Phospholipid transfer protein (PLTP) plays an important role in human plasma HDL metabolism. Clinical data have recently indicated that plasma PLTP activity and mass were both higher in diabetic patients concomitant with hyperglycemia. The present study shows that high glucose increases both PLTP mRNA and functional activity in HepG2 cells, due to a significant increase in the promoter activity of human PLTP gene. The glucose-responsive elements are located between −759 and −230 of the PLTP 5′-flanking region, within which two binding motifs (−537 to −524 and −339 to −327) for either peroxisome proliferator-activated receptor or farnesoid X-activated receptor are involved in this glucose-mediated transcriptional regulation. This finding suggests that high glucose upregulates the transcription of human PLTP gene via nuclear hormone receptors. In addition, high glucose increases mRNA levels for several genes that are functionally important in HDL metabolism, including human ATP-binding cassette transporter A1, apolipoprotein A-I, scavenger receptor BI, and hepatic lipase. The functional promoter activities of these genes are enhanced by high glucose in three cell lines tested, indicating that glucose may also regulate these genes at the transcriptional level. Our findings provide a molecular basis for a role of hyperglycemia in altered HDL metabolism. 

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The leading cause of death for individuals with diabetes is cardiovascular disease. It has been shown that diabetic patients have altered HDL metabolism and this may account in part for the increased incidence of coronary disease, because epidemiological data have demonstrated an inverse relationship between coronary disease and plasma HDL level and size (1,2). HDL plays a pivotal role in “reverse cholesterol transport,” a process by which excess cholesterol from the tissue is returned to the liver (3,4). Cellular cholesterol efflux involves the interaction of HDL with specific receptors, such as ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor BI (SR-BI) (5,6). Several studies suggest that the plasma phospholipid transfer protein (PLTP) also plays an important role in HDL-mediated reverse cholesterol transport (7–13). PLTP can modulate HDL particle size and lipid composition (7,8), and generate prebeta HDL as the earliest acceptor of cellular cholesterol in reverse cholesterol transport (9). Transgenic mice overexpressing human PLTP or PLTP knockout mice provide additional evidence to suggest that PLTP not only regulates HDL metabolism, but also acts as an antiatherogenic factor preventing cellular cholesterol overload by generation of prebeta HDL in vivo (10–12). Furthermore, PLTP has been shown to enhance HDL interaction with cholesterol-loaded cells and the efflux of excess cholesterol (13). Higher plasma PLTP activity has consistently been found accompanied by hyperglycemia in diabetes (14–17). Plasma PLTP activity and mass were found to be higher by ~120 and 170%, respectively, in type 2 diabetic patients than in nondiabetic subjects (14). Furthermore, increased plasma PLTP activity was found to be associated with insulin resistance in obese type 2 diabetic patients (17). These findings suggest that the regulation of plasma PLTP activity may be altered under conditions of hyperglycemia, although the mechanism responsible for this alteration has yet to be delineated. In addition, higher hepatic lipase (HL) activity, associated with lower levels of and a smaller particle size of HDL, was observed in patients with diabetes (18–20), suggesting that the increased HL may be attributed, in part, to hyperglycemia.

The regulation of lipoprotein metabolism at the tran-
GLUCOSE REGULATES TRANSCRIPTION OF HDL GENES

...criptional level has been intensively investigated in recent years, because transcriptional regulation plays a central role in connecting the influence of environmental and physiological regulators with the response of the corresponding genes. The transcriptional regulation of the human PLTP gene was initially studied in our laboratory (21,22) and recently followed by other investigators (23,24). The PLTP gene is transcriptionally regulated by fenofibrate (22) and bile acid (24) via the responsive elements for nuclear hormone receptors, peroxisome proliferator–activated receptor (PPAR) (22), and farnesoid X-activated receptor (FXR) (23,24), respectively. The aim of this study was to investigate the role of glucose in the transcriptional regulation of PLTP and other genes that are involved in the metabolism of HDL.

