Glucose does not stimulate apoprotein B secretion from HepG2 cells because of insufficient stimulation of triglyceride synthesis

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Abstract **We have previously demonstrated a close relationship between oleic acid (OA)-stimulated triglyceride (TG) synthesis and apolipoprotein B (apoB) secretion in HepG2 cells. However, other investigators studying the association between glucose-stimulated TG synthesis and apoB secretion have reported variable results. The present study was carried out to answer the question: does TG derived from glucose have different effects on apoB secretion from HepG2 cells compared to TG derived from oleate? We observed that incubations of HepG2 cells for as long as 48 h in 30 mm glucose did not increase apoB secretion. We then demonstrated that the failure of glucose to stimulate apoB secretion from HepG2 cells results from insufficient stimulation of TG synthesis by glucose. Thus, incorporation of [^{3}H]glycerol into [^{3}H]TG in the presence of 30 mm glucose for up till 48 h was not increased compared to basal conditions. The inability of glucose to stimulate TG synthesis was also evidenced by the inability of both 8-h and 24-h incubations with 30 mm glucose to increase cell TG mass; similar incubations with OA increased TG mass 50–100%. Additional studies demonstrated that glucose conversion to either glycerol or fatty acids was minimal; this accounted for the lack of stimulation of TG synthesis. We conclude that in HepG2 cells, availability of high glucose levels in the media for as long as 48 h does not stimulate triglyceride synthesis, and that this is the basis for the failure of glucose to stimulate apoB secretion.—**Jiang, H., H. N. Ginsberg, and X. Wu. **Glucose does not stimulate apolipoprotein B secretion from HepG2 cells because of insufficient stimulation of triglyceride synthesis. *J. Lipid Res.* 1998.** 39: **2277–2285.**

Supplementary key words apoprotein B • HepG2 cells • triglyceride • glucose • fatty acids • oleic acid • palmitic acid • secretion

Apolipoprotein B-100 (apoB), a highly hydrophobic secretory protein containing 4536 amino acids, carries lipids (triglyceride [TG], cholesterol, cholesteryl ester [CE], and phospholipids) from the liver to the circulation in the forms of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) (1–3). Secretion of newly synthesized apoB from HepG2 cells, a widely used model for the study of assembly and secretion of apoB-containing lipoproteins from the liver (4), is regulated mainly at post-translational or post-transcriptional levels (5–12). Thus, the majority of newly synthesized apoB in HepG2 cells undergoes rapid, co-translational targeting for intracellular degradation via the ubiquitin–proteasomal pathway (4, 13–18), and only a small fraction of newly synthesized apoB is eventually secreted as lipoprotein particles (19, 20).

The availability of newly synthesized lipids is critical in determining apoB particle assembly and secretion. Dixon, Furukawa, and Ginsberg (21) demonstrated that by incubating HepG2 cells with oleic acid (OA), intracellular degradation of newly synthesized apoB was significantly decreased. Oleic acid (OA) treatment results in a significant increase in TG synthesis, suggesting that TG plays an important role in the secretion of apoB-containing lipoproteins. The association between TG synthesis and apoB secretion has been confirmed using exogenous VLDL, eicosapentaenoic acid, or docosahexaenoic acid as sources of TG (22–24). As a result, we have proposed that newly synthesized TG, possibly by stimulating interaction between microsomal triglyceride transfer protein and nascent apoB (25), plays a critical role in co-translationally targeting apoB away from degradation and to secretion (18).

Cianflone et al. (26), using inhibitors of hydroxymethylglutaryl-CoA reductase (HMG-CoAR) and acyl-CoA:cholesterol acyltransferase (ACAT), presented data supporting a critical role for CE in apoB secretion (26). That finding has been confirmed in some but not all studies using various ACAT inhibitors (22, 27–31). More recently, using glucose as a substrate for TG formation, Cianflone et al.

Abbreviations: apoB, apolipoprotein B; OA, oleic acid; PA, palmitate; TG, triglyceride; BSA, bovine serum albumin; HMG-CoAR, hydroxymethylglutaryl-coenzyme A reductase; ACAT, acylcholesterol acyl transferase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; MEM, minimum essential medium; PBS, phosphate-buffered saline; CE, cholesteryl ester.

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(32) reported that apoB secretion from HepG2 cells was not changed, although TG synthesis and, accordingly, the size of secreted apoB-containing particles was significantly increased. Those results indicated that either TG was not important for the initial co-translational targeting of apoB for secretion from HepG2 cells, or that TG derived from glucose had a different effect on apoB secretion than TG derived from exogenous fatty acids. The goal of the present studies was to address these issues.

