similarly, the LDL concentration was decreased by 68% ($P < 0.01$) and 45% ($P < 0.01$) in basal and oleate-supplemented media, respectively, upon the addition of the hormone (Fig. 3). In contrast, there was no change in the concentration of HDL secreted into either basal or oleate-supplemented medium in the presence of insulin (Fig. 3).

Effects of insulin and oleate on the composition of secreted lipoprotein density classes

In the basal medium, triglycerides were the major neutral lipids in both VLDL and LDL, whereas unesterified cholesterol was the main neutral lipid of HDL (**Table 5**).
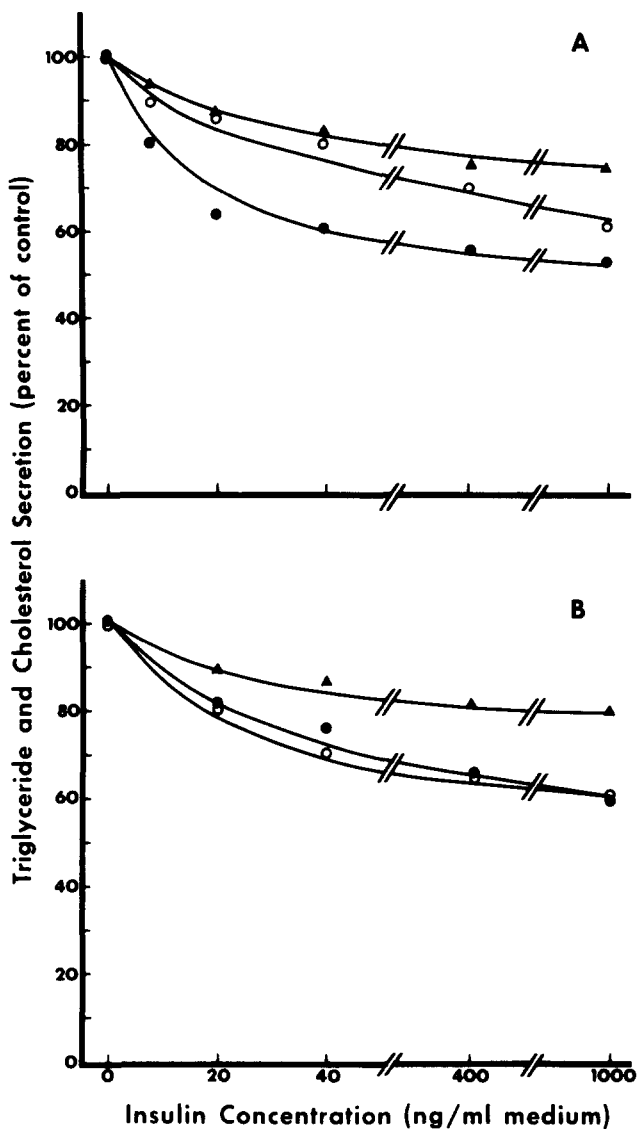
Fig. 2. Effect of insulin on the concentrations of triglyceride, unesterified and esterified cholesterol in the medium. Hep G2 cells were cultured under conditions described in Fig. 1. The maintenance medium was removed, cells were washed, and fresh medium was added. The effect of various concentrations of insulin on the secretion of triglycerides (●), unesterified cholesterol (▲), and cholesteryl esters (○) by Hep G2 cells incubated for 24 hr in basal medium (A) or basal medium containing 0.4 mM oleate-1.5% bovine serum albumin (B) was determined. Each value is the mean of triplicate plates.

ApoB was the major apolipoprotein of lipoproteins with d < 1.063 g/ml and accounted for 51% of total VLDL apolipoproteins and 84% of total LDL apolipoproteins (**Table 6**). We have previously shown (54) that in HepG2 cells apoB-100 was the only form of apoB present in lipoproteins with d < 1.063 g/ml. ApoA-I was the main apolipoprotein of HDL and accounted for 41% of the total apolipoproteins; apoE was the next most abundant apolipoprotein (Table 6). The decreased concentration of total lipoproteins with d < 1.063 g/ml by insulin (Fig. 3) was

due not only to a lower concentration of lipids but also to decreased levels of apolipoproteins; the apolipoprotein contents of VLDL and LDL were reduced by 53% and 56%, respectively. In addition, insulin altered the composition of VLDL and LDL by producing particles that had a significantly lower content of triglycerides (Table 5), contained less apoB, and were deficient in apoE (Table 6). On the other hand, insulin had no significant effect on the neutral lipid composition of HDL (Table 5), and did not affect their total apolipoprotein concentration or composition (Table 6).

The addition of oleate to the culture medium not only increased the total concentration of VLDL, it also altered their neutral lipid composition. In the presence of oleate, the secreted VLDL particles contained significantly lower percentages of unesterified cholesterol and cholesteryl esters and a higher percentage of triglycerides when compared to basal medium (Table 5). On the other hand, the HDL particles had a significantly higher content of unesterified cholesterol and a lower content of cholesteryl ester as compared to basal medium (Table 5). The addition of oleate did not alter the neutral lipid composition of LDL (Table 5). There were small to moderate elevations in the concentration of total apolipoproteins in VLDL, LDL, and HDL; these changes were similar to those observed in the total medium. The major apolipoprotein in VLDL and LDL was apoB, while HDL contained similar concentrations of apolipoproteins A-I and E (Table 6). The insulin administration resulted in the secretion of VLDL and LDL particles containing slightly lower percentages of triglycerides and HDL with a slightly higher percentage of unesterified cholesterol (Table 5). There was a 41–51% decrease in the apolipoprotein concentration of VLDL and LDL by insulin. The VLDL particles secreted in the presence of insulin contained lower concentrations of apolipoproteins B and E, while the LDL particles were characterized by lower levels of apolipoproteins A-I and B. A slight reduction in the total apolipoprotein content of HDL was due to a decrease in the apoB concentration (Table 6).

