Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression

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Abstract The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein that is required for the assembly and secretion of apoB-containing lipoproteins. In this study, four factors that modulate lipid and lipoprotein metabolism were tested for their ability to regulate MTP levels in HepG2 cells. Of the factors tested, only insulin ($\geq 10^{-9}$ M), and high concentrations of glucose (>30 mM) were found to decrease MTP large subunit mRNA levels. Oleate and glucagon had no effect on MTP mRNA levels. The insulin effect was dose- and time-dependent and was mediated through the insulin receptor. In addition, insulin also decreased protein disulfide isomerase (the small subunit of MTP) mRNA levels, although to a lesser extent. Due to the slow turnover rate of MTP ($t_{1/2}$ = 4.4 days), short-term insulin treatment (24 h) did not change MTP activity levels, indicating that the regulation of MTP mRNA levels by insulin is unrelated to insulin's acute inhibition of apoB secretion in HepG2 cells. In summary, MTP mRNA levels are acutely regulated by insulin in HepG2 cells; however, sustained changes in MTP mRNA levels would be required to affect MTP protein levels.—**Lin, M. C. M., D. Gordon, and J. R. Wetterau.** Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. *J. Lipid Res.* 1995. **36:** 1073–1081.

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It is well established that elevated levels of plasma cholesterol lead to premature coronary heart disease. The majority of this cholesterol is transported within low density lipoproteins (LDL). Abnormal cholesterol levels may result from either increased production or decreased removal of LDL from the blood (1, 2). Whereas elevated LDL levels resulting from impaired catabolism through the LDL receptor pathway are well characterized (3, 4), the various mechanisms involved in the overproduction of LDL and its precursor, very low density lipoprotein (VLDL), are only beginning to be elucidated (5). In addition to a requirement for apolipoprotein B (apoB), the major structural protein of VLDL and LDL, the availability of triglyceride, phospholipid, and possibly cholesteryl ester plays an important role in regulating lipoprotein production in cell culture models (for recent reviews, see refs. 6–11).

Microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein composed of a unique large subunit of molecular weight 97,000 and the multifunctional enzyme, protein disulfide isomerase (PDI) (12–15). It is found in the lumen of microsomes isolated from liver and intestine (16). Recent studies have demonstrated that MTP is essential for VLDL synthesis and secretion. An absence of functional MTP causes abetalipoproteinemia (17–19), a rare autosomal recessive disease characterized by a defect in the assembly and secretion of plasma VLDL and chylomicrons (5). Due to its central role in lipoprotein assembly, one may speculate that MTP levels affect lipoprotein production.

We have previously investigated MTP regulation in hamsters and found that MTP mRNA levels are modulated by dietary manipulation. A high-fat diet caused an increase in both the hepatic and intestinal MTP large subunit mRNA levels, while a high-sucrose diet caused an increase in the hepatic MTP large subunit mRNA levels (20). In these studies, positive correlations between MTP mRNA levels and plasma cholesterol and triglyceride levels were observed. To identify the specific factors that may be affecting the regulation of MTP in vivo, an investigation of MTP regulation in HepG2 cells, a human liver-derived cell line, was initiated. These cells, which synthesize and secrete apoB-containing lipoproteins, have been used extensively to investigate factors that regulate the synthesis of lipoproteins and their components (21–24).

Abbreviations: MTP, microsomal triglyceride transfer protein; PDI, protein disulfide isomerase; apoB, apolipoprotein B; VLDL, very low density lipoprotein; LDL, low density lipoprotein; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium with 5 mM glucose; SDS, sodium dodecyl sulfate; ELISA, enzyme-linked immunosorbent assay; bp, base pairs; IGF-1, insulin-like growth factor-1.

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MATERIALS AND METHODS

Materials

A monoclonal anti-human insulin receptor antibody that recognizes the extracellular domain of the human insulin receptor, monoclonal anti-human apoB antibody, and goat anti-human apoB polyclonal antibody were purchased from Biodesign International (Kennebunkport, ME). Cell culture media were purchased from Gibco (Grand Island, NY). Bovine insulin, glucagon (extracted from a mixture of bovine and porcine pancreas), fatty acid-free bovine serum albumin (BSA), glucose, and sodium oleate were obtained from Sigma (St. Louis, MO). [^{35}S]methionine was supplied by DuPont New England Nuclear (Boston, MA).