RESEARCH DESIGN AND METHODS

Cell culture. Human hepatoma HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 4 mM l-glutamine, 1 mM l-pyruvate, 0.05 mg/ml penicillin, and 0.05 mg/ml streptomycin supplemented with 10% fetal calf serum (FCS) (BioWhittaker, Walkersville, MD) as previously described (21,22), except that three different glucose concentrations (5.5, 12.5, and 25 mM) were used to grow cells. Human choriocarcinoma BeWo cells were maintained in a F12 nutrient mixture (Ham's medium) (Life Technologies, Gaithersburg, MD) containing 4 mM l-glutamine, 0.05 mg/ml penicillin, and 0.05 mg/ml streptomycin supplemented with 5% FCS. Green monkey kidney CV-1 cells were cultured in DMEM containing 10% FCS, 4 mM l-glutamine, 0.05 mg/ml penicillin, and 0.05 mg/ml streptomycin. The growing medium was changed every other day to supply the cells with sufficient glucose for energy purposes. These cells were incubated at 37°C in a humidified atmosphere of 5% CO2/95% air and were routinely split about every 4–5 days.

Measurement of human PLTP mRNA and activity in HepG2 cells. The HepG2 cells that were grown and maintained in the medium with different glucose concentrations for more than five passages were used to measure the mRNA level and functional activity of PLTP. Upon reaching confluence, the cells were washed two times with the phosphate-buffered saline, and total RNA was extracted from the cells following the protocols of the TRIzol Reagent (Life Technologies). Northern blotting analysis was carried out by electrophoresis of 30 μg of total RNA on a 1% agarose/7% formaldehyde gel, followed by transfer to a nylon membrane and hybridization with 32P-labeled full-length human PLTP cDNA as previously described (8). For normalization, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (Clontech Laboratories, Palo Alto, CA) was used for the same blotting analyses separately. The transcripts were quantitated by densitometric tracing of autoradiographs.

The relative amounts of PLTP mRNA among three cell lines were computed by the intensity obtained with PLTP mRNA adjusted to the respective GAPDH mRNA. We used primers specific to the cDNA encoding the following mRNAs: the promoterless pGL2-Basic vector (Promega) to create the fused gene promoter/luciferase vector; the internal control pRL-TK in the transfection experiments was used to determine the normalized promoter activity. The promoterless pGL2-Basic (vector only) and pGL2-promoter were also included as a negative and positive external control, respectively.

Measurement of human apolipoprotein A-I and A-II, SR-BI, HL, and ABCA1 mRNA levels in HepG2 cells. Reverse transcriptase–polymerase chain reaction (RT-PCR) was used to obtain the cDNA probes of human apolipoprotein (apo) A-I (0.47 kilobase pairs [kb]), apo A-II (0.33 kb), SR-BI (1.25 kb), HL (1.5 kb), ABCA1 (1.35 kb), cholesterol ester transfer protein (CETP) (0.78 kb), and lecithin-cholesterol acyltransferase (LCAT) (1.35 kb). We used 1 μg of total RNA from HepG2 cells with specific primers for RT-PCR following the standard protocol of OneStep RT-PCR kit (Qiagen, Valencia, CA). Specific primers corresponding to the cDNA are 5′-GGGCCAGATGAC-TCTGTCTTCTGGG-3′ and 5′-CAGGCTGGTGGATCTGCGAGAAAAGG-3′ for ABCA1 (25), 5′-CCGAGACAGATGGGTCCTCCAAAGG-3′ and 5′-ACCCCTAGTTT-GCTGCTTCTGACGACAG-3′ for SR-BI (26), 5′-GGAGTGAAGTCTGCGAC-TGAGCTC-3′ and 5′-AGGGCTGGACGCTAGTACCAT-3′ for CETP (27), 5′-GGAGCTGAAGTAGAACTGAGGCTCC-3′ and 5′-GAGTCGACGCCAGCTGATG-3′ for HL (28), 5′-GGAGGCTGAGTGAAGGGGGCCGGCCGC-3′ and 5′-GGATTTTATCGGAGGGGGCCGGGC-3′ for LCAT (29), 5′-GGAAGITGAAGGCCAAGTGACGAGCACC-3′ and 5′-GCTCTCTGACGGGCGACAGCCC-3′ for apo A-I (30), and 5′-GGCCCTTCCCTGATTACAC-3′ and 5′-GGAACCTTACATTGCTGGTCGAGGT-3′ for apo A-II (31). The cDNA probes were verified by their DNA sequences. These probes were then used for the Northern blotting analyses of the corresponding mRNAs separately following the same protocol as described above for the measurement of human PLTP mRNA. The relative amounts of these mRNAs among three cell lines were computed by the intensity obtained with the mRNA adjusted to the respective GAPDH mRNA after scanning the Northern blot Autorad with a Hewlett-Packard Desk-Scan II.