Effects of insulin and oleate on the distribution of neutral lipids and apolipoproteins between lipoprotein density classes

In basal medium, the major amounts of cholesterol, triglyceride, and apoB were associated with VLDL + LDL (d < 1.063 g/ml), while 87% of apolipoproteins A-I and A-II and 58% of apoE were recovered in lipoproteins with d > 1.063 g/ml (**Table 7**). Analysis of lipoproteins with d < 1.063 g/ml showed that approximately 15% of total neutral lipids, 7% of apoA-I, and 10–15% of apolipoproteins B and E were associated with VLDL. Whereas apoB was not detected in VHDL (d > 1.21 g/ml), approximately 10% of apoE and 20–40% of apoA-I

TABLE 3. Effect of insulin on the secretion of triglycerides and cholesterol by Hep G2 cells in oleate-supplemented medium

	Insulin	Triglycerides	Unesterified Cholesterol	Cholesteryl Esters	Total Cholesterol
1 μg/ml medium			*μg/g cell protein per hr*		
Medium + oleate	−	679.88 ± 170.87	382.22 ± 47.36	103.02 ± 18.24	443.81 ± 57.99
Medium + oleate	+	380.75 ± 72.29	316.33 ± 37.83	60.89 ± 9.72	353.76 ± 42.73
% decrease		44.0	17.0	40.9	20.3
P^a		NS[b]	NS[b]	< 0.05	NS[b]

The concentrations of triglycerides and cholesterol in the medium were measured after 24-hr incubation with MEM containing 0.4 mM oleate–1.5% albumin with or without insulin. Values are means ± SEM of six experiments.
[a]Determined by Student's *t*-test.
[b]NS, not significant ($P > 0.05$).

were recovered in this lipid-poor fraction. Addition of insulin resulted in the recovery of major part of cholesterol and 96% of apoE in lipoproteins with d >1.063 g/ml (Table 7). The effect of insulin on VLDL was more pronounced than on LDL. Only 3–5% of total neutral lipids and apoB were present in VLDL.

When culture medium was supplemented with oleate, 95% of total triglyceride was recovered in lipoproteins with d < 1.063 g/ml **(Table 8)** and this was distributed equally between VLDL and LDL. The VLDL particles contained 30% of the total cholesteryl esters; there were also slight to moderate increases in the apolipoproteins A-I (14% of total), B and E (22% of total) contents of VLDL under these conditions. The effect of insulin on the distribution of neutral lipids and apolipoproteins between lipoprotein density classes (Table 8) was similar, although less pronounced, to that observed in basal medium (Table 7).

Effect of insulin on the incorporation of [1-¹⁴C]oleate into cellular and secreted lipids

To evaluate the effects of insulin on the de novo synthesis of lipids, the rate of incorporation of [1-¹⁴C]oleate into phospholipids and neutral lipids was determined. As shown in **Table 9,** insulin significantly decreased the secretion of newly synthesized labeled phospholipids, triglycerides, and cholesteryl esters. In contrast to

decreased secretion, insulin increased the accumulation of most cellular lipids. With the exception of cholesteryl esters, which remained unchanged, there was a 19% increase in the accumulation of newly synthesized phospholipids and a highly significant elevation of 82% in cellular triglycerides in the presence of insulin (Table 9). The total lipid esters in the medium and cells were increased from 111 nmol of oleate converted/mg of cell protein in the control system to 172 nmol of oleate converted/mg of cell protein in the presence of insulin. Thus, insulin enhanced the synthesis of total lipid esters (medium and cellular) by 55%, decreased their secretion into the medium by 36%, and enhanced the cellular accumulation of these newly synthesized lipids by 61%.

DISCUSSION

The present study was designed to establish the rates of secretion and composition of neutral lipids, apolipoproteins, and lipoproteins in the basal culture medium of HepG2 cells in the presence of glucose and then in the same medium supplemented with oleic acid. The next set of experiments was designed to examine the effect of insulin on both of these processes.

The maximum rates of triglyceride and cholesterol production by HepG2 cells grown in the basal culture

TABLE 4. Effect of insulin on the secretion of apolipoproteins by Hep G2 cells in oleate-supplemented medium

	Insulin	Apolipoproteins			
		A-I	A-II	B	E
1 μg/ml medium			*μg/g cell protein per hr*		
Medium + oleate	−	132.50 ± 28.76	56.46 ± 19.89	398.52 ± 52.19	80.36 ± 19.12
Medium + oleate	+	121.55 ± 29.66	59.03 ± 21.44	318.05 ± 36.65	63.91 ± 16.74
% change		− 8.3	+ 4.6	− 20.2	− 20.5
P^a		NS[b]	NS[b]	NS[b]	NS[b]

Experimental conditions are described in Table 3. Values are means ± SEM of six experiments.
[a]Determined by Student's *t*-test.
[b]NS, not significant ($P > 0.05$).
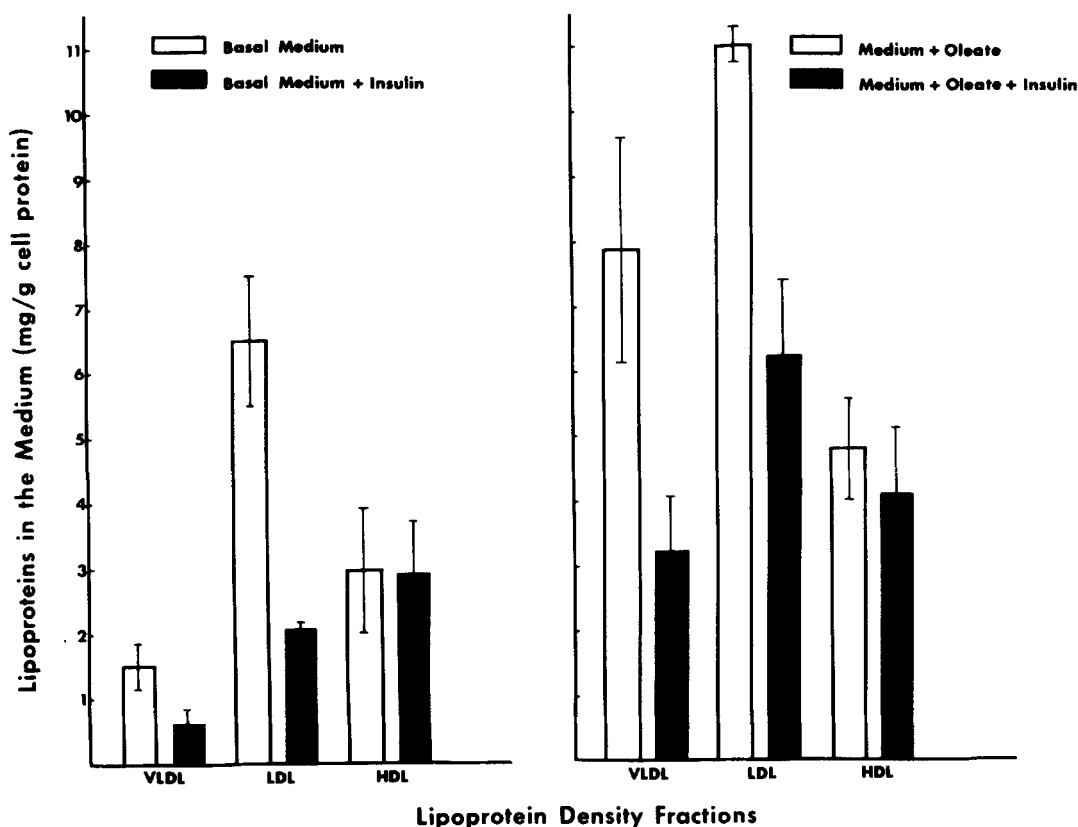
Fig. 3. Effects of oleate and insulin on the accumulation of lipoprotein density classes in the medium. After 5–6 days in culture, the maintenance medium was removed, cells were washed, and basal medium (left panel) or basal medium containing 0.4 mM oleate-1.5% bovine serum albumin (right panel) was added, and cells were incubated for 24 hr in the presence or absence of insulin (1 μg/ml). The concentrations of neutral lipids and apolipoproteins in VLDL (d < 1.006 g/ml), LDL (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) isolated from culture media were determined. Values are expressed as total concentration of lipoproteins and represent mean ± SEM of four experiments.