Cell culture and experimental conditions

HepG2 cells were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium with 5 mM glucose (DMEM) supplemented with 10% fetal bovine serum under standard culture conditions (5% CO_2, 37°C). In a typical experiment, cells were seeded into 100-mm culture dishes and allowed to grow to 80% confluence. Cells were then acclimated in control medium (serum-free DMEM plus 3% BSA) for 24 h. Immediately prior to the experiment, cells were washed twice with phosphate-buffered saline and 10 ml experimental media was added. Cells were then incubated at 37°C for the indicated time, harvested by trypsin-EDTA digestion, and recovered for analysis. Experimental media were prepared by adding hormone or nutrient supplements to the control medium. To maintain the concentration of the factors tested, cells were replenished with fresh experimental media every 6 h. Sodium oleate was complexed to 3% BSA according to the method described by Dixon, Furukawa, and Ginsberg (23).

RNA isolation

Total RNA was isolated by the guanidinium thiocyanate method (TRISOLV, Cinna Biotex Labs., Houston, TX). The integrity of the RNAs was verified by 1% agarose, 2.2% formaldehyde gel electrophoresis and ethidium bromide staining. Only RNA preparations that showed distinct major bands of 18 and 28S r-RNA were analyzed further.

Quantitation of MTP large subunit and PDI mRNA levels by DNA excess solution hybridization assays

Fifty bp and 35 bp oligonucleotide probes complementary to human MTP large subunit cDNA (64–113 bp downstream of the translation start site) (18) or human PDI (−17 to +18 bp corresponding to the translation start site) (25) were synthesized and labeled on the 5' end with T4 polynucleotide kinase and [γ-^{32}P]ATP. Samples of HepG2 total cellular RNA (50–200 μg) were adjusted to 200 μg with yeast *t*-RNA and hybridized with 240 pg radiolabeled probe at 68°C for 48 h in the presence of 2× sodium chloride/sodium citrate buffer, 40 mM piperazine-N,N'-bis(2-ethanesulfonic acid), pH 6.4, and 10 μg salmon sperm DNA in a total volume of 100 μl. Unhybridized probe and excess RNA were removed by digestion at 37°C for 30 min with 60 units of S1 nuclease as described previously (20). To the digested mixture, 200 μg salmon sperm DNA was added and the protected RNA:DNA fragments were precipitated with 9% trichloroacetic acid at 0°C for 10 min and collected on Whatman GF/C filters. Radioactivity was quantitated by liquid scintillation counting. In the blank (200 μg yeast *t*-RNA), less than 0.4% of total radioactive probe remained undigested. After subtracting the blank, relative mRNA levels in each sample were calculated as the % of the radioactive probe resistant to S1 nuclease digestion per 100 μg of total cellular RNA. The results obtained were within the linear range of the assay (1–20% S1 protection). The data were then normalized to the average of the control cells in the experiment.

MTP activity assay

Cell pellets were homogenized, treated with deoxycholate to release MTP from the microsomes, and MTP activities were quantitated by measuring the rate of transfer of ^{14}C-labeled triglyceride from donor small unilamellar vesicles to acceptor small unilamellar vesicles as described previously (17). The percent triglyceride transferred from donor to acceptor membranes per h per mg of cell protein was calculated. The results were normalized to the average of the control cells in each experiment. All of the samples in a single experiment were assayed simultaneously to facilitate comparison of the results.

ApoB secretion rate

Human apoB was quantitated by a sandwich ELISA using a monoclonal anti-human apoB antibody, goat anti-human apoB polyclonal antibody, and rabbit anti-goat IgG conjugated to alkaline phosphatase as primary, secondary, and tertiary antibodies, respectively. The amount of apoB was quantitated using the *p*-nitrophenyl-phosphate colorimetric reaction. Samples of unknown concentration were measured in triplicate against a standard curve of purified human LDL. Concentrations of standard ranged from 1.25 to 40.0 ng LDL protein in a twofold dilution series. Within this concentration range, the absorbance response of the assay was linear. Unknowns were diluted to concentrations within the linear range.

