Effects of oleate and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells

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Abstract We have reported previously that, in HepG2 cells, there is a lack of coordinate induction of triglyceride and apolipoprotein (apo) production by oleate and that insulin inhibits the production of triglyceride-rich, apoB-containing lipoproteins. The aim of the present study was to determine whether the effects of oleate and insulin on the net accumulation of apolipoproteins, specifically apoB, were related to their cellular mRNA concentrations. It was first established that the production of triglyceride-rich, apoB-containing lipoproteins and the concentration of mRNA for apolipoproteins A-I, A-II, B, and E were not affected by increasing the glucose concentration of medium from 5.5 to 20 mM. Oleate and insulin had no effect on either the accumulation in the medium or the cellular mRNA concentration of apolipoproteins A-I and A-II. On the other hand, the addition of oleate caused a two- to threefold increase in the accumulation of triglycerides in the medium without significantly affecting either the rates of accumulation or cellular mRNA levels of apolipoproteins B and E. In the presence of insulin, there was a dose-dependent decrease in the net accumulation of triglycerides and apoB and, to a lesser extent, cholesteryl esters and apoE. This inhibitory effect of insulin on the accumulation of triglycerides and apoB was partially abolished after a prolonged exposure of cells to insulin. Under these experimental conditions and at all concentrations tested, insulin had no effect on the cellular concentration of mRNA for either apoB or apoE. These results demonstrate that the previously reported effect of oleate and insulin on the net accumulation of triglyceride-rich, apoB-containing lipoprotein particles by HepG2 cells is not mediated by changes in the mRNA concentration of apoB. — **Dashti, N., D. L. Williams, and P. Alaupovic.** Effects of oleate and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells. *J. Lipid Res.* 1989. **30:** 1365–1373.

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Hepatic lipoprotein production is markedly influenced by nutritional and hormonal states of the animal. It has been shown that the secretion of triglycerides (1,2) and apolipoproteins (3,4) by the liver is enhanced in fed animals and is decreased after fasting. Various studies have demonstrated the stimulatory effect of glucose (5,6) and long-chain fatty acids (7–11) on the hepatic secretion of triglycerides. However, a number of observations in cultured rat (12,13) and chicken (14) hepatocytes as well as in humans (15) have shown that the secretion rates of VLDL triglycerides and apolipoproteins in response to fatty acid provision do not always change in parallel, indicating that these two processes are not tightly coordinated.

The role of insulin in the regulation of triglyceride secretion is controversial. Although insulin is generally considered to stimulate the de novo synthesis of fatty acids (16), it has been shown that, in the rat, the secretion of hepatic VLDL and apolipoprotein B (apoB) is either increased (5,17) or decreased (6,18,19). This divergent effect of insulin has also been observed in humans, where both increased hepatic triglyceride secretion (20,21) and inhibition in this process (22) have been reported. The inhibitory effect is less pronounced in rat hepatocytes preincubated with insulin (23), an observation ascribed to the down-regulation of insulin receptors (23). As an anabolic hormone, insulin stimulates net protein synthesis at both the transcriptional level in the liver (24–29) and translational level in extrahepatic tissues (30–32). There is no information about the molecular mechanism by which insulin exerts its effect on hepatic production of apolipoproteins.

In view of the essential role of apoB in the assembly and secretion of hepatic VLDL particles and the interaction of LDL with cellular receptors, a better understanding of mechanism(s) involved in regulation of its synthesis and

Abbreviations: apo, apolipoprotein; MEM, minimum essential medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; VLDL, very low density lipoprotins; EDTA, ethylenediaminetetraacetic acid; LDL, low density lipoproteins.

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secretion is of considerable biochemical and clinical interest. Previous studies from this laboratory (33) have demonstrated the lack of a coordinated enhancement in the secretion of triglycerides and apoB by HepG2 cells in response to the fatty acid provision. We also provided evidence for the direct inhibitory effect of insulin on the secretion of triglyceride-rich, apoB-containing lipoproteins by HepG2 cells (33). The main goal of the present study was to determine whether the effects of oleate and insulin on the secretion of apolipoproteins, specifically apoB, were mediated through changes in their cellular mRNA concentrations. Part of this work has been reported in abstract form (34).