MATERIALS AND METHODS

Reagents

l-[4,5-^{3}H]leucine (135 Ci/mmol, catalog number, TRK. 683) and [2-^{3}H]glycerol (1.0 Ci/mmol, catalog number, TRA.118) were purchased from Amersham Corp (Arlington Heights, IL). Monospecific anti-human apoB antiserum was raised in a rabbit. Protein A-Sepharose CL 4B was obtained from Pharmacia LKB Biotechnology, Inc. (Uppsala, Sweden). Minimum essential medium (MEM), nonessential amino acids, sodium pyruvate, and penicillin/streptomycin were from GIBCO Laboratories (Grand Island, NY). Leucine-free medium was generated from a minimum essential select-amine kit (GIBCO, catalog number, 300 9050AV). Heat-inactivated fetal bovine serum was from Gemini Bioproducts Inc. Leupeptin and pepstatin A were from Peninsula Laboratories, Inc. (Belmont, CA). Bovine serum albumin (BSA) (essentially fatty acid-free), oleic acid (sodium salt) (catalog number, O7501), and palmitic acid (sodium salt) (catalog number, I-3380) were from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity available.

Growth of cells

HepG2 cells, obtained from American Type Culture Collection, were grown in 6-well dishes. The cells were maintained in MEM containing 0.1 mm nonessential amino acids, 1 mm sodium pyruvate, penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% FBS for 4 days with medium replenishment at day 3. The medium was then changed to serum-free MEM experimental medium containing 1.5% BSA as described below.

Treatment and labeling of cells

HepG2 cells were washed twice with phosphate-buffered saline (PBS) and subjected to several types of protocols. To study the short-term effects of glucose on apoB secretion, the cells were preincubated with or without glucose for 4 h and then labeled with [^{3}H]leucine in the same media for up to 8 h. To study the long-term effects of glucose, the cells were incubated with [^{3}H]leucine in media with or without glucose for up to 48 h. In some experiments, media was removed every 8 h and replaced with new label and glucose. After labeling, the medium was collected for the determination of apoB secretion. To estimate intracellular degradation of newly synthesized apoB, the cells were pre-incubated with glucose for 4 h, pulse-labeled with [^{3}H]leucine for 10 min, and chased in serum-free medium for up to 60 min. The cells were lysed at each chase time point, and intracellular apoB was determined. Only a small percent of synthesized apoB (usually between 10 and 20%) was secreted during a 60-min chase.

Immunoprecipitation

Immunoprecipitation of apoB in medium and cell homogenate was carried out exactly according to the method of Dixon et al. (21). After completion of the labeling or chase period, medium was transferred to a tube containing a protease inhibitor mixture (1 mm benzamidine, 5 mm EDTA, 0.86 mm phenylmethylsulfonyl fluoride, 100 kallikrein-inactiving units/ml of aprotinin, and 10 mm HEPES, pH 8.0) . The cells were solubilized in lysis buffer (150 mm NaCl, 5 mm EDTA, 50 mm Tris, pH 7.4, 0.0625 m sucrose, 0.5% Triton X-100, 0.5% sodium deoxycholate, 50 μg/ml leupeptin, and 50 μg/ml pepstatin A in addition to the protease inhibitor mixture).

Aliquots of cell extract or medium were combined with NET buffer (150 mm NaCl, 5 mm EDTA, and 50 mm Tris, pH 7.4, 0.5% Triton X-100, and 0.1% SDS) and an excess amount of monospecific antihuman apoB antiserum, and the mixture was incubated for 10 h at 4°C on a rocking platform. One hundred μl of a 5% solution of protein A-Sepharose in NET buffer was then added to the reaction mixture and the incubation was continued for an additional 3 h. At the end of incubation, the pellets were washed 5 times with 1 ml NET buffer and resuspended in sample buffer (0.125 m Tris HCl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol). ApoB was extracted from the pellet by boiling for 4 min. An aliquot of each sample was analyzed by SDS-PAGE (3–15% gradient gel). The gel was treated with Autofluor (National Diagnostics, Atlanta, GA) and, after drying, was exposed to a film (Kodak, X OMAT AR) at −80°C. Except when noted in the figure legend, when apoB secretion rates were quantitated, the bands on the gel were excised, dissolved in scintillation fluid, and counted directly in a liquid scintillation counter.

Analysis of cellular TG synthesis and mass

To determine TG synthesis, cells were labeled with [^{3}H]glycerol (5 μCi/ml) or [^{14}C]OA (0.01 mm) for several hours. After labeling, the medium was removed and the cells were washed twice with cold PBS. The cellular lipids were extracted with hexane–isopropanol 3:2 and the organic solvent was evaporated under a stream of N_2. The dried lipids were re-suspended in a small volume of hexane and run on a thin-layer chromatography (TLC) plate. Lipid spots were visualized with iodine, scraped off the plate, and counted in a liquid scintillation counter. To measure TG mass in HepG2 cells, lipids were extracted from cell monolayers with hexane–isopropanol 3:2, and TG mass was determined with either a Sigma kit (UV334) or a Boehringer kit (Cat. 450032).

Statistics

Results are shown as means ± SD. Significance of means between two groups was examined with Student's *t*-test.