MTP protein half life

Subconfluent cultures of HepG2 cells in 6-well plates were pulse-labeled with [^{35}S]methionine as follows. Cells

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were treated with methionine-free, serum-free RPMI-1640 medium for 30 min, followed by labeling medium (methionine-free, serum-free RPMI 1640 containing 190 μCi/ml [^{35}S]methionine) for 15 min, followed by chase medium (RPMI 1640, 50 mM methionine, 10% fetal bovine serum) for the indicated times. At each time point, duplicate cultures were processed for immunoprecipitation by rinsing three times with phosphate-buffered saline and quantitatively scraping the cells into 1.5 ml homogenization buffer (0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.005 M sodium ethylenediaminetetraacetate, 0.0625 M sucrose, 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.001 M phenylmethylsulfonyl fluoride, 0.001 M benzamidine, 50 mg/ml aprotinin, 0.1 mg/ml pepstatin, and 0.1 mg/ml leupeptin). After 16 h incubation at 4°C with gentle agitation, the cellular debris was removed by centrifugation at 10,000 *g* for 10 min. Dilutions of the cell supernatant were immunoprecipitated with 10 μl of either polyclonal anti-bovine MTP antiserum (13) or pre-immune serum. After 16 h incubation at 4°C, antibody-antigen complexes were isolated using protein A-Sepharose beads. The immunoprecipitated proteins were dissolved in sample buffer (0.125 M Tris, pH 6.8, 1% sodium dodecyl sulfate (SDS), and 1% beta-mercaptoethanol final concentration), and analyzed on a 4–12% SDS polyacrylamide gradient gel. Radioactivity in the band corresponding to the large subunit of MTP was quantitated by direct counting on a Betascope radioimage analyzer (Betagen, Boondock, MA). Background from the matched preimmune samples was subtracted and a decay curve was calculated. The signal:noise ratio ranged from 6.8 at time = 0 to 3.6 at time = 8 days. When the immunoprecipitation was performed with pre-immune serum, a band corresponding to the MTP large subunit was not apparent.

Statistical analysis

Data were pooled from experiments performed on different days and an analysis of variance model was used to compare groups. Data are presented as mean ± SD. Significant differences were determined by using an unpaired Student's *t*-test. For multiple comparisons, a Bonferroni-adjusted two-tailed Student's *t*-test was used.

RESULTS

Regulation of MTP large subunit mRNA levels

To identify factors that regulate MTP expression, HepG2 cells were grown for 24 h in control medium (serum-free DMEM, 3% BSA) or control medium supplemented with glucose (50 mM final concentration), oleic acid (0.8 mM), insulin (1×10^{-7} M), or glucagon (10^{-7} M). As shown in **Table 1,** insulin and glucose decreased MTP large subunit mRNA levels 31 and 23%, respectively, while oleate and glucagon had no effect. In these experiments, the concentration of the factors tested was maintained by replacing the media with fresh media every 6 h. The collected media were retained, pooled, and the total apoB secreted into the media was quantitated by an ELISA. Oleate addition to the growth media increased apoB secretion, while insulin decreased apoB secretion. Similar effects on apoB secretion have been reported by other investigators (for recent review, see ref. 11). Glucose and glucagon had no effect on apoB secretion.

Effect of glucose on MTP large subunit mRNA levels

HepG2 cells were grown in the presence of 5–50 mM glucose for 24 h and the effects on MTP mRNA levels were determined. At glucose concentrations ranging from 5 (the concentration in control media) to 30 mM, there was no effect on MTP mRNA levels. Fifty mM glucose resulted in a moderate trend towards decreased MTP mRNA levels (**Fig. 1A**). To determine the time course of the glucose effect, HepG2 cells were grown in either 5 mM glucose or 50 mM glucose for 6, 12, and 24 h. Cells grown in the 5 mM glucose maintained constant MTP mRNA levels for 24 h (Fig. 1B). Fifty mM glucose caused

TABLE 1. Effects of insulin, glucagon, glucose, or oleate on MTP mRNA levels and apoB secretion rates

Hormones or Nutrients	MTP mRNA Levels	ApoB Secretion Rate
	% of control	
Insulin, 1×10^{-7} M (9)	69 ± 13[a]	57 ± 16[a]
Glucagon, 1×10^{-7} M (6)	105 ± 26	87 ± 26
Glucose, 50 mM (6)	77 ± 12[a]	108 ± 6
Oleate, 0.8 mM (9)	96 ± 15	228 ± 23[a]
Control (9)	100 ± 10	100 ± 8

HepG2 cells were grown in the presence of hormone or nutrient supplements for 24 h. To maintain the concentrations of the hormones or nutrients, media were replaced with fresh media every 6 h. The apoB secretion rate and MTP mRNA levels were determined as described in Materials and Methods. Data were normalized against the average value obtained from cells grown in the control media in the same experiment and presented as percentage of control. Numbers in parentheses represent the total number of plates used in two to three separate experiments, (three plates/experiment). Values represent means ± SD.