MATERIALS AND METHODS

Materials

Minimum essential medium (MEM), fetal bovine serum (FBS), trypsin, sodium pyruvate, L-glutamine, and MEM vitamin solution were purchased from Grand Island Biological Company (Grand Island, NY). Oleic acid was from Sigma Chemical Co. (St. Louis, MO). Fatty acid-free bovine serum albumin was purchased from Miles Laboratories (Elkhart, IN). [^{32}P]dCTP was from Amersham Corp. (Arlington Heights, IL). Bovine insulin was obtained from Calbiochem-Behring (La Jolla, CA) and human insulin (Novolin R) was from Squibb-Novo, Inc. (Princeton, NJ).

Cell culture

Cells were seeded in 100-mm diameter dishes in 12 ml MEM supplemented with 2 mM glutamine, 1 mM sodium pyruvate, MEM vitamin solution, and 10% FBS. In all experiments the medium was changed 48 h after plating, unless otherwise stated. Four days after seeding, the maintenance medium was removed, monolayers were washed twice with phosphate-buffered saline (PBS), and 12 ml of serum-free MEM was added to each dish. In studies where the effect of fatty acid was to be assessed, the medium was supplemented with 0.8 mM oleate bound to 3% bovine serum albumin. Bovine insulin, prepared as previously described (9), and human insulin were added as indicated for each experiment. At the end of each incubation, the conditioned medium was removed and, to prevent oxidative and proteolytic damage, preservative cocktail (35) was added to obtain final concentrations of 500 units/ml penicillin-G, 50 μg/ml streptomycin sulfate, 20 μg/ml chloramphenicol, 1.3 mg/ml ϵ-amino caproic acid, 1 mg/ml EDTA, and 0.2 mg/ml glutathione. The medium was centrifuged at 2000 rpm for 30 min at 4°C to remove small amounts of cells and debris. The supernatant fraction was concentrated approximately 10- to 15-fold with either polyvinylpyrolidone (PVP) or sucrose placed outside the dialysis bag (5000 mol wt cut-off).

Determination of neutral lipids and apolipoproteins

Triglycerides, unesterified cholesterol and cholesteryl ester contents of concentrated culture medium were determined by gas-liquid chromatography (36). The concentrations of apolipoproteins A-I, A-II, B, and E were measured by electroimmunoassays developed in this laboratory (37–39).

Isolation and characterization of cellular RNA

RNA was isolated from HepG2 cells by the guanidine thiocyanate method (40). The integrity of all RNA samples was verified by electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde (41).

Preparation of probes and DNA-excess solution hybridization assays

Hybridization probes were prepared from human apolipoprotein A-I, A-II, B, and E cDNA fragments subcloned in bacteriophage M13 vectors. The human apolipoprotein A-I, B, and E cDNA clones were provided by Dr. J. Breslow (42–44). The preparation of single-stranded cDNA probes and the hybridization assays for apoA-I and apoE mRNAs were carried out exactly as described (45–47). The apoB-100 cDNA probe corresponding to nucleotides + 12,898 to + 13,106 was prepared from a PstI-HindIII fragment of pB8 (44) subcloned in M13 mp8 (48). Probe was isolated by digestion with AvaII after DNA synthesis and purified as described (46). The apoA-II cDNA was provided by Dr. H. B. Brewer (49). ApoA-II cDNA probe corresponding to nucleotides encoding residues − 1 to − 10 of the prepro region, the entire sequence of mature protein, and 71 nucleotides from 3'-untranslated region (49) was prepared from a PstI-SstI fragment subcloned in M13 mp19. Probe was isolated by digestion with PstI after DNA synthesis and purified as described (46). Hybridization assays for apoB-100 and apoA-II mRNAs were carried out as described (45–47) and used the respective M13-apoDNA template as hybridization standards to calculate absolute mRNA concentrations (46). All assays were carried out using RNA from triplicate dishes and four mRNA measurements were made on RNA prepared from each dish, and thus the values represent the mean of 12 determinations for both control and treated cells.