RESULTS

Short-term incubation with glucose does not affect either secretion or intracellular degradation of newly synthesized apoB in HepG2 cells

To examine the effect of short-term glucose treatment on apoB secretion, HepG2 cells were pre-incubated with either BSA (1.5%) or BSA plus glucose (30 mm) for 4 h. BSA-treated cells were then labeled with [^{3}H]leucine in leucine-free MEM in the presence of either BSA or BSA plus OA (0.4 mm). Glucose-treated cells were labeled with [^{3}H]leucine in the presence of glucose. After the cells were labeled for either 1 or 2 h, medium was collected for determination of apoB secretion. **Figure 1A** represents a typical autoradiograph. ApoB secretion after 2 h labeling was quantitated and is shown in Fig. 1B. When compared to BSA (1.5%) alone, 30 mm glucose treatment caused no

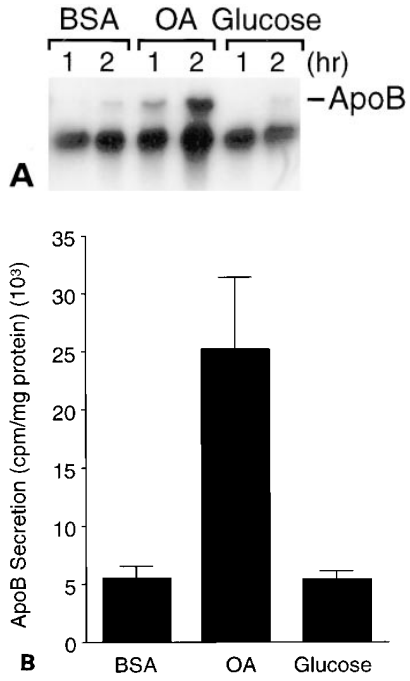

Fig. 1. Short-term incubation with glucose does not increase the secretion of apoB from HepG2 cells. HepG2 cells were pre-incubated with either BSA (1.5%) or BSA plus glucose (30 mm) for 4 h. BSA-treated cells were then labeled with [^{3}H]leucine in leucine-free MEM in the presence of either BSA or OA (0.4 mm). Glucose-treated cells were labeled with [^{3}H]leucine in the presence of glucose. After the cells were labeled for 1 or 2 h, medium was collected for determination of apoB secretion. When compared to BSA (1.5%) alone, 30 mm glucose treatment caused no increase in apoB secretion. OA, on the other hand, increased apoB secretion in a time-dependent manner (panels A and B). ApoB bands at 2 h labeling were cut from the gels and counted in a scintillation counter and the results from three experiments are shown in panel B as means ± SD.

increase in apoB secretion (P = NS, glucose vs. BSA). OA, a known stimulator of apoB secretion (5, 6, 19, 21), increased apoB secretion significantly ($P < 0.01$, OA vs. BSA). Pulse-chase studies were carried out to determine the effects of short-term incubation with glucose on intracellular decay of apoB. Glucose had no effect compared to BSA, whereas treatment with OA significantly slowed intracellular decay of apoB (data not shown).

ApoB secretion from cells incubated with glucose at concentrations ranging between 10 mm and 120 mm was found to be identical to that from control BSA cells (data not shown). Therefore, 30 mm glucose was used in the following experiments to study other aspects of the regulation of apoB secretion.

Short-term incubation with glucose does not affect either synthesis or secretion of TG in HepG2 cells

The contrasting effects of short-term glucose and OA on apoB secretion and intracellular degradation in HepG2 cells was unexpected, considering that glucose is assumed to be a substrate for the synthesis of fatty acids, which are, in turn, substrates for TG synthesis. Therefore, we carried out a series of experiments to determine whether glucose stimulated TG synthesis in HepG2 cells.

HepG2 cells were labeled with [^{3}H]glycerol in the presence of either BSA, BSA plus OA (0.4 mm), or BSA plus 30 mm glucose for 1 to 8 h, and the cell content of [^{3}H]TG was determined. As shown in **Fig. 2A**, glucose treatment caused no significant increase in [^{3}H]TG synthesis over control at any time point (P = NS, glucose vs. BSA). On the other hand, a time-dependent increase in [^{3}H]TG synthesis was observed when HepG2 cells were incubated with 0.4 mm OA ($P < 0.01$, OA vs. BSA at every time point). [^{3}H]TG secretion after 8 h labeling was not increased by glucose (Fig. 2B; P = NS, glucose vs. BSA). OA treatment significantly increased the secretion of [^{3}H]TG