[a] $P < 0.05$, significantly different from the control group as judged by Bonferroni adjusted *t*-test.

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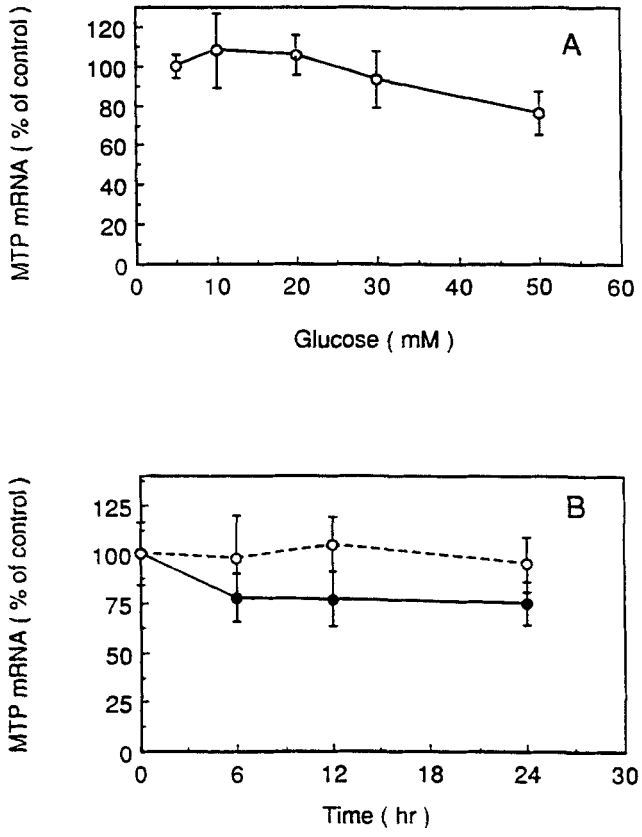

Fig. 1. Effect of glucose on MTP large subunit mRNA levels. Panel A: cells were incubated for 24 h in control media (5 mM glucose) or control media supplemented with additional glucose to a final concentration of 10–50 mM. At the end of the incubation, cells were harvested, total RNA was extracted, and MTP mRNA levels were determined as described in Materials and Methods. MTP mRNA levels were normalized against the average value from cells incubated in control media. Panel B: cells were incubated with either control media (open circle) or control media supplemented with glucose to a final concentration of 50 mM (close circle) for the time indicated. MTP mRNA levels were determined as described in Materials and Methods, then normalized against the average value from cells harvested at the beginning of the experiment. Data are expressed as means ± SD (n = 3).

a trend towards decreased (approximately 25%) MTP mRNA levels within 6 h. This decrease was maintained for an additional 18 h. However, none of the effects of 50 mM glucose were significant unless the results from two experiments were combined for an n = 6 (Table 1).

Effect of insulin on PDI and MTP large subunit mRNA levels

It has been previously reported that insulin decreases hepatic PDI mRNA levels in streptozotocin-induced diabetic rats (26). These results, in conjunction with our results, suggest that both MTP subunits may be coordinately regulated by insulin. To test this possibility, the effect of 10^{-7} M insulin on both PDI and MTP large subunit mRNA levels was investigated (**Fig. 2**). An 80% decrease in MTP large subunit mRNA levels was observed 3 and 6 h after initiating the insulin treatment. At 12 and 24 h, MTP large subunit mRNA levels gradually increased to 40 and 60% of the control levels, respectively. In these experiments, insulin concentrations were maintained by replacing the medium every 6 h, thus the reduced effect of insulin cannot be attributed to a decreased insulin concentration in the culture medium over time (24). The pattern observed for PDI was similar to that observed for the MTP large subunit, except the magnitude of the insulin effect was smaller.

To investigate whether the insulin effect is mediated through the insulin receptor or the insulin-like growth factor-1 (IGF-1) receptor, the concentration dependence of the insulin effect was compared to that of IGF-1. HepG2 cells were grown in either control medium or control medium supplemented with various concentrations of insulin or IGF-1 for 6 h (a time when the maximal insulin effect is observed) and the effect on MTP large subunit mRNA levels was determined (**Fig. 3**). At 1×10^{-9} M insulin, a significant 30% decrease in MTP mRNA levels was observed. A maximal 80% decrease in message levels was observed at 1×10^{-8} M insulin. Although IGF-1 also decreased MTP mRNA levels in a dose-dependent manner, at least 10-fold higher concentrations were required for the same effect, suggesting that the insulin effect is mediated through the insulin receptor.