TABLE 5. Effects of oleate and insulin on the neutral lipids composition of lipoprotein density classes secreted by Hep G2 cells

	Medium			Medium + Oleate		
	Unesterified Cholesterol	Cholesteryl Esters	Triglycerides	Unesterified Cholesterol	Cholesteryl Esters	Triglycerides
			% of total neutral lipids			
Control						
d < 1.006 g/ml	22.9 ± 0.6	15.4 ± 1.8	61.7 ± 2.1	13.9 ± 1.7[a]	7.8 ± 0.9[b]	78.3 ± 2.1[a]
d 1.006–1.063 g/ml	23.1 ± 2.7	11.1 ± 1.4	65.8 ± 2.9	24.3 ± 1.6	8.2 ± 1.3	67.5 ± 1.6
d 1.063–1.21 g/ml	47.0 ± 6.2	23.2 ± 2.9	29.8 ± 5.6	74.0 ± 9.0[c]	10.4 ± 2.9[b]	15.6 ± 8.1
Insulin						
d < 1.006 g/ml	34.2 ± 8.0	21.4 ± 8.4	44.4 ± 3.2[d]	17.4 ± 2.9	8.9 ± 2.3	73.6 ± 4.7
d 1.006–1.063 g/ml	37.6 ± 3.9[e]	13.7 ± 1.5	48.7 ± 4.6[e]	30.8 ± 3.9	7.0 ± 0.4	62.2 ± 4.1
d 1.063–1.21 g/ml	65.5 ± 2.1	15.2 ± 6.7	19.3 ± 6.6	83.21 ± 2.7	8.9 ± 2.6	7.9 ± 1.1

Experimental conditions are described in Table 1. The neutral lipid composition of lipoprotein density classes secreted by Hep G2 cells in minimum essential medium with or without 0.4 mM oleate-1.5% albumin was determined in the presence or absence of insulin. Values are means ± SEM of four experiments.

[a]The difference between the control and oleate-supplemented medium was significant at $P < 0.001$.
[b]The difference between the control and oleate-supplemented medium was significant at $P < 0.02$.
[c]The difference between the control and oleate-supplemented medium was significant at $P < 0.05$.
[d]The difference between the control and insulin treatment was significant at $P < 0.005$.
[e]The difference between the control and insulin treatment was significant at $P < 0.02$.

TABLE 6. Effects of oleate and insulin on the apolipoprotein composition of lipoprotein density classes secreted by Hep G2 cells

	Medium				Medium + Oleate			
	A-I	A-II	B	E	A-I	A-II	B	E
	% of total apolipoproteins							
Control								
d < 1.006 g/ml	19 ± 7	10 ± 4	51 ± 9	20 ± 5	26 ± 5	6 ± 3	50 ± 8	18 ± 10
d 1.006–1.063 g/ml	9 ± 4	ND[a]	84 ± 5	7 ± 3	12 ± 5	ND[a]	80 ± 6	8 ± 4
d 1.063–1.21 g/ml	41 ± 4	16 ± 1	12 ± 6	31 ± 7	35 ± 2	15 ± 4	21 ± 8	29 ± 6
Insulin								
d < 1.006 g/ml	56 ± 19	27 ± 4	17 ± 17	ND[a]	36 ± 6	5 ± 2	46 ± 6	13 ± 7
d 1.006–1.063 g/ml	11 ± 5	ND[a]	88 ± 6	1 ± 1	13 ± 6	ND[a]	82 ± 6	5 ± 2
d 1.063–1.21 g/ml	40 ± 5	23 ± 4	14 ± 8	23 ± 7	39 ± 4	16 ± 4	15 ± 6	30 ± 6

Experimental conditions are described in Table 5. Values are mean ± SEM of five experiments.
[a]ND, not detectable.

medium containing glucose were similar to the secretion rates of neutral lipids observed in a previous study with rat hepatocytes in primary culture (15). The major neutral lipid secreted by HepG2 cells was triglyceride followed by unesterified and esterified cholesterol; similar results were reported in studies with rat perfused liver (55) and cultured hepatocytes (15). The high percentage of unesterified cholesterol, which was not affected by either oleate or insulin, was similar to that observed in the rat perfused liver system (55) and cultured hepatocytes (9, 15). The low concentration of cholesteryl esters in culture medium, in comparison to that in human plasma, reflects the low activity of lecithin:cholesterol acyltransferase (LCAT) in the culture medium (56). ApoB was the major apolipoprotein in the culture medium followed, in the order of decreasing concentrations, by apolipoproteins A-I, E, A-II, and apoC peptides. Thus, the composition of apolipoproteins in the culture medium differs from that in

plasma where apoA-I is the major apolipoprotein (57); the ratio of apolipoproteins A-I/A-II in the culture medium (2.5:1) was also greater than in plasma (2:1) (57). Another important difference in the apolipoprotein composition was the higher percent content of apoE in the culture medium (13%) when compared to that in plasma (3.5%) (57). These differences could be explained by the intestinal contribution of apolipoproteins A-I and A-II (13) and different residence time of apolipoproteins in the plasma compartments.

The major lipoprotein density class secreted by HepG2 cells in basal culture medium was LDL which contained the major part of total neutral lipids and apoB. On the other hand, the contribution of VLDL to the total lipoproteins was minimal accounting for only 10–15% of neutral lipids, apoB, and apoE. This reduced rate of VLDL production is in agreement with those reported by Ellsworth, Erickson, and Cooper (58) and Thrift et al.