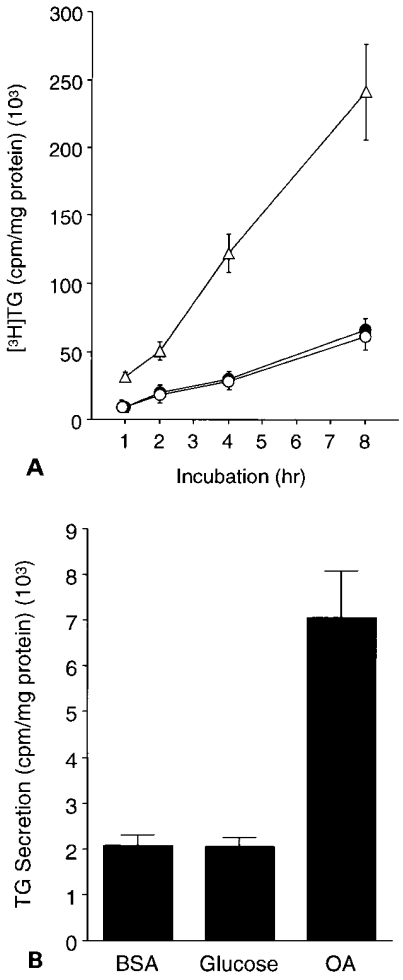

Fig. 2. Glucose is a poor substrate for TG synthesis in HepG2 cells. HepG2 cells were labeled with [^{3}H]glycerol in the presence of either BSA, BSA plus OA (0.4 mm), or BSA plus 30 mm glucose for up to 8 h, and the content of [^{3}H]TG in the cells was determined. As shown in panel A, glucose treatment (solid circle) caused no significant increase in [^{3}H]TG synthesis over control (open circle) at any time point (n = 3). On the other hand, a time-dependent increase in [^{3}H]TG synthesis was observed when HepG2 cells were incubated with OA (triangle) (n = 3). Secretion of [^{3}H]TG into the incubation medium after labeling for 8 h was identical in BSA-treated cells and in glucose-treated cells (panel B; n = 3). OA treatment resulted in significant increase in the secretion of [^{3}H]TG (panel B; n = 3). The results are shown as means ± SD.

over the 8-h incubation period. The results presented in Fig. 1B, together with those in Fig. 2B, also indicate that TG content per apoB particle (i.e., the density of apoB-containing lipoprotein particles) was not changed by glucose treatment.

Long-term incubation with glucose does not affect either secretion of apoB or synthesis of TG in HepG2 cells

All of the experiments above were of durations of 8 h or less. Although the effects of OA on TG synthesis and apoB secretion are clearly seen after very short incubation periods, the effects of glucose might require longer incubation periods. In order to determine the long-term effects of glucose incubation, we conducted an additional series of experiments. In the first experiment, cells were incubated with either BSA, BSA plus OA (0.4 mm), or BSA plus glucose (30 mm) for periods of 4 to 48 h in media containing either [^{3}H]leucine or [^{3}H]glycerol, and TG synthesis and apoB secretion were measured. It is clear in **Fig. 3A** that although labeled apoB secreted into the media from all three groups of cells increased for at least 24 h, the amount of apoB in the media of cells treated with glucose did not differ from apoB in the media of BSA-treated cells. In contrast, there was always much more apoB in the media of OA-treated cells than in the media of either BSA- or glucose-treated cells. Figure 3B depicts the mean results of three different experiments in which both 8-h and 24-h media samples were analyzed. Similarly, cellular TG synthesis increased during the first 24 h with much more synthesis in the OA-treated cells compared to either the BSA-treated and glucose-treated cells (Fig. 3C). The latter two groups showed the same TG synthesis.

These results indicated the unlike OA, long-term exposure to very high levels of glucose did not affect either apoB secretion or TG synthesis. We were concerned, however, that the tracers, added at the start of the incubation, may not have been representative of metabolic activities late in the long-term incubations. Additionally, as glucose would have been constantly metabolized over the course of the experiment (33), we could have underestimated the effects of "true" chronic hyperglycemia. These concerns were heightened by the plateauing of both apoB secretion and TG synthesis after the first 24 h of incubation. Therefore, we next conducted an experiment in which we changed the media every 8 h, adding new glucose and new radiolabeled leucine or glycerol. The results in **Fig. 4A** show that only OA stimulated apoB secretion during each 8 h of incubation with new media over a total incubation period of 24 h. The last three lanes of the gel show the results of pooling aliquots from each 8-h period; this gives a direct measure of total apoB secreted by each group of cells during the entire 24 h (with replenished media every 8 h). Glucose did not stimulate apoB secretion compared to BSA while OA did. Figure 4B depicts the