Insulin treatment caused a dose-dependent decrease in PDI mRNA levels in HepG2 cells; however, a significant 20–50% decrease was observed only at 1×10^{-8} to

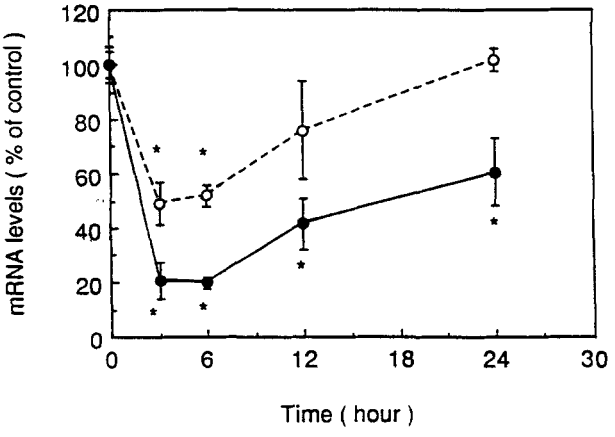

Fig. 2. Time course of insulin effect on PDI and MTP large subunit mRNA levels. Cells were incubated in either control media or control media supplemented with 1×10^{-7} M insulin for the time indicated. PDI (open circle) or MTP large subunit (solid circle) mRNA levels were determined and normalized against the average values obtained from cells grown in the control media and harvested at the same time. Under the control culture condition at 3, 6, 12, and 24 h, PDI and MTP large subunit mRNA levels were 100 ± 16, 100 ± 14, 109 ± 17, 102 ± 11 and 100 ± 6, 99 ± 23, 105 ± 15, 95 ± 15 percent the zero time value, respectively. Data are expressed as means ± SD (n = 3). Asterisks denote $P < 0.05$, significantly different from the control values.

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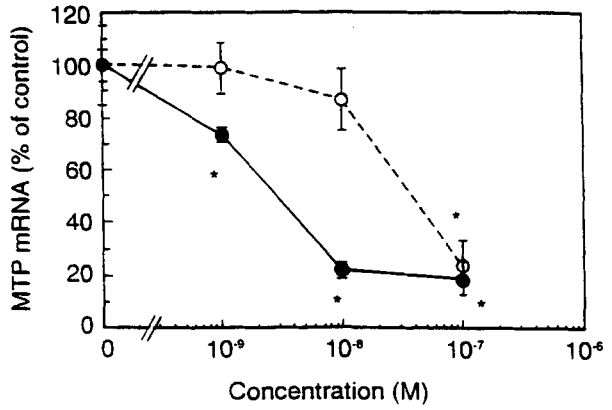

Fig. 3. Effect of insulin or IGF-1 concentration on MTP large subunit mRNA levels. Cells were incubated in control media or control media supplemented with various concentrations of either insulin (solid circle) or IGF-1 (open circle) for 6 h. MTP mRNA levels were determined and then normalized against the average value obtained from cells grown in the control media. Data are expressed as means ± SD (n = 3). Asterisks denote $P < 0.05$, significantly different from the control values.

1×10^{-7} M concentrations, respectively (**Fig. 4**). IGF-1 at 1×10^{-7} M also caused a 30% decrease in PDI mRNA levels. When compared to the regulation of the MTP large subunit, the regulation of PDI is less sensitive to insulin.

Monoclonal anti-human insulin receptor antibodies that specifically recognize the extracellular domain of the insulin receptor have been shown to initiate the insulin signal transduction pathway by binding to the receptor (27, 28). This effect was utilized to further investigate whether the insulin effect is mediated through the insulin receptor. As shown in **Fig. 5**, the antibody that recognizes the extracellular domain of the insulin receptor at a 1:5000 dilution caused a significant 60% decrease in MTP large subunit mRNA levels when compared to control levels. Higher antibody concentrations (1:1000 or 100 dilutions) further decreased MTP large subunit mRNA levels to less than 10% of control levels. The ability of anti-insulin receptor antibodies to mimic the insulin effect is consistent with the insulin effect being mediated through the insulin receptor. Anti-insulin receptor antibodies also decreased PDI mRNA levels (Fig. 5). The maximal decrease observed at 1:1000 or 100 antibody dilutions was only 50%, consistent with PDI mRNA levels being less responsive to insulin than the MTP large subunit mRNA levels.