TABLE 7. Effect of insulin on the distribution of neutral lipids and apolipoproteins between lipoprotein density classes secreted by Hep G2 cells in minimum essential medium

	Neutral Lipids			Apolipoproteins			
	Unesterified Cholesterol	Cholesteryl Esters	Triglycerides	A-I	A-II	B	E
	% distribution						
Control							
d < 1.063 g/ml	64 ± 7	64 ± 10	86 ± 4	12 ± 3	13 ± 6	80 ± 4	42 ± 6
d > 1.063 g/ml	36 ± 7	36 ± 10	14 ± 4	88 ± 3	87 ± 6	20 ± 4	58 ± 6
Insulin							
d < 1.063 g/ml	36 ± 8[a]	48 ± 14	62 ± 7[b]	13 ± 3	10 ± 5	56 ± 7[b]	4 ± 4[c]
d > 1.063 g/ml	64 ± 8[a]	52 ± 14	38 ± 7[b]	87 ± 3	90 ± 5	44 ± 7[b]	96 ± 4[c]

Experimental conditions are described in Table 5. Values are means ± SEM of four experiments.
[a]The difference between the control and insulin treatment was significant at P < 0.05.
[b]The difference between the control and insulin treatment was significant at P < 0.02.
[c]The difference between the control and insulin treatment was significant at P < 0.005.

TABLE 8. Effect of insulin on the distribution of neutral lipids and apolipoproteins between lipoprotein density classes secreted by Hep G2 cells in oleate-supplemented medium

	Neutral Lipids			Apolipoproteins			
	Unesterified Cholesterol	Cholesteryl Esters	Triglycerides	A-I	A-II	B	E
	% distribution						
Oleate							
d < 1.063 g/ml	51 ± 2	66 ± 8	95 ± 3	27 ± 5	13 ± 9	87 ± 4	41 ± 6
d > 1.063 g/ml	49 ± 2	34 ± 8	5 ± 3	73 ± 5	87 ± 9	13 ± 4	59 ± 6
Oleate + insulin							
d < 1.063 g/ml	47 ± 9	67 ± 12	94 ± 2	20 ± 5	2 ± 2	83 ± 9	28 ± 4
d > 1.063 g/ml	53 ± 9	33 ± 12	6 ± 2	80 ± 5	98 ± 2	17 ± 9	72 ± 4

Experimental conditions are as described in Table 5. Values are means ± SEM of four experiments.

(59). These observations, however, are at variance with results obtained with the human liver in the organ culture (60), hepatocytes in primary culture (61), or HepG2 cells (62), which all demonstrated that the majority of apoB is associated with the VLDL fraction. It is possible that this discrepancy in results may be due to the presence of specific hormones and/or metabolites in FCS-containing medium used in the above studies (60–62) rather than the methodology for quantifying apoB as recently suggested (59). The results of the present study do not completely rule out the possibility that the concentration and composition of the accumulated lipoproteins might reflect postsecretory modification, e.g., catabolism and/or lipolysis of newly synthesized particles. Although the catabolism of de novo synthesized lipoproteins was not examined directly, a time study on the secretion of apoB-containing particles showed that lipoprotein B was not a degradation product of lipoprotein B:E (Dashti, N. and G. Wolfbauer, unpublished data). Furthermore, no lipolytic activity could be detected in the medium indicating the lack of a precursor–product relationship between particles present in VLDL and LDL. The lack of any significant lipolytic activities (34) or re-uptake of newly secreted VLDL has

also been demonstrated in rat cultured hepatocytes (34, 35).

Direct hepatic synthesis of LDL, which appears to be operative in HepG2 cells, is a controversial issue. The general consensus, based on studies with several animal species using a liver perfusion system (3, 63–69), cultured hepatocytes (8–10), and isolated Golgi apparatus (70–72), is that VLDL are the major and HDL the minor lipoproteins, and that the contribution of LDL is minimal. Moreover, it has been suggested that the LDL-like particles found in liver perfusate of rabbit (69), guinea pig (67), and rhesus monkey (68) are LDL contaminants from serum. On the other hand, a direct hepatic secretion of LDL has been demonstrated in the rat (73), miniature pig (74), and nonhuman primates (75–77). While direct synthesis of LDL-like particles does not appear to be limited to HepG2 cells, the underlying cause for their enhanced secretion by this cell line remains to be elucidated.

The major portion of neutral lipids and apoB was associated with lipoproteins with d < 1.063 g/ml. Both VLDL and LDL secreted by HepG2 cells were triglyceride-rich and cholesteryl ester-poor lipoproteins containing apoB as their major and apolipoproteins A-I, A-II, and E as

TABLE 9. Effects of insulin on the incorporation of [1-¹⁴C]oleic acid into cellular and secreted lipids

	Incorporation of [1-¹⁴C]Oleate into Secreted Lipids			Incorporation of [1-¹⁴C]Oleate into Cellular Lipids		
	PL	TG	CE	PL	TG	CE
	nmol/mg cell protein					
Control	2.84 ± 0.16	3.04 ± 0.41	1.03 ± 0.03	29.27 ± 4.29	70.78 ± 11.14	4.15 ± 0.34
+ Insulin	1.92 ± 0.09	1.66 ± 0.10	0.86 ± 0.03	34.76 ± 3.60	128.86 ± 6.57	3.82 ± 0.45
% change	− 32.6	− 45.3	− 16.5	+ 18.7	+ 82.1	− 8.1
P^a	< 0.01	< 0.05	< 0.02	NS[b]	< 0.005	NS[b]

The incorporation of 0.4 mM [1-¹⁴C]oleate–1.5% bovine serum albumin in MEM into cellular and secreted lipids was measured after 4 hr incubation at 37°C in the presence and absence of insulin. Abbreviations: PL, phospholipids; TG, triglycerides; CE, cholesteryl esters. Values are means ± SEM of triplicate plates.
[a]Determined by Student's *t*-test.
[b]NS, not significant ($P > 0.05$).

their minor protein moities; both were deficient in apoC peptides. This is in agreement with properties of nascent hepatic VLDL from several species which are triglyceride-rich and cholesteryl ester-poor (3, 9, 63, 66, 68, 69, 72) with apolipoproteins B and E as the major and apoC as minor protein constituents (7, 9, 11, 12, 16, 63, 66, 72). The only exception appears to be a relatively high percent content of apolipoproteins A-I and A-II in VLDL particles secreted by HepG2 cells. Similar to the composition of LDL from the basal medium of HepG2 cells, rat hepatic LDL particles produced under normal conditions seem to be triglyceride-rich and to contain both apolipoproteins B and E (65, 66). In contrast to triglyceride-rich LDL particles secreted by HepG2 cells, their plasma counterparts are rich in cholesteryl esters; this compositional difference might be the reason why triglyceride-rich LDL particles may not be taken up by the HepG2 cells. It can be postulated that, in plasma, these nascent triglyceride-rich LDL-like particles will interact with apoC-peptides, rendering them a suitable substrate for lipoprotein lipase. The cholesteryl ester-rich lipoprotein B particles produced by the action of lipoprotein lipase are then directed to their sites of degradation including the liver.