RESULTS

Effect of glucose concentration on secretion of neutral lipids and apoB accumulation and mRNA levels

There is general consensus that glucose stimulates triglyceride synthesis (5,50,51). To determine whether the ac-

cumulation rate of apoB-containing lipoproteins rich in triglycerides was related to the concentration of glucose in the medium, HepG2 cells were incubated with MEM containing either 5.5 mM or 20 mM glucose. Results showed that increased concentration of glucose in the medium caused only a small increase (9.5%) in the net accumulation of triglycerides and had no effect on either the accumulation of apolipoproteins in the medium or their cellular mRNA levels (data not shown). Thus, under the culture conditions described, 5.5 mM glucose present in MEM is sufficient for the optimal neutral lipid and apolipoprotein production by HepG2 cells.

Effect of oleate on the cellular apoB mRNA levels

We have demonstrated previously that oleate-induced stimulation in net accumulation of triglycerides was not concurrent with similar changes in the apolipoproteins, specifically apoB (33). To verify this divergent effect of fatty acids on the accumulation of lipoprotein constituents, we measured the concentration of mRNA for apolipoproteins in HepG2 cells incubated in medium containing 3% bovine serum albumin with or without 0.8 mM oleate. There were no changes in either the accumulation rates or cellular mRNA levels of apolipoproteins A-I, A-II, and E or net accumulation of total cholesterol subsequent to fatty acid addition (data not shown). As shown in **Table 1**, the addition of oleate resulted in a threefold increase in triglyceride concentration, but had no effect on the accumulation of apoB in the medium. The lack of oleate effect on apoB level in the medium was corroborated by the finding that cellular content of apoB mRNA remained unchanged in the presence of this fatty acid (Table 1).

Effects of human insulin on the accumulation of neutral lipids in the culture medium

Our previous studies have shown that incubation of HepG2 cells in the presence of 0.2–25 mU/ml of bovine insulin resulted in decreased accumulation of neutral lipids in the medium (33). The effect of bovine insulin was dose-dependent and reached maximum at 25 mU/ml (33). To determine the effectiveness of the homologous hormone on this process, a semi-synthetic human insulin (Novolin R) was used. Consistent with previous results using bovine insulin (33), a 14-h incubation of HepG2 cells with human insulin caused a dose-dependent inhibition in the accumulation of all neutral lipids in the culture medium. In agreement with previous results (33), the major inhibitory effect of human insulin was on the accumulation of triglycerides and cholesteryl esters which were inhibited by 40% at 25 mU insulin/ml of medium; the accumulation of unesterified cholesterol in the medium was less sensitive to insulin.

Effects of human insulin on the accumulation of apolipoproteins in the culture medium

The effect of a single addition of human insulin on the accumulation of apolipoproteins A-I, A-II, B, and E in the culture medium during a 14-h incubation is shown in **Fig. 1**. Incubation of cells in the presence of insulin at 0.1–25 mU/ml of medium resulted in a dose-dependent decrease in the accumulation of apolipoproteins B and E in culture medium (Fig. 1). The decrease in apoB net accumulation was more prominent than that of apoE, becoming apparent at as low a dose as 0.1 mU insulin/ml of medium and reaching maximum reduction (52%) at 25 mU/ml of medium (Fig. 1). ApoE accumulation in the medium was also reduced by the addition of human insulin, but this reduction was moderate (20%) reaching a plateau at 10 mU insulin/ml of medium (Fig. 1). In contrast to our previous results with bovine insulin showing a moderate inhibition in the production of apoA-I and apoA-II at a pharmacological concentration (25 mU/ml of medium) of this hormone (33), the rates of accumulation of apolipoproteins A-I and A-II were not altered by the addition of human insulin up to as high as dose as 25 mU/ml (Fig. 1).

Long-term effects of human insulin on the accumulation of triglycerides and apolipoproteins

In view of the possibility that acute and chronic addition of insulin might have opposing effects on lipoprotein synthesis and secretion, cells were also grown in the presence of insulin for several generations. HepG2 cells were seeded in the presence and absence of insulin at 10 mU/ml of medium, a

TABLE 1. Effect of oleate on net accumulation of triglycerides and apolipoprotein B and on cellular apolipoprotein B mRNA content

	Triglycerides	Apolipoprotein B	
Medium	Accumulation in Medium	Accumulation in Medium	Cellular mRNA Content
	μg/g cell protein/h		*pg/μg RNA*
Control	92.2 ± 1.1	107.6 ± 8.7	455.1 ± 61.3
Oleate	321.3 ± 28.7	110.6 ± 12.6	450.5 ± 75.2

Cells were grown in MEM containing 10% FBS. After 4 days in culture, the maintenance medium was removed, monolayers were washed twice with PBS and incubated with serum-free MEM with or without 3% BSA–0.8 mM oleate. Values are mean ± SEM of triplicate dishes.