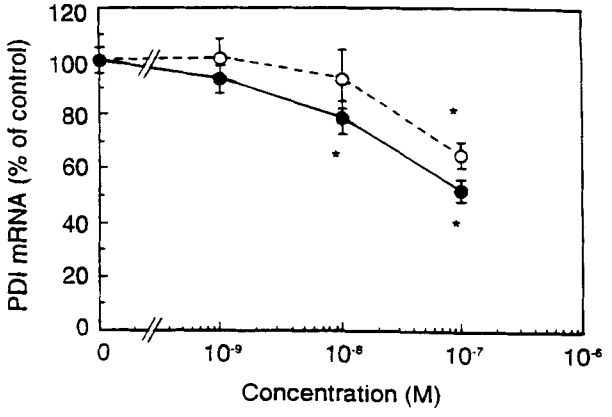

Fig. 4. Effect of insulin or IGF-1 concentration on PDI mRNA levels. Cells were incubated in control media or control media supplemented with various concentrations of either insulin (solid circle) or IGF-1 (open circle) for 6 h. PDI mRNA levels were determined and normalized against the average value obtained from cells grown in the control media. Data are expressed as means ± SD (n = 3). Asterisks denote $P < 0.05$, significantly different from the control values.

Half life of MTP

To determine the effect of changes in MTP mRNA levels on MTP activity levels, the effect of insulin, glucose, oleate, and glucagon treatment on MTP activity levels was investigated. None of the four treatments affected MTP activity levels even though insulin, and to a lesser extent, glucose decreased MTP mRNA levels (**Table 2**). These somewhat surprising results indicated that within the time frame of the experiment, MTP activity levels are not controlled by gene expression.

The discrepancy between the regulation of MTP mRNA levels and MTP activity levels in HepG2 cells may be explained if the MTP protein has a long half-life. To determine the half-life of MTP, total HepG2 protein was pulse labeled with [^{35}S]methionine and then chased for varying times. **Figure 6** shows the decay curve over an

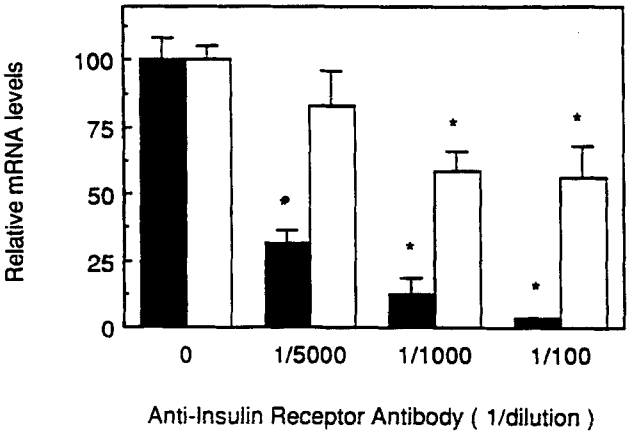

Fig. 5. Effect of anti-insulin receptor antibody on PDI and MTP large subunit mRNA levels. Cells were incubated for 6 h in control media supplemented with 1:5000 to 1:100 dilutions of a monoclonal antibody that specifically recognizes the extracellular domain of the insulin receptor, and then the PDI (open bar) or MTP large subunit (solid bar) mRNA levels were determined. The values were normalized against the average value obtained from cells grown in the control media alone. Data are expressed as means ± SD (n = 3). Asterisks denote $P < 0.05$, significantly different from the control values.

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8-day chase period. Assuming first order kinetics, the calculated half-life of MTP is 4.4 days. Thus, in a 24-h experiment, a 30–80% decrease in mRNA levels should have a minimal effect on MTP protein if there are no other regulatory mechanisms present. The long half-life for the MTP large subunit is consistent with our observation that acute modulation of MTP mRNA levels had no effect on MTP activity levels.

DISCUSSION

In this study, various factors that affect lipid and lipoprotein metabolism were tested for their ability to regulate MTP in HepG2 cells. A modest decrease in MTP large subunit mRNA levels was observed when 50 mM glucose was added to HepG2 growth media. No effect of glucose on MTP activity or apoB secretion was observed. Cianflone et al. (29) have reported similar findings for apoB secretion. In their study, an increase in triglyceride synthesis and secretion into the media was observed when HepG2 cells were grown in glucose concentrations of 30 mM or higher. It was concluded that glucose does not affect the number of lipoproteins secreted, but rather, their composition. Normal human fasting plasma glucose levels are around 5 mM. Glucose levels shown to affect MTP mRNA in HepG2 cells (50 mM) are only found in very severe diabetic subjects. These results suggest that glucose plays little or no role in regulating hepatic MTP in vivo.