The major amounts of apolipoproteins A-I and A-II occurred in lipoproteins with d > 1.063 g/ml. The HDL particles secreted by HepG2 cells were deficient in cholesteryl esters and triglycerides and were enriched in unesterified cholesterol, probably due to the low levels of LCAT in cell culture medium. A previous study from our laboratory (56) has demonstrated the presence of LCAT in the HepG2 cell homogenate. In contrast, the cell culture medium showed only a weak reaction with antiserum to human plasma LCAT (56). Although Chen et al. (78) have reported the synthesis and secretion of LCAT by HepG2 cells, it has been suggested (59) that the secreted LCAT may be inhibited under culture conditions. Alternatively, the elevated concentration of unesterified cholesterol in HDL might reflect a possible cell turnover, resulting in release of membrane fragments into the medium. In contrast to plasma HDL, lipoproteins with d 1.063–1.21 g/ml secreted by HepG2 cells were enriched in apoE. The production of HDL particles with similar properties has been demonstrated in LCAT deficiency (79), in hypercholesterolemic animals (77, 80), and in the absence of LCAT in liver perfusate (3, 11, 63). Whereas no apoB was detected in the d > 1.21 g/ml fraction of culture medium, approximately 10% of apoE and 20–40% of total apoA-I were present in this fraction. Our findings are consistent with those in rat perfusate in which LCAT was inhibited (12), in HepG2 cells (59), and in patients with familial LCAT deficiency (81).

Total triglycerides and unesterified cholesterol increased approximately twofold when the culture medium was supplemented with oleate. The increase in unesterified cholesterol could partly be due to the presence of bovine serum albumin in the system or possibly a higher cell turnover rate. Previous studies from our laboratory (15) and by others (25, 55) have demonstrated that, in contrast to stimulation in triglyceride secretion, the addition of fatty acid did not significantly affect the hepatic secretion of unesterified cholesterol or cholesteryl esters. The oleate-induced increase in the secretion of triglyceride molecular species with 57 carbon atoms (TG-57) is indicative of the incorporation of this fatty acid into newly synthesized and secreted lipoprotein triglycerides. In contrast to the marked enhancement of the triglyceride and cholesterol accumulation in the medium, the provision of oleate caused only small to moderate increases in the concentration of apolipoproteins. These results, demonstrating a lack of coordinate induction in the synthesis of neutral lipids and apolipoproteins, are in agreement with results reported in studies using rat hepatocytes (24, 25) or perfused liver (26). However, contrary to the unchanged rates of apoB synthesis reported in these studies, our findings showed that oleate increased the secretion of this apolipoprotein by 28%.

The oleate-induced increases in total triglyceride and cholesteryl ester concentrations were reflected mainly in a 425% stimulation of VLDL secretion. This response to fatty acid substrate is similar to that observed in perfused liver (55), rat cultured hepatocytes (10, 15, 23), and HepG2 cells (58). The addition of oleate altered the composition of VLDL by increasing the percent content of triglycerides and slightly enhancing the levels of apolipoproteins A-I, B, and E. Similar compositional changes have occurred in VLDL particles secreted by cultured rat hepatocytes incubated in the presence of oleate (24, 25) and in VLDL isolated from rat liver perfusate (82). Oleate had no major effect on the composition of LDL. The HDL particles produced under this condition were characterized by decreased percent contents of cholesteryl esters and triglycerides and increased relative contents of unesterified cholesterol.

It appears that the oleate-induced changes in the concentration and composition of VLDL particles were brought about by an increased association of apolipoproteins with larger, triglyceride-rich lipoproteins. These particles contained a higher ratio of triglyceride to apoB (4.90 ± 0.67) than the corresponding particles isolated from basal medium (1.06 ± 0.20). When considered in conjunction with a twofold increase in triglyceride/apoB ratio in the total medium, this finding clearly demonstrates that in HepG2 cells, as in cultured rat (24) and chick (83) hepatocytes, the VLDL apolipoproteins may assemble into secretory vesicles with variable amounts of lipids.

The addition of insulin to the culture medium produced

significant decreases in the secretion of all neutral lipids, especially triglycerides and cholesteryl esters. There was a concurrent reduction in the accumulation of all apolipoproteins with apoB being reduced to a greater extent than apolipoproteins A-I, A-II, and E. The effect of insulin on the secretion of lipids and apolipoproteins in oleate-supplemented medium was less pronounced. These results differ from those indicating an increase in triglyceride secretion in rat perfused liver (31) by insulin, but are in agreement with the inhibitory effects of this hormone observed in cultured rat hepatocytes (34, 35).

The lipid composition of both VLDL and LDL was markedly altered by insulin. These lipoproteins contained less triglyceride and more cholesterol in agreement with similar changes reported for VLDL particles secreted by rat cultured hepatocytes (34, 35). The total concentrations of apoB were markedly reduced in VLDL and LDL; these particles were deficient in apoE resulting in a complete shift of apoE from lipoproteins with d $<$ 1.063 g/ml to lipoproteins with d $>$ 1.063 g/ml. The low apoE content in lipoproteins with d $<$ 1.063 g/ml reported by Thrift et al. (59) most probably is due to the presence of insulin in the culture medium. Since there was little effect on the concentration and composition of HDL particles, it appears that insulin specifically inhibited the secretion of triglyceride-rich particles with apolipoproteins B and E as their main protein constituents. Insulin added to oleate-supplemented medium decreased the triglyceride, cholesterol, and apolipoproteins in lipoproteins with d $<$ 1.063 g/ml without affecting those with d $>$ 1.063 g/ml. As in the basal medium, VLDL secreted into oleate-supplemented medium were the major lipoprotein density class altered by insulin. However, insulin decreased the secretion of triglyceride-rich, apolipoprotein B- and E-containing lipoprotein particles in the oleate-supplemented medium to a lesser degree than in basal medium. The decreased concentration of ^{14}C-labeled triglycerides, phospholipids, and cholesteryl esters in the medium concomitant with a simultaneous increase in the concentrations of these lipids in the cells suggest that, while enhancing the synthesis of lipids, insulin reduces their rate of secretion. Our studies and those reported by others (36) have demonstrated that the insulin-related inhibition in the secretion of triglyceride-rich apoB-containing particles was maintained in the presence and absence of fatty acid substrate. These results suggest that the observed reduction in hepatic triglyceride secretion in vivo subsequent to insulin administration (32, 33) may not be due entirely to decreased concentration of plasma fatty acids, but may reflect a direct action of insulin on hepatic lipoprotein secretion.