Measurement of human apo A-I, SR-BI, HL, and ABCA1 promoter activities in HepG2, BeWo, and CV-1 cells. Polymerase chain reaction (PCR) was used to amplify the proximal 5′-flanking region of human ABCA1 (32), SR-BI (33), and HL (34) DNA sequences. These probes were then used for the Northern blotting analyses of the corresponding mRNAs separately following the same protocol as described above for the measurement of human PLTP mRNA. The relative amounts of these mRNAs among three cell lines were computed by the intensity obtained with the mRNA adjusted to the respective GAPDH mRNA after scanning the Northern blot Autorad with a Hewlett-Packard Desk-Scan II.

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RESULTS

HepG2 cells that were grown in the medium with 5.5, 12.5, and 25 mmol/l glucose for more than five passages were used in the study of the long-term effect of glucose on the expression of human PLTP. The PLTP activity secreted by these cells was measured by phospholipid transfer activity assay. The results indicate that the PLTP activities (% transfer \* 100 \* 15 min^{-1}) of 13.9 ± 1.2 (n = 4, P < 0.01) and 11.7 ± 0.4 (n = 4, P < 0.01), respectively, for the cells grown with 25 and 12.5 mmol/l glucose are ~1.6-fold higher than the PLTP activity of 7.1 ± 1.4 (n = 4) for the cells grown with 5.5 mmol/l glucose. In addition, the increase in PLTP activity was associated with an ~30% increase in PLTP mRNA level (Fig. 1).

To test whether glucose affects the functional activity of PLTP promoter and further locate the region or regions responsible for the glucose effect, various PLTP gene promoter/luciferase constructs were transfected into HepG2 cells for the measurement of promoter activity in the presence of 5.5, 12.5, and 25 mmol/l glucose. The results show that increased glucose concentrations in the medium had little influence (<10% change) on promoter activity for the constructs with DNA sequences from –230, –205, –159, and –72 each to +15 (Fig. 2A). We have previously reported that the promoter region (–230 to –159) consists of the DNA sequences that are crucial for the basal transcription of human PLTP gene (21). This finding suggests that the effect of high glucose on PLTP gene expression is not related to the basic transcription, which involves transcription factors Sp1, AP-2, and glucocorticoid receptor (21). In contrast, an ~25% increase in activity was observed for the –377/15 construct in the HepG2 cells with 25 mmol/l glucose as compared with cells with either 5.5 or 12.5 mmol/l glucose (Fig. 2A). The effect of high glucose was even greater for constructs –759/15 and –2,800/15. When compared with the activity for the constructs –759/15 and –2,800/15 in the cells with 5.5 mmol/l glucose, there was 33 and 20%, respectively, higher activity in those with 12.5 mmol/l glucose, and 230 and 190%, respectively, higher activity in those with 25 mmol/l glucose. These findings suggest that the high glucose-mediated increase of the PLTP promoter activity in the HepG2 cells is primarily attributed to the DNA sequences between –759 and –230 in the PLTP gene.

Two recent studies demonstrated that the mRNA levels and binding affinity of PPAR nuclear proteins in macrophages and pancreatic β-cells are influenced by high glucose (37,38). We have previously identified the binding motifs for PPAR (–537 to –524, –339 to –327, and –318 to –302) in the PLTP gene promoter (22), which are located exactly within the glucose-responsive region (–759 to –230). The mutants with mutations on each of the PPAR-binding areas were therefore used to study whether these PPAR sites are responsible for the effect of high glucose on the PLTP promoter activity. The mutation at the area (–318 to –302) exhibited no change of the promoter activity in response to high glucose as compared with that for the wild type (Fig. 2B). Thus, this PPAR site appears not to be involved in the regulation by glucose. In contrast, the mutation at the area (–339 to –327) showed slightly higher promoter activity than the wild type in the cells with low glucose, but it showed only a 1.7-fold increase of the activity in response to high glucose, as compared with a 2.3-fold increase for the wild type (Fig. 2B). This suggests that the PPAR site (at –339 to –327) contributes partially to the upregulation of the PLTP gene by high glucose. In addition, this PPAR site is completely responsible for the difference in the high glucose–enhanced promoter activity between the constructs (–377/15) and (–300/15). Interestingly, this PPAR site was also identified as a binding site for nuclear hormone receptor FXR (23,24). However, it has never been reported whether FXR is involved in glucose-mediated gene regulation. Furthermore, the mutation at the area (–537 to –524) showed only a 1.5-fold increase of the promoter activity in response to high glucose, as compared with a 2.3-fold increase for the wild type (Fig. 2B), indicating that this area is also partially responsible for the regulation of the PLTP gene by high glucose. The finding that the increase of the PLTP promoter activity by high glucose was partially abolished when the two PPAR binding areas (–537 to –524 and –339 to –327) were mutated indicates that the response ele-
In the present study, the glucose-responsive elements identified at the proximate 5'-flanking region of the PLTP gene are overlapping with two PPAR-binding sites, indicating that PPARs are very likely involved in the increased expression of human PLTP gene by high glucose.

In addition to the increase of the PLTP mRNA level, the HepG2 cells that were grown and maintained in higher glucose concentrations exhibited higher mRNA levels to various extents for several other HDL-relevant genes. As shown in Fig. 1, a significant increase in mRNA level was observed for apo A-I in the cells grown with high glucose (25 mmol/l glucose) as compared with those grown with low glucose. This high glucose–induced increase in mRNA level was also found to various extents for ABCA1, SR-BI, and HL. There was no significant change of mRNA level for either apo A-II, LCAT, or CETP among the cells grown in 25 mmol/l glucose; and open bars for those expressed in the cells grown with 12.5 mmol/l glucose; and solid bars for those expressed in the cells grown with 5.5 mmol/l glucose. Data shown are the means ± SE of at least three independent experiments. Values for constructs in 25 or 12.5 mmol/l glucose are statistically compared with those for corresponding controls in 5.5 mmol/l glucose (*P < 0.05, **P < 0.01).

DISCUSSION
In diabetic patients, the plasma lipid and lipoprotein parameters, along with the activities of lipolytic enzymes and lipid transfer proteins, are quite different from those in nondiabetic individuals (39). In addition to the possible effects of diabetes per se, the contribution of altered gene expression of these proteins has not been fully explored. Hyperglycemia could represent one of the key elements that initiate the alteration of gene expression in diabetes. The alteration of functional activities of HDL-processing proteins and receptors and their effects on HDL metabolism in diabetes concomitant with hyperglycemia are also poorly understood. In recent clinical studies, PLTP and HL have been consistently reported as higher in diabetic patients (14–20), which is a finding in agreement with the ex vivo data in the present study, showing that PLTP and HL are increased in cultured hepatocytes by high glucose. Our data also suggest that ABCA1, apo A-I, and SR-BI are upregulated by high glucose in the cultured hepatocytes. It is therefore reasonable to speculate that hyperglycemia may also alter the expression of these HDL receptors, which could contribute to the abnormal HDL metabolism that occurs in diabetes.

We have previously delineated several pathways for the regulation of the transcription of human PLTP gene (21,22,40). In the present study, the glucose-responsive elements identified at the proximate 5'-flanking region of the PLTP gene are overlapping with two PPAR-binding sites, indicating that PPARs are very likely involved in the
regulation of human PLTP gene by high glucose. The PPAR-responsive elements are present in the promoter of many genes implicated in lipoprotein metabolism (41). Recent findings have demonstrated that PPARs are involved in several metabolic diseases (such as dyslipidemia, insulin resistance, and chronic inflammation) that predispose to atherosclerosis (42). Evidence also indicates that a high-glucose environment can modulate the expression of PPAR nuclear proteins, which subsequently affects lipid metabolism in macrophage-derived