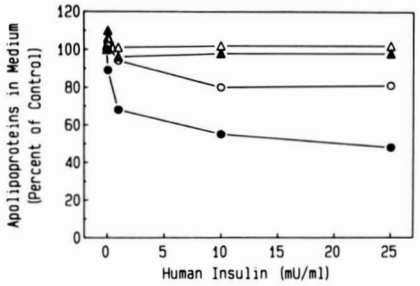

Fig. 1. Insulin-induced changes in the accumulation of apolipoproteins in HepG2 culture medium. Cells were cultured in minimum essential medium containing 10% FBS. After 4 days, the maintenance medium was removed, monolayers were washed twice with PBS, and serum-free medium was added. The effect of increasing concentration of human insulin on the accumulation of apoA-I (△), apoA-II (▲), apoB (●), and apoE (○) in culture medium after a 14-h incubation was determined. Each value is the mean of triplicate dishes.

concentration at which the major effects of hormone were observed (Fig. 1). In addition, this initial high concentration of insulin was used to avoid a possible depletion of the hormone during the 14- to 16-h incubation time used in these studies. Determination of insulin concentration in the HepG2 culture medium showed that only 8–10% of the initial level of insulin remained after a 16-h incubation, indicating that similarly to rat hepatocytes (19), insulin is rapidly degraded by HepG2 cells. The cells were passaged every 4 days and culture medium was changed every 2 days during the course of this experiment. Thus, insulin was added every 2 days at 10 mU/ml of medium and, considering its rate of degradation by HepG2 cells, this would provide insulin concentration similar to that of postprandial portal vein level (19,52). The effect of chronic addition of insulin on the accumulation of apolipoproteins A-I, A-II, and E in the medium during a 16-h incubation is shown in **Fig. 2**. Long-term incubation of cells with insulin produced no changes in the accumulation of apolipoproteins A-I and A-II when compared to parallel cultures grown under the same conditions but without insulin (Fig. 2). The inhibitory effect of insulin on apoE accumulation was less apparent upon prolonged incubation with the hormone in that only a 10% decrease in apoE production was observed after a 13-day hormone treatment (Fig. 2). The most interesting difference between acutely and chronically treated cells was in their response to the inhibitory effect of insulin on triglyceride and apoB net accumulation. After 1, 5, and 13 days incubation with insulin, there was a 40, 30, and 20% inhibition in the accumulation of triglycerides and 52, 35, and 25% reduction in the accumulation of apoB (**Fig. 3**). Even after 30 days of incubation in the presence of insulin, there was no stimulation in the triglyceride and apoB net accumulation in HepG2 culture medium (data not shown). Thus, there was an inverse relationship between the duration of insulin treatment and the effectiveness of the hormone in inhibiting triglyceride and apoB accumulation in the medium. Similar results were obtained after long term incubation of cells with 25 mU insulin/ml of medium (data not shown).

Effect of bovine and human insulin on the level of apolipoprotein mRNAs in HepG2 cells

To determine whether the inhibitory effect of insulin on the net accumulation of apoB, and to a lesser degree apoE, was caused by decreased levels of corresponding mRNAs, we examined the effect of insulin on apolipoproteins A-I, A-II, B, and E mRNA levels in HepG2 cells. Since the maximum effect of insulin was observed after an acute hormone treatment (Figs. 2 and 3), in this series of experiments the cells were incubated for 16 h in the presence of both physiological (1.0 mU/ml) and pharmacological (25 mU/ml) doses of insulin. These studies were repeated three times and showed a high degree of reproducibility. The results of a representative experiment are shown in **Table 2** and **Table 3**. Consistent with unimpaired accumulation rates of apolipoproteins A-I and A-II in the presence of insulin (Fig. 1), there were no changes in their corresponding cellular mRNA levels (Table 2). The observed 20% decrease in the accumulation of apoE caused by human or bovine insulin was not due to its reduced cellular mRNA level (Table 2). It should be pointed out that the control values for apoA-I and apoE mRNA levels in the present study were higher than those reported previously (46). This difference might be due to variation in culture conditions under which the HepG2 cells were maintained. In the present study, the cells were grown under defined conditions and RNA was isolated from cells incubated for 16 h in the absence of FBS which might influence the mRNA levels. Although the addition of human insulin at a dose of 1.0 mU/ml of medium inhibited the net accumulation of apoB by 26%, there were no detectable changes in its mRNA levels. Even at the highest concentration of insulin (25 mU/ml) causing a 50% reduction in apoB accumulation in the

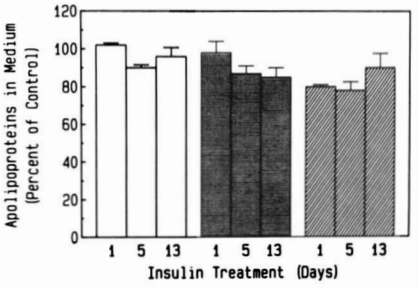

Fig. 2. Acute and chronic effects of insulin on apolipoprotein net accumulation. HepG2 cells were cultured as described in the legend to Fig. 1. The effect of a 16-h incubation with human insulin on net accumulation of apoA-I (open bars), apoA-II (speckled bars), and apoE (diagonal bars) in the culture medium of HepG2 grown in the absence (indicated as day 1) or presence (for 5–13 days) of insulin was determined. Values are mean ± SEM of triplicate dishes.

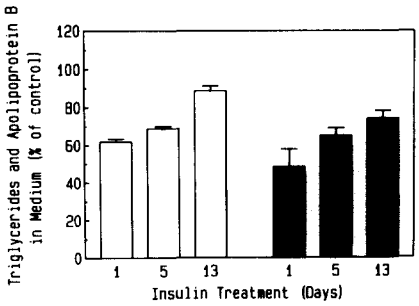

Fig. 3. Extent of inhibition in accumulation of triglyceride-rich, apoB-containing lipoproteins by acute and chronic insulin treatment. HepG2 cells were cultured with or without insulin as described in the legend to Fig. 2. The effect of a 16-h incubation of HepG2 cells with human insulin on the accumulation of triglycerides (open bars) and apoB (solid bars) was determined. Values are mean ± SEM of triplicate dishes.

medium, the cellular apoB mRNA levels remained unchanged (Table 3).

DISCUSSION

To assure that, under the experimental conditions used in this study, the concentration of glucose in MEM is not a limiting factor for the optimal rate of accumulation of triglyceride-rich, apoB-containing lipoprotein particles, we increased the medium concentration from 5.5 mM to 20 mM. The results of this experiment showed that increased concentration of glucose had no significant effect on the accumulation of apoB-containing lipoproteins in the medium or the cellular concentration of apoB mRNA. Thus, the 5.5 mM glucose concentration in MEM provided sufficient substrate for the synthesis and secretion of triglyceride-rich lipoprotein particles.

The addition of oleate resulted in a two- to threefold increase in the accumulation of triglyceride in the medium without significantly altering total cholesterol production. The oleate-induced increase in total triglycerides was reflected mainly in the stimulation of VLDL production as previously reported (33). This response to fatty acid substrate is in agreement with reported accumulation of cytoplasmic lipid storage droplets in the cells (53) and increased VLDL secretion (54) by HepG2 cells subsequent to the inclusion of oleic acid in the medium. In the present study, incubation of cells with 0.8 mM oleate resulted in a threefold increase in the accumulaiton of triglyceride in medium without any significant effect on the apoB production. The inability of oleate to stimulate apoB net accumulation was commensurate with the unchanged level of cellular apoB mRNA observed under these conditions. Since similar results were obtained with apoE, the present work clearly demonstrates that in HepG2 cells intracellular apolipoprotein synthesis is not accelerated by increased availability of fatty acids. The fact that oleate did not result in coordinated accumulation of triglycerides and apolipoproteins, as verified by their unaltered mRNA levels, provides strong support for the concept that in HepG2 cells, as in rat (12,13) and chicken (14) hepatocytes, these processes are not tightly coupled. The present study clearly demonstrates that, although oleic acid causes composition changes in the VLDL particles secreted by the liver (12,13,33,55), parallel enhancement in the synthesis of triglycerides and apolipoproteins B and E is not a prerequisite for their assembly into secretory vesicles. The disproportionate increase in triglycerides and apolipoprotein levels of VLDL produced by HepG2 cells incubated with oleic acid suggests that this fatty acid increases the size rather than the number of VLDL particles.

TABLE 2. Direct effect of insulin on cellular mRNA levels for apolipoproteins A-I, A-II, and E

Insulin		Apolipoprotein		
Source	mU/ml	A-I	A-II	E
			pg/μg RNA	
	0	24.7 ± 3.2	34.1 ± 2.9	22.9 ± 2.1
Human	1	25.9 ± 1.2	31.8 ± 3.0	21.4 ± 0.6
Bovine	1	28.2 ± 3.5	33.2 ± 3.3	24.8 ± 3.8
Human	25	29.7 ± 1.1	37.6 ± 0.4	24.7 ± 0.1
Bovine	25	29.9 ± 3.6	40.3 ± 2.8	24.6 ± 2.8

HepG2 cells were grown in MEM containing 10% FBS. After 4 days in culture, the maintenance medium was removed, cells were washed twice with PBS and incubated for 16 h with serum-free medium containing either human or bovine insulin. Values are mean ± SEM of triplicate dishes. The mean for each dish represents four determinations. The values for mRNA expressed in terms of molecules per cell for the control group were apoA-I = 696 ± 90; apoA-II = 2235 ± 190; apoE = 620 ± 57.

TABLE 3. *Direct effect of insulin on apolipoprotein B accumulation in medium and cellular mRNA content*

Insulin		Apolipoprotein B		
Source	mU/ml	Accumulation	Cellular mRNA Content	
		% inhibition	*pg/μg RNA*	*molecules/cell*
	0	0	392.6 ± 30.8	863.7 ± 67.8
Human	1	26.2 ± 7.1	374.6 ± 28.8	824.1 ± 63.4
Human	25	42.6 ± 9.8	376.7 ± 20.9	828.7 ± 46.0
Bovine	25	50.5 ± 4.2	354.1 ± 24.5	779.0 ± 53.9

HepG2 cells were grown under experimental conditions described in Table 2. Values are mean ± SEM of three experiments. The mean for each experiment represents triplicate dishes. The cellular mRNA content of each dish represents four determinations.

Consistent with our previous studies using bovine insulin (33), incubation of HepG2 cells with human insulin resulted in diminished accumulation of neutral lipids and apolipoproteins in the medium. The decrease was particularly marked in the case of apoB, triglyceride and cholesteryl esters, and, to a much lesser degree apoE and nonesterified cholesterol. The inhibitory effect of human insulin already evident at 0.1 mU/ml of medium became more pronounced as a function of increasing insulin concentration. In contrast to bovine insulin which caused a moderate inhibition in the accumulation of apolipoproteins A-I and A-II when added at pharmacological concentrations (33), human insulin exhibited no significant effect at any of the concentrations tested. These results indicate that insulin specifically inhibits the net accumulation of triglyceride-rich lipoprotein particles containing apoB and apoE, but has no effect on the accumulation of apoA-containing lipoproteins.

Recent studies by Bartlett and Gibbons (56) have indicated that, in contrast to short-term inhibitory effect of insulin on VLDL secretion, the long-term treatment of rat hepatocytes results in the stimulation of VLDL secretion. In view of the potential importance of this possibly diverse effect of insulin on VLDL secretion, HepG2 cells were grown with insulin for a prolonged time. The accumulation of apolipoproteins A-I and A-II in the medium remained unchanged regardless of the duration of hormone treatment, indicating that the synthesis of apoA-containing particles is not regulated by insulin. The most interesting difference between short- and long-term treatment of HepG2 cells with insulin was in their production rates of triglycerides and apoB. The inhibitory effect of insulin on the accumulation of triglycerides and apoB in the medium decreased with increasing time of exposure to the hormone. Similar, although less pronounced, changes were also observed in the apoE accumulation. These results are consistent with studies by Patsch, Gotto, and Patsch (23) and indicate that insulin resistance of HepG2 cells after prolonged incubation times most probably is due to the down-regulation of insulin receptors (57). Thus, under the present experimental conditions, we were not able to demonstrate stimulation of VLDL production by prolonged treatment of HepG2 cells with human insulin. Although in the present study the removal of apoB-containing lipoproteins by insulin-treated HepG2 cells was not determined, studies using rat cultured hepatocytes (6,18) have demonstrated that insulin does not affect the degradation of ^{125}I-labeled VLDL significantly, suggesting that insulin causes decreased secretion rather than increased uptake of newly synthesized triglyceride-rich lipoproteins.

The molecular mechanism by which insulin exerts its inhibitory effect on apoB secretion is unknown. In general, insulin has a positive effect on the net protein production through both stimulation of protein synthesis (30,58,59) and inhibition of protein degradation (60). In liver, insulin promotes fatty acid synthesis (16) by increasing the level of mRNA for glucokinase (29), pyruvate kinase (24), malic enzyme (26), and fatty acid synthetase (25), while decreasing the hepatic mRNA content for phosphoenolpyruvate carboxykinase (61). Insulin has been reported to exert both positive (27,28) and negative (62) effects on hepatic mRNA levels for albumin. Therefore, the inhibitory effect of insulin on apoB net production by HepG2 cells could potentially be due to decreased level of mRNA. The lack of effect of human insulin on the accumulation of apoA-I and apoA-II was supported by the finding that mRNA concentrations for these apolipoproteins had not changed. However, the marked reduction in apoB net accumulation caused by insulin could not be ascribed to decreased cellular concentration of mRNA for this apolipoprotein. Similarly, the moderate inhibitory effect of insulin on apoE accumulation was not related to its mRNA levels. It appears, therefore, that the insulin-suppressed net accumulation of apoB is due either to the decreased translation of mRNA or altered assembly and subsequent secretion of apoB-containing lipoproteins caused by post-translational modification of the protein. Alternatively, insulin might exert its effect by accelerating the intracellular degradation of

apoB. Support for such mechanisms is provided by studies of Nakanishi, Goldstein, and Brown (63) demonstrating that the inhibitory effect of mevalonate on 3-hydroxy-3-methylglutaryl coenzyme A reductase was due to a combined decrease in translation of mRNA and increase in the rate of degradation of enzyme rather than decline in levels of mRNA. Evidence for a possible post-translational modification leading to altered assembly and/or secretion of apoB-containing lipoproteins is provided by studies of Davis et al. (64) demonstrating the phosphorylation of apoB and of Sparks et al. (65) showing that this process is altered in hepatocytes of diabetic rats. It has been suggested (64,65) that phosphorylated apoB may play a role in the intracellular assembly and transport of lipoproteins by allowing apoB to dissociate from membrane lipids for subsequent incorporation into VLDL particles. Since apoB phosphorylation is significantly increased in diabetic rats (65), it is conceivable that insulin might exert its inhibitory effect by decreasing apoB phosphorylation and ultimately its secretion.

Previous results obtained with diabetic rats (5) and humans (66,67) are in contrast to the direct inhibitory effects of insulin on hepatic triglyceride-rich lipoprotein secretion observed in this study and in studies with cultured rat hepatocytes (6,18,19,56). One of the main reasons for this discrepancy, as discussed by Gibbons (68), may be due to the in vivo hepatic insulin resistance in type II diabetes. The present study demonstrating diminished sensitivity of HepG2 cells to insulin after long-term incubation with hormone supports this hypothesis. Under conditions where the normal regulation of hepatic lipoprotein secretion is reversed, such as in type II diabetes, overproduction of triglyceride and apoB would be expected to occur. Recent studies by Alaupovic et al. (69) have shown a significant increase in the plasma concentration of triglyceride-rich, apoB-containing lipoproteins in type II diabetes. Further studies are needed to determine the direct effects of insulin on translation of apoB mRNA, possible modification of the protein, and ultimately assembly and secretion of apoB-containing lipoprotein particles.

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