Since the intracellular concentrations of apolipoproteins were not determined, no conclusion could be made regarding the effect of insulin on the synthetic rates of apolipoproteins. Direct effects of insulin on the mRNA levels and de novo synthesis of apolipoproteins remain to be established. The increased accumulation of triglycerides in the cells suggests that insulin either inhibits the synthesis of apolipoproteins or affects the assembly of lipoproteins and, therefore, their secretion. The divergent effects of insulin on the synthesis and secretion of triglycerides in the presence of oleate suggest that its function in HepG2 cells, as in rat hepatocytes (34), may be to increase the stored pool of triglyceride for future utilization.

The present study has clearly demonstrated the ability of HepG2 cells to synthesize and secrete all the major neutral lipid and apolipoprotein constituents of human lipoprotein density classes. Furthermore, the responsiveness of HepG2 cells to the addition of fatty acids and insulin documented in the present study provides further evidence for the usefulness of this cell line in studying the regulation of hepatic lipoprotein processing. ■

REFERENCES

1. Haft, D. E., P. S. Roheim, A. White, and H. A. Eder. 1962. Plasma lipoprotein metabolism in perfused rat livers. I. Protein synthesis and entry into the plasma. *J. Clin. Invest.* **41:** 842–849.
2. Windmueller, H. G., and A. E. Spaeth. 1967. De novo synthesis of fatty acid in perfused rat liver as a determinant of plasma lipoprotein production. *Arch. Biochem. Biophys.* **122:** 362–369.
3. Marsh, J. B. 1974. Lipoproteins in a nonrecirculating perfusate of rat liver. *J. Lipid Res.* **15:** 544–550.
4. Sundler, R., B. Akesson, and A. Nilsson. 1973. Triacylglycerol secretion in very low density lipoproteins by isolated rat liver parenchymal cells. *Biochem. Biophys. Res. Commun.* **55:** 961–968.
5. Gravela, E., G. Poli, E. Albano, and M. U. Dianzani. 1977. Studies on fatty liver with isolated hepatocytes. I. The action of colchicine, phalloidin, cytochalasin B, and cyclohexamide on protein and triglyceride synthesis and secretion. *Exp. Mol. Pathol.* **27:** 339–352.
6. Edwards, P. A., D. Lemongello, and A. M. Fogelman. 1979. The effect of glucagon, norepinephrine, and dibutyryl cyclic AMP on cholesterol efflux and on the activity of 3-hydroxy-3-methylglytaryl CoA reductase in rat hepatocytes. *J. Lipid Res.* **20:** 2–7.
7. Jeejeebhoy, K. N., J. Ho, C. Breckenridge, A. Bruce-Robertson, G. Steiner, and J. Jeejeebhoy. 1975. Synthesis of

VLDL by isolated rat hepatocytes in suspension. *Biochem. Biophys. Res. Commun.* **66:** 1147–1153.

8. Tarlow, D. M., P. A. Watkins, R. E. Reed, R. S. Miller, E. E. Zwergel, and M. D. Lane. 1977. Lipogenesis and the synthesis and secretion of very low density lipoprotein by avian liver cells in nonproliferating monolayer culture. Hormonal effects. *J. Cell Biol.* **73:** 332–353.

9. Davis, R. A., S. C. Engelhorn, S. H. Pangburn, D. B. Weinstein, and D. Steinberg. 1979. Very low density lipoprotein synthesis and secretion by cultured rat hepatocytes. *J. Biol. Chem.* **254:** 2010–2016.

10. Bell-Quint, J., and T. Forte. 1981. Time-related changes in the synthesis and secretion of very low density, low density and high density lipoproteins by cultured rat hepatocytes. *Biochim. Biophys. Acta.* **663:** 83–98.

11. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a non-circulating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.

12. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18:** 465–473.

13. Wu, A. L., and H. G. Windmueller. 1979. Relative contribution by liver and intestine to individual plasma apolipoproteins in the rat. *J. Biol. Chem.* **254:** 7316–7322.

14. Blue, M. L., A. A. Protter, and D. L. Williams. 1980. Biosynthesis of apolipoprotein B in rooster kidney, intestine, and liver. *J. Biol. Chem.* **255:** 10048–10051.

15. Dashti, N., W. J. McConathy, and J. A. Ontko. 1980. Production of apolipoproteins E and A-I by rat hepatocytes in primary culture. *Biochim. Biophys. Acta.* **618:** 347–358.

16. Kempen, H. J. 1980. Lipoprotein secretion by isolated rat hepatocytes: characterization of the lipid-carrying particles and modulation of their release. *J. Lipid Res.* **21:** 671–680.

17. Fukuda, N., and J. A. Ontko. 1984. Interaction between fatty acid synthesis, oxidation, and esterification in the production of triglyceride-rich lipoproteins by the liver. *J. Lipid Res.* **25:** 831–842.

18. Nestel, P. J., and D. Steinberg. 1963. Fate of palmitate and of linoleate perfused through the isolated rat liver at high concentrations. *J. Lipid Res.* **4:** 461–469.

19. Kohout, M. B. Kohoutova, and M. Heimberg. 1971. The regulation of hepatic triglyceride metabolism by free fatty acids. *J. Biol. Chem.* **246:** 5067–5074.

20. Heimberg, M., and H. G. Wilcox. 1972. The effect of palmitic and oleic acids on the properties and composition of the very low density lipoproteins secreted by the liver. *J. Biol. Chem.* **247:** 875–880.

21. Schonfeld, G., and B. Pfleger. 1971. Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers of carbohydrate-fed rats. *J. Lipid Res.* **12:** 614–621.

22. Rose, H., M. Vaughan, and D. Steinberg. 1984. Utilization of fatty acids by rat liver slices as a function of medium concentration. *Am. J. Physiol.* **206:** 345–356.

23. Davis, R. A., S. C. Engelhorn, D. B. Weinstein, and D. Steinberg. 1980. Very low density lipoprotein secretion by cultured rat hepatocytes. Inhibition by albumin and other macromolecules. *J. Biol. Chem.* **255:** 2039–2045.