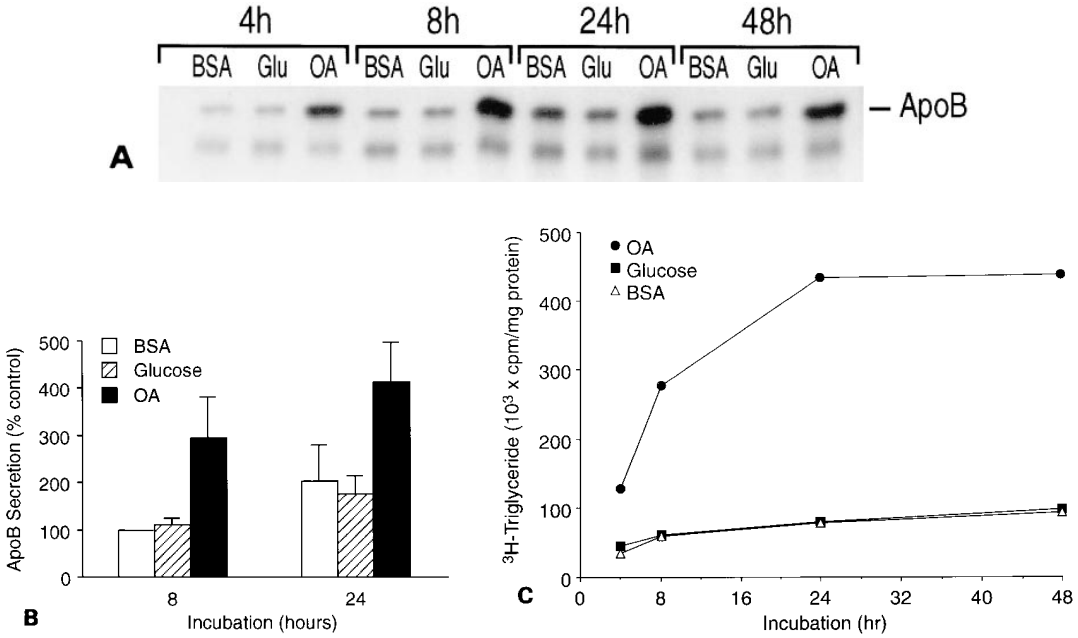

Fig. 3. Long-term incubation with glucose does not increase apoB secretion or TG synthesis in HepG2 cells. HepG2 cells were incubated with either BSA, BSA plus OA (0.4 mm), or BSA plus glucose (30 mm) for periods of 4 to 48 h in media containing either [^{3}H]leucine or [^{3}H]glycerol and apoB secretion and TG synthesis were measured on aliquots of media removed at 4, 8, 24, and 48 h. Panel A: ApoB secretion into the media increased in all three groups up to 24 h and then plateaued. OA treatment induced more secretion than either BSA or glucose treatment at every time point. This gel is representative of three experiments with similar time courses. Panel B: 8- and 24-h incubations were used in all three experiments and the individual gels were scanned using a Molecular Dynamic Densitometer. The data were averaged and are presented as means ± SD. OA-treated cells secreted more apoB than either BSA- or glucose-treated cells at both 8 and 24 h. Glucose treatment did not stimulate apoB secretion above that seen in BSA-treated cells. Panel C: TG synthesis increased in all three groups up to 24 h and then plateaued. OA-treated cells had much greater rates of TG synthesis at all time points; glucose did not stimulate TG synthesis at any time point compared to BSA. The data shown are representative of two experiments.