Physiological (1×10^{-9} M) and higher concentrations of insulin decreased MTP large subunit mRNA levels within 6 h in a concentration-dependent fashion. Insulin also decreased PDI mRNA levels; however, the effect was not as dramatic as that observed for the MTP large subunit. Prolonged treatment of HepG2 cells with insulin (more than 6 h) decreased the magnitude of the insulin effect. This may have resulted from an insulin-stimulated down-regulation of the insulin receptor (30, 31) or the generation of an MTP mRNA stimulatory factor(s). Thus, MTP is another example of a protein involved in lipid metabolism that has its gene expression regulated by insulin. Other such proteins include lipoprotein lipase (32–34) and fatty acid synthetase (35).

Two approaches were used to investigate the signaling pathway for the insulin response in HepG2 cells. A monoclonal anti-insulin receptor antibody that binds the extracellular domain of the receptor decreased MTP large subunit message levels, indicating that the insulin effect is mediated through the insulin receptor. This was further supported by comparing the insulin response to an IGF-1 response. Insulin and IGF-1 share sequence homology and each cross-reacts with the others' receptor, but with a significantly lower affinity (36). HepG2 cells express insulin, IGF-1, and insulin/IGF-1 hybrid receptors (37). A decrease in MTP large subunit mRNA levels was observed at insulin concentrations at least tenfold lower than the IGF-1 concentrations required to see a comparable response. This is consistent with the insulin regulation of MTP large subunit mRNA being mediated mainly by the insulin receptor rather than the IGF-1 or insulin/IGF-1 hybrid receptor which, like the IGF-1 receptor, also has a higher affinity for IGF-1 (36). PDI mRNA levels were also

TABLE 2. Effect of insulin, glucagon, glucose, or oleate on MTP activity levels

Hormones and Nutrients	MTP Activity
	% of control
Insulin, 1×10^{-7} M (9)	106 ± 19
Glucagon, 1×10^{-7} M (3)	118 ± 6
Glucose, 50 mM (6)	110 ± 11
Oleate, 0.8 mM (9)	88 ± 25
Control (9)	100 ± 14

HepG2 cells were incubated in media supplemented with hormones or nutrients for 24 h. To maintain the concentrations of the hormones or nutrients, media were replaced with fresh media every 6 h. At the end of the incubation, cells were harvested and cellular MTP activity was determined as described in Materials and Methods. MTP activities were expressed as percentage of the activity obtained from the cells grown in the control media in a given experiment. Numbers in parentheses represent the total number of plates used in one to three separate experiments, (three plates/experiment). Values represent means ± SD.

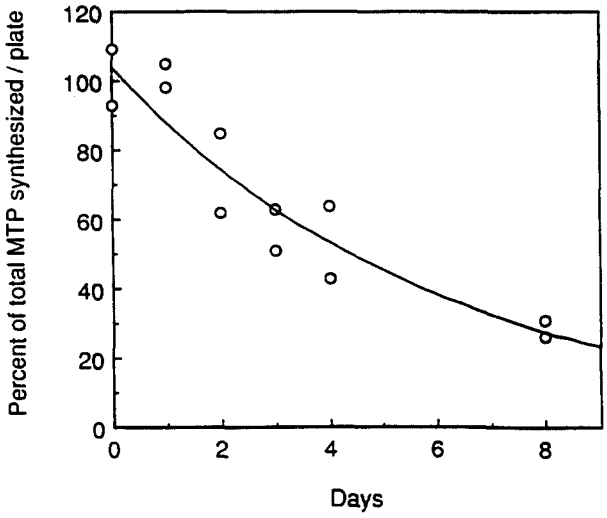

Fig. 6. Decay curve of [^{35}S]MTP large subunit in HepG2 cells. Multiple plates of cells were pulsed with [^{35}S]methionine for 15 min and chased for various times up to 8 days. [^{35}S]MTP large subunit per plate was determined at the times indicated as described in Materials and Methods, and normalized against the [^{35}S]MTP large subunit at the beginning of the chase (time = 0). Each circle represents data from a single plate and duplicate plates were used at each time point. Using a plot of time in days (x) versus log of the fraction of radiolabeled MTP large subunit remaining (y), an MTP half-life of 4.4 days was determined. This linear plot was described by equation $y = 0.017 - 0.072\ x$ and had an r of 0.95.