24. Patsch, W., T. Tamai, and G. Schonfeld. 1983. Effect of fatty acids on lipid and apoprotein secretion and association in hepatocyte cultures. *J. Clin. Invest.* **72:** 371–378.

25. Davis, R. A., and J. R. Boogaerts. 1982. Intrahepatic assembly of very low density lipoproteins. Effect of fatty acids on triacylglycerol and apolipoprotein synthesis. *J. Biol. Chem.* **257:** 10908–10913.

26. Patsch, W., and G. Schonfeld. 1981. The degree of sialylation of apoC-III is altered by diet. *Diabetes.* **30:** 530–534.

27. Farquhar, J. W., A. Frank, R. C. Gross and G. M. Reaven. 1966. Glucose, insulin, and triglyceride responses to high and low carbohydrate diets in man. *J. Clin. Invest.* **45:** 1648–1656.

28. Reaven, G. M., R. L. Lerner, M. P. Stern, and J. W. Farquhar. 1967. Role of insulin in endogenous hypertriglyceridemia. *J. Clin. Invest.* **46:** 1756–1767.

29. Topping, D. L., and P. A. Mayes. 1972. The immediate effects of insulin and fructose on the metabolism of the perfused liver. Changes in the lipoprotein secretion, fatty acid oxidation and esterification, lipogenesis and carbohydrate metabolism. *Biochem. J.* **126:** 295–311.

30. Woodside, W. F., and M. Heimberg. 1976. Effect of anti-insulin serum, insulin, and glucose in output of triglycerides and in ketogenesis by the perfused rat liver. *J. Biol. Chem.* **251:** 13–23.

31. Laker, M. E., and P. A. Mayes. 1984. Investigations into the direct effects of insulin on hepatic ketogenesis, lipoprotein secretion and pyruvate dehydrogenase activity. *Biochim. Biophys. Acta.* **795:** 427–430.

32. Sadur, C. N., and R. H. Eckel. 1982. Insulin stimulation of adipose tissue lipoprotein lipase. Use of euglycemic clamp technique. *J. Clin. Invest.* **69:** 1119–1125.

33. Alcindor, L.-G., R. Infante, C. Soler-Argilaga, and J. Polonovski. 1973. Effect of a single insulin administration on the hepatic release of triglycerides into the plasma. *Biochim. Biophys. Acta.* **306:** 347–352.

34. Durrington, P. N., R. S. Newton, D. B. Weinstein, and D. Steinberg. 1982. Effects of insulin and glucose on very low density lipoprotein triglyceride secretion by cultured rat hepatocytes. *J. Clin. Invest.* **70:** 63–73.

35. Patsch, W., S. Franz, and G. Schonfeld. 1983. Role of insulin in lipoprotein secretion by cultured rat hepatocytes. *J. Clin. Invest.* **71:** 1161–1174.

36. Patsch, W., A. M. Gotto, Jr., and J. R. Patsch. 1986. Effects of insulin on lipoprotein secretion in rat hepatocyte cultures. The role of the insulin receptor. *J. Biol. Chem.* **261:** 9603–9606.

37. Aden, D. P., A. Fogel, S. Plotkin, I. Damjanov, and B. B. Knowles. 1979. Controlled synthesis of HB$_s$Ag in a differentiated human liver carcinoma-derived cell line. *Nature.* **282:** 615–616.

38. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209:** 497–499.

39. Schwartz, A. L., S. E. Fridovich, B. B. Knowles, and H. F. Lodish. 1981. Characterization of the asialoglycoprotein receptor in a continuous hepatoma line. *J. Biol. Chem.* **256:** 8878–8881.

40. Morris, K. M., G. Goldberger, H. R. Colten, D. P. Aden, and B. B. Knowles. 1982. Biosynthesis and processing of a human precursor complement protein, pro-C3, in a hepatoma-derived cell line. *Science.* **215:** 399–400.

41. Rash, J. M., G. H. Rothblat, and C. E. Sparks. 1981. Lipoprotein apolipoprotein synthesis by human hepatoma cells in culture. *Biochim. Biophys. Acta.* **666:** 294–298.

42. Zannis, V. I., J. L. Breslow, T. R. San Giacomo, D. P. Aden, and B. B. Knowles. 1981. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* **20:** 7089–7096.

43. Dashti, N., and G. Wolfbauer. 1985. Production of lipopro-

tein and apolipoproteins by human hepatoma cell line HepG2: effect of oleic acid and insulin. *Arteriosclerosis.* 5: 535a.

44. Dashti, N., G. Wolfbauer, and P. Alaupovic. 1985. Binding and degradation of human high-density lipoproteins by human hepatoma cell line HepG2. *Biochim. Biophys. Acta.* 833: 100–110.

45. Alaupovic, P., D. M. Lee, and W. J. McConathy. 1972. Studies on the composition and structure of plasma lipoproteins. Distribution of lipoprotein families in major density classes of normal human plasma lipoproteins. *Biochim. Biophys. Acta.* 260: 689–707.

46. Kuksis, A., J. J. Myher, L. Marai, and K. Geher. 1975. Determination of plasma lipid profiles by automated gas chromatography and computerized data analysis. *J. Chromatogr. Sci.* 13: 423–430.

47. Curry, M. D., P. Alaupovic, and C. A. Suenram. 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassay. *Clin. Chem.* 22: 315–322.

48. Curry, M. D., A. Gustafson, P. Alaupovic, and W. J. McConathy. 1978. Electroimmunoassay, radioimmunoassay, and radial immunodiffusion assay evaluated for quantification of human apolipoprotein B. *Clin. Chem.* 24: 280–286.

49. Curry, M. D., W. J. McConathy, J. D. Fesmire, and P. Alaupovic. 1981. Quantitative determination of apolipoproteins C-I and C-II in human plasma by separate electroimmunoassay. *Clin. Chem.* 27: 543–548.

50. Curry, M. D., W. J. McConathy, J. D. Fesmire, and P. Alaupovic. 1980. Quantitative determination of human apolipoprotein C-III by electroimmunoassay. *Biochim. Biophys. Acta.* 617: 503–513.

51. Curry, M. D., W. J. McConathy, P. Alaupovic, J. H. Ledford, and M. Popovic. 1976. Determination of human apolipoprotein E by electroimmunoassay. *Biochim. Biophys. Acta.* 439: 413–425.

52. Dashti, N., J. L. Kelley, R. H. Thayer, and J. A. Ontko. 1983. Concurrent inductions of avian hepatic lipogenesis, plasma lipids, and plasma apolipoprotein B by estrogen. *J. Lipid Res.* 24: 368–380.

53. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.