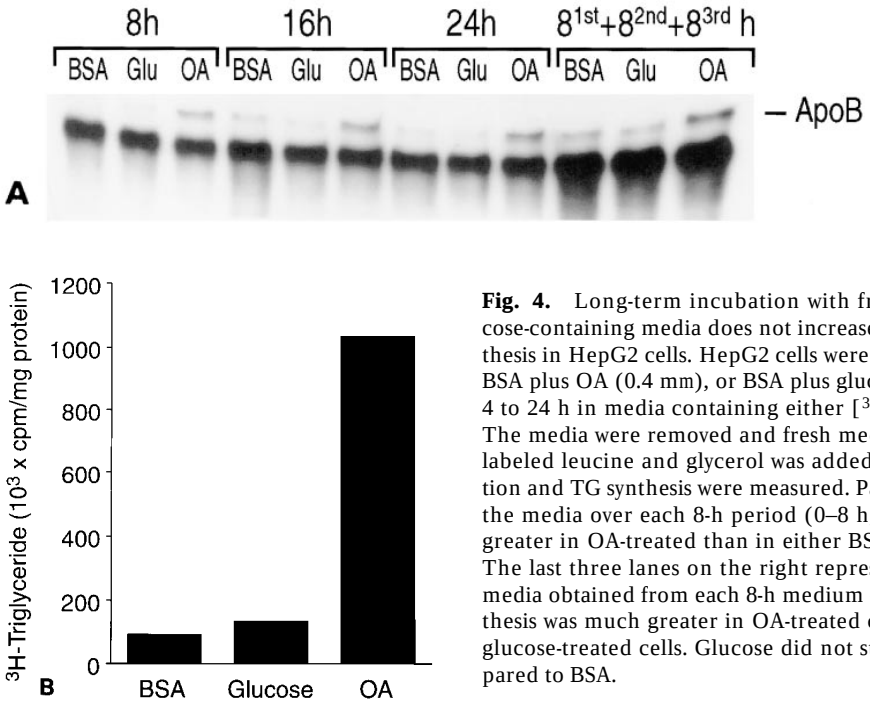

Fig. 4. Long-term incubation with frequently replenished glucose-containing media does not increase apoB secretion or TG synthesis in HepG2 cells. HepG2 cells were incubated with either BSA, BSA plus OA (0.4 mm), or BSA plus glucose (30 mm) for periods of 4 to 24 h in media containing either [^{3}H]leucine or [^{3}H]glycerol. The media were removed and fresh medium with new glucose and labeled leucine and glycerol was added every 8 h and apoB secretion and TG synthesis were measured. Panel A: ApoB secretion into the media over each 8-h period (0–8 h, 8–16 h, and 16–24 h) was greater in OA-treated than in either BSA- or glucose-treated cells. The last three lanes on the right represent the sum of aliquots of media obtained from each 8-h medium collection. Panel B: TG synthesis was much greater in OA-treated cells than in either BSA- or glucose-treated cells. Glucose did not stimulate TG synthesis compared to BSA.

data for TG synthesis over the 24 h of incubation (lysates from cells labeled with fresh leucine in fresh glucose every 8 h for 24 h total); only OA stimulated TG synthesis in the HepG2 cells.

Neither short- nor long-term incubation with glucose affected TG mass in HepG2 cells

Finally, we determined directly the effects of both short-term and long-term incubation with 30 mm glucose on the cellular mass of TG in the HepG2 cells. TG mass was not significantly increased after 8 h incubation with 30 mm glucose (**Fig. 5A**; $P > 0.05$, glucose vs. BSA). In contrast, TG mass was significantly increased after 8 h incubation with 0.4 mm OA ($P < 0.01$, OA vs. BSA). Essentially the same results were obtained when the cells were incubated with 30 mm glucose for 48 h (Fig. 5, panel B). Together these results clearly indicated that nonsignificant amounts of TG were formed de novo in HepG2 cells after glucose treatment.

DISCUSSION

ApoB-containing lipoproteins secreted from hepatocytes are precursors of the major apoB-containing lipoprotein,

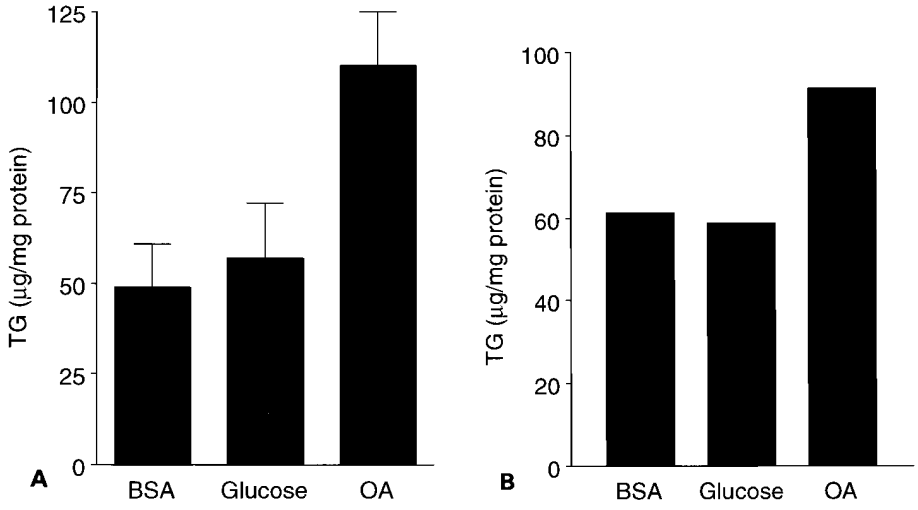

Fig. 5. Neither short-term nor long-term incubation with glucose-containing media increases cell TG mass in HepG2 cells. HepG2 cells were incubated in media containing BSA, BSA plus 0.4 mm OA, or BSA plus 30 mm glucose for either 8 h (panel A) or 24 h (panel B). Cells were extracted with hexane–isopropanol 3:2 and TG mass was determined. Consistent with the above TG synthesis results, TG mass was only slightly increased after 8 h incubation with 30 mm glucose (panel A; n = 6). OA treatment significantly increased TG mass (n = 6). Essentially the same results were obtained in two experiments carried to 24 h (panel B: representative results shown).

LDL, in the circulation (1–3, 34). It has been suggested that overproduction of apoB-containing lipoproteins from the liver is responsible for a large percentage of patients with hyperlipidemia (35–38). Thus, regulation of apoB-containing lipoprotein secretion from hepatocytes has been under extensive investigation, and HepG2 cells are widely used as a model for this purpose. It has been shown that the apoB gene is constitutively expressed in HepG2 cells (5), and significant changes in apoB secretion in several studies were not accompanied by any significant changes in apoB mRNA content. Some studies have reported that apoB mRNA is affected by certain stimuli, such as 25-hydroxycholesterol and thyroxine (39, 40). A large body of evidence, however, has convincingly shown that apoB secretion from HepG2 cells is regulated mainly at post-translational sites (5–12) where newly synthesized apoB molecules are either targeted for rapid intracellular degradation or secretion (41, 42).

As apoB is a large hydrophobic protein, it has to be incorporated into a lipoprotein particle consisting of a hydrophobic neutral lipid core (triglyceride and cholesteryl ester) covered by amphipathic lipids (phospholipids and free cholesterol) before secretion. Therefore, a major effort has been made to characterize the importance of lipids in the regulation of apoB secretion. Studies by Dixon et al. (21) demonstrated that OA increased secretion of apoB, and this was associated with a remarkable reduction of intracellular degradation of newly synthesized apoB. OA is a lipid precursor that has the potential to be incorporated into triglyceride, cholesteryl ester, and phospholipids. Exogenous lipoproteins were also shown to stimulate apoB secretion from HepG2 cells (22, 43), and in one study this was clearly linked to an increase in cell TG content (22). Based on these and other studies (28), we concluded that newly synthesized TG was the critical lipid-ligand for targeting of apoB to secretion.

The role of cholesteryl ester in the regulation of the secretion of apoB-containing lipoproteins from HepG2 cells has also been studied extensively (26, 32). For example, Cianflone et al. (26) reported that in OA-treated HepG2 cells, inhibition of either cholesterol synthesis with the HMG-CoAR inhibitor, lovastatin, or cholesteryl ester synthesis by the ACAT inhibitor, Sandoz 58-035, was associated with a decrease in apoB secretion. The ability of an ACAT inhibitor to reduce apoB secretion was confirmed by Musanti et al. (29), while Graham, Wood and Russell (30) demonstrated variable findings with different inhibitors. Taking several approaches, Wu et al. (22,28) demonstrated that in HepG2 cells, neither cholesteryl ester synthesis nor mass was associated with apoB secretion. Thus, inhibition of triglyceride synthesis by Triascin D, a specific inhibitor of fatty acyl CoA synthetase (44), significantly decreased apoB secretion from HepG2 cells treated with either BSA, OA, or exogenous VLDL; Triacsin D had no effect on cholesteryl ester synthesis. By contrast, nearly complete inhibition of cholesteryl ester formation by Sandoz 58-035 did not affect apoB secretion from HepG2 cells. In addition, increased cholesteryl ester synthesis and content in HepG2 cells transfected with HMG-CoA reductase cDNA was not associated with changes in apoB secretion from HepG2 cells. Consistent with Wu et al. (22, 28), a study by Furukawa and Hirano (27) reported that OA stimulation of apoB secretion was not associated with significant change in cholesteryl ester synthesis in HepG2 cells. More recently, Benoist and Grand-Perret (31) demonstrated that in HepG2 cells, newly synthesized TG was correlated with apoB secretion, and inhibition of ACAT was without effect. On the other hand, in vivo studies in miniature pigs, with either HMG-CoAR or ACAT inhibitors, have resulted in reduced apoB secretion into plasma (45, 46), and HMG-CoAR inhibitors appear to reduce hepatic secretion of apoB-containing lipoproteins in humans (35, 47, 48).

Using HepG2 cells as a model, Cianflone et al. (32) reported that treatment with glucose, a potential substrate for various lipids in cells, resulted in a significant increase in triglyceride synthesis but no change in apoB secretion. Cholesteryl ester was not changed by glucose treatment in that study. The authors used these results to support their view that CE synthesis, but not TG synthesis, was necessary for increased apoB secretion. Additionally, their findings of a dissociation of TG synthesis and secretion from apoB secretion appeared to provide a cellular basis for the long-standing, in vivo, observation that carbohydrate-induced hypertriglyceridemia in humans is associated with increased secretion of VLDL TG without changes in VLDL apoB secretion (49). The results of Cianflone et al. (32) also suggested that triglyceride derived from different precursor sources (glucose vs. fatty acids) could have different effects on the assembly and secretion of apoB secretion from HepG2 cells. In the present studies, we attempted to address these issues by comparing the effects of OA and glucose on apoB secretion, and on both TG synthesis and secretion during either short-term or long-term incubations of HepG2 cells. The results from the our studies confirmed the finding of Cianflone et al. (32) that glucose does not stimulate apoB secretion from HepG2 cells. However, in contradiction to Cianflone et al. (32), we found that glucose did not stimulate TG synthesis or secretion. Our present results suggest that the inability of glucose to increase apoB secretion from HepG2 cells is due to the inability of glucose to sufficiently increase triglyceride synthesis.

It is difficult to explain the differences between the present study and that by Cianflone et al. (32), who reported that glucose was efficiently converted to TG in HepG2 cells. In both studies, the cells were cultured in MEM. They used 50 mm glucose and we used 30 mm glucose, but we also studied the effects of higher concentrations of glucose and observed the same results. They incubated for 24 h; we conducted studies of 4 h to 48 h duration. Their study design utilized [^{3}H]glucose as a tracer, and the specific activity of [^{3}H]TG was used to estimate synthesis of TG from glucose. However, [^{3}H]glucose has to be initially metabolized to [^{3}H]acetyl-CoA before being incoporated into [^{3}H]fatty acids and finally, into [^{3}H]TG. Unless one knows the fraction of label that is actually converted into TG, it is impossible to calculate the amount of TG that is derived from glucose. Thus, the TG

mass generated from [^{3}H]glucose could have been overestimated in their study. On the other hand, it does appear that Cianflone et al. (32) also measured cell TG directly and found a glucose-induced increase. We do not have, at this time, a basis for the differences in our findings.

A review of other studies of the effects of glucose on apoB secretion from HepG2 cells also shows variable findings. Dashti, Williams, and Alaupovic (7) reported that 20 mm glucose had no effect on apoB secretion. There were no data for TG synthesis or cell TG mass reported in that study, and length of incubation with glucose was not stated. Surprisingly, those authors did not see an effect of OA on apoB secretion. Semenkovich, Coleman, and Goforth (50) observed that apoB secretion was not affected significantly after 12 h of incubation in 25 mm glucose; no TG data were provided. On the other hand, Arrol et al. (51) found that increasing glucose treatment for 24 h resulted in increased apoB secretion. Unfortunately, no data for TG synthesis or cell mass was given by Arrol et al. (51). Finally, Kempen et al. (33) reported that glucose increased apoB secretion after incubations of 48 and 72 h, but not after a 24-h incubation. Kempen et al. (33) stated that cell TG content was also increased after prolonged glucose treatment and, most importantly, demonstrated that inhibition of lipogenesis by either 8-Br-cyclic AMP or 5-(tetradecyloxy)-2-furancarboxylic acid inhibited apoB secretion. Of interest, Kempen et al. (33) conducted their studies in DMEM and found that when the HepG2 cells were incubated in MEM, basal rates of apoB secretion were higher, but the response to glucose, although still significant, was blunted. Kempen et al. (33) commented that Dashti et al. (7) and Cianflone et al. (32) both used MEM, whereas Arrol et al. (51) used DMEM. Our use of MEM in the present studies could account, at least in part, for our inability to reproduce the effects seen by Kempen et al. (33). However, both our present data (negative for glucose effects on both TG synthesis and apoB secretion), and the data of Kempen et al. (positive for effects of glucose on both TG synthesis and apoB secretion) demonstrate concordance between glucose effects on TG synthesis and apoB secretion. This is in accord with our prior findings for OA-induced apoB secretion (21, 22, 28).

In searching for a mechanism for the lack of stimulation of TG synthesis by glucose, we found that very little glucose is converted into either glycerol or, more importantly, fatty acids used for TG synthesis (data not shown). These findings are in accord with two studies in humans conducted using stable isotopes. Hellerstein et al. (52) demonstrated that very little de novo synthesized fatty acid (<2%) is incorporated into VLDL TG during either the fasting or the fed state. More recently, Aarsland, Chinkes, and Wolfe (53) confirmed that finding in the basal state, and found that even after 4 days of a hypercaloric high carbohydrate diet, de novo synthesized fatty acids made up only about 20% of secreted VLDL TG-fatty acids. Both authors suggested that during high carbohydrate feeding of humans, increased TG synthesis may result from shunting of fatty acids into TG synthesis concomitant with increased use of glucose for oxidation.

In conclusion, our new experimental results with glucose provide further evidence that, in the HepG2 cell model of assembly and secretion of apoB-containing lipoproteins, TG synthesis is tightly linked to apoB secretion. These results are in accord with those of Kempen et al. (33) who under somewhat differing conditions (DMEM rather than MEM in the culture media) found parallel increase in both apoB and TG secretion and with the recent report from Benoist and Grand-Perret (31). This model still leaves us without an explanation for the dissociation of apoB and TG secretion observed in vivo, in humans fed high carbohydrate diets, by Melish et al. (49). We would like to speculate about a potential explanation: Studies by Sparks and coworkers (54–56) in rat hepatocytes and rat liver cell lines have demonstrated that increased levels of insulin can simultaneously increase TG synthesis but decrease apoB secretion; the latter effect occurs via increased post-translational degradation of apoB. Lewis et al. (57, 58) and Malstrom et al. (59, 60) recently confirmed the findings in liver cells by demonstrating that hyperinsulinemia can reduce VLDL apoB secretion in humans. We propose, therefore, that with chronic delivery of large quantities of glucose to the liver (during high carbohydrate diets), in the presence of hyperinsulinemia, fatty acids will be preferentially incorporated into TG, TG synthesis will be increased, and the initial steps in the assembly of apoB into VLDL will be stimulated. However, hyperinsulinemia, by increasing post-translational degradation of apoB, will favor the generation of larger, TG-rich VLDL carrying more TG per particle.

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