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decreased by the anti-insulin receptor antibody. In contrast to the MTP large subunit, the IGF-1 and insulin dose responses were similar for PDI. This latter observation may, in part, be related to a poor PDI response to insulin and the possibility that PDI responds to IGF-1.

Insulin may exert its effect on PDI or MTP large subunit mRNA levels by decreasing either gene transcription or mRNA stability. Insulin has been reported to regulate PDI at the transcriptional level in rats (26). Using various MTP large subunit promoter/reporter gene constructs, Hagan et al. (38) found that the MTP large subunit promoter is negatively regulated by insulin. However, the effect was observed at insulin concentrations almost two orders of magnitude higher than the concentrations at which we observed effects on the expression of the endogenous gene. Although the reason for this discrepancy is not clear, it may be related to the temporal nature of the insulin effect. The promoter analysis was done after 48 h insulin treatment. When we determined the insulin dose response after 24 h insulin treatment (data not shown), the dose–response curve was shifted to insulin concentrations over tenfold higher than that observed for the dose–response curve determined at 6 h (Fig. 3). Lower concentrations of insulin may be sufficient for a promoter response if shorter insulin treatments are used. These findings suggest that the endogenous MTP large subunit gene is regulated at the transcriptional level.

Of the four compounds tested for their ability to modulate MTP activity in HepG2 cells, none, including glucose and insulin which decreased MTP mRNA levels, affected MTP activity levels. The apparent discrepancy between the regulation of MTP mRNA and activity levels suggested that within the time frame of the experiment (24 h), MTP protein levels were not controlled by MTP mRNA levels. The half-life of the MTP large subunit is 4.4 days, indicating that HepG2 cells would need sustained changes in mRNA levels to affect MTP protein levels. PDI in rat liver has been reported to have a half-life of 7 days and thus, like MTP, is probably not acutely regulated by insulin (39). Although insulin and oleate did not affect MTP activity levels in HepG2 cells, they did affect apoB secretion significantly. These findings indicate that, under the conditions investigated in this study, MTP regulation does not play a role in the acute regulation of lipoprotein production.

Insulin plays an important role in regulating lipoprotein production. Acute studies in humans (40) have shown that insulin administration decreases hepatic secretion of VLDL. Insulin treatment has also been reported to decrease apoB secretion rates in hepatocytes (41–45) by directly regulating apoB translation rates (41, 42). Our results indicate that this is by a mechanism independent of a change in MTP levels. It has been reported that type II diabetics have increased apoB secretion rates (reviewed in ref. 46). This, in part, has been attributed to the lack of the normal down-regulation of apoB synthesis by insulin due to hepatic insulin resistance. By analogy, an impaired insulin response caused by hepatic insulin resistance should also result in increased MTP levels. A more direct effect of insulin in regulating MTP may be found in type I diabetes. In an animal model for type I diabetes, the streptozotocin-treated rat, we found that hepatic MTP large subunit message levels were decreased upon insulin injection (M. C. M. Lin and J. R. Wetterau, unpublished results). This finding strongly suggests that insulin plays a role in regulating MTP in vivo.

We have previously reported that in hamsters, a 31-day high-fat or high-sucrose diet resulted in elevated hepatic MTP large subunit mRNA levels (20). An acute hepatic response to a high-fat diet was not observed. This latter observation suggested that the increase in MTP message levels observed at 31 days was not a direct effect of the high-fat diet, but rather the result of a long term metabolic response to the diet. Based upon the findings of our study in HepG2 cells, we speculate that the metabolic response that caused an elevation in hamster hepatic MTP large subunit mRNA levels may have been hepatic insulin insensitivity resulting from the chronic high-fat (or possibly high-sucrose) diet. High-fat diets (47), and in particular, diets enriched in saturated and monounsaturated fats (48), have been reported to induce insulin resistance in rats fed experimental diets for time periods comparable to those used in the hamster study. However, the effect of insulin insensitivity may have been diminished by increased insulin secretion by the pancreatic beta cells, which frequently occurs in the early stages of insulin resistance (49). The role of insulin in chronic regulation of MTP levels in vivo under normal and insulin-resistant conditions requires further investigation.

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