54. Dashti, N., D. M. Lee, and T. Mok. 1986. Apolipoprotein B is a calcium-binding protein. *Biochem. Biophys. Res. Commun.* 137: 493–499.

55. Wilcox, H. G., G. D. Dunn, and M. Heimberg. 1975. Effect of several common long chain fatty acids on the properties and lipid composition of the very low density lipoproteins secreted by the perfused rat liver. *Biochim. Biophys. Acta.* 398: 39–54.

56. Karen, E., W. J. McConathy, A. G. Lacko, and B. Knowles. 1985. Detection of lecithin:cholesterol acyltransferase (LCAT) in a human hepatoma cell line. *Cancer Biochem. Biophys.* 7: 301–307.

57. Alaupovic, P. 1983. Serum apolipoprotein profiles of dyslipoproteinemias. *In* Dietary Fats and Health. E. G. Perkins and W. J. Visek, editors. American Oil Chemists' Society, Champaign, IL. 574–597.

58. Ellsworth, J. L., S. K. Erickson, and A. D. Cooper. 1984. Regulation of lipoprotein (LP) synthesis and secretion in the human hepatoma cell line, Hep G2. *Federation Proc.* 43: 153la.

59. Thrift, R. N., T. M. Forte, B. E. Cahoon, and V. G. Shore. 1986. Characterization of lipoproteins produced by the hu-
man liver cell line, Hep G2, under defined conditions. *J. Lipid Res.* 27: 236–250.

60. Edge, S. B., J. M. Hoeg, P. D. Schneider, and H. B. Brewer, Jr. 1985. Apolipoprotein B synthesis in humans: liver synthesizes only apolipoprotein B-100. *Metabolism.* 34: 726–730.

61. Kosykh, V. A., S. N. Preobrazhensky, I. V. Fuki, O. E. Zaikina, V. P. Tsibulsky, V. S. Repin, and V. N. Smirnov. 1985. Cholesterol can stimulate secretion of apolipoprotein B by cultured human hepatocytes. *Biochim. Biophys. Acta.* 836: 385–389.

62. Tam, S-P., T. K. Archer, and R. G. Deeley. 1985. Effect of estrogen on apolipoprotein secretion by the human hepatocarcinoma cell line, Hep G2. *J. Biol. Chem.* 260: 1670–1675.

63. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* 58: 667–680.

64. Dolphin, P. J., L. Wong, and D. Rubinstein. 1978. A comparison of some immunological characteristics of very low density lipoproteins of normal and hypercholesterolemic rat sera. *Can J. Biochem.* 56: 673–683.

65. Marsh, J. B., and C. E. Sparks. 1979. Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. *J. Clin. Invest.* 64: 1229–1237.

66. Dolphin, P. J. 1981. Serum and hepatic nascent lipoproteins in normal and hypercholesterolemic rats. *J. Lipid Res.* 22: 971–989.

67. Guo, L. S. S., R. L. Hamilton, R. Ostwald, and R. J. Havel. 1982. Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* 23: 543–555.

68. Jones, L. A., T. Teramoto, D. J. Juhn, R. B. Goldberg, A. H. Rubenstein, and G. S. Getz. 1984. Characterization of lipoprotein produced by the perfused rhesus monkey liver. *J. Lipid Res.* 25: 319–335.

69. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* 80: 6096–6100.

70. Mahley, R. W., R. L. Hamilton, and V. S. LeQuire. 1969. Characterization of lipoprotein particles isolated from the Golgi apparatus of rat liver. *J. Lipid Res.* 10: 433–439.

71. Nestruck, A. C., and D. Rubinstein. 1976. The synthesis of apoproteins of very low density lipoproteins isolated from the Golgi apparatus of rat liver. *Can. J. Biochem.* 54: 617–628.

72. Swift, L. L., N. R. Manowitz, G. D. Dunn, and V. S. LeQuire. 1980. Isolation and characterization of hepatic Golgi lipoproteins from hypercholesterolemic rats. *J. Clin. Invest.* 66: 415–425.

73. Fidge, N. H., and P. J. Poulis. 1978. Metabolic heterogeneity in the formation of low density lipoprotein from very low density lipoprotein in the rat: evidence for the independent production of a low density lipoprotein subfraction. *J. Lipid Res.* 19: 342–349.

74. Huff, M. W., and D. E. Telford. 1984. Direct synthesis of low-density lipoprotein apoprotein B in the miniature pig. *Metabolism.* 34: 36–42.

75. Goldberg, I. J., N-A. Le, H. N. Ginsberg, J. R. Paterniti, Jr., and W. V. Brown. 1983. Metabolism of apoprotein B in cynomolgus monkey: evidence for independent production of low-density lipoprotein apoprotein B. *Am. J. Physiol.* 244: E196–E201.

76. Illingworth, D. R. 1975. Metabolism of lipoproteins in nonhuman primates. Studies on the origin of low density lipoprotein apoprotein in the plasma of the squirrel monkey. *Biochim. Biophys. Acta.* **388:** 38–51.

77. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. *J. Clin. Invest.* **72:** 221–236.

78. Chen, C-H., T. H. Forte, B. E. Cahoon, R. N. Thrift, and J. J. Albers. 1986. Synthesis and secretion of lecithin-cholesterol acyltransferase by the human hepatoma cell line Hep G2. *Biochim. Biophys. Acta.* **877:** 433–439.

79. Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. 1980. Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **21:** 625–634.

80. Krul, E. S., and P. J. Dolphin. 1982. Isolated hepatocytes from hypercholesterolemic rats secrete discoidal lipoproteins. *FEBS Lett.* **139:** 259–264.

81. Norum, K. R., J. A. Glomset, A. V. Nichols, T. Forte, J. J. Albers, W. C. King, C. D. Mitchell, K. R. Applegate, E. L. Gong, V. Cabana, and E. Gjone. 1975. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: effects of incubation with lecithin:cholesterol acyltransferase in vitro. *Scand. J. Clin. Lab. Invest.* **Suppl. 142:** 31–55.

82. Salam, W. R., H. G. Wilcox, and M. Heimberg. 1985. Stimulation by oleic acid of incorporation of L-[4,5-^{3}H]leucine into very low density lipoprotein apoprotein by the isolated perfused rat liver. *Biochem. Biophys. Res. Commun.* **132:** 28–34.

83. Siuta-Mangano, P., D. R. Janero, and M. D. Lane. 1982. Association and assembly of triglyceride and phospholipid with glycosylated and unglycosylated apoproteins of very low density lipoprotein in the intact liver cell. *J. Biol. Chem.* **257:** 11463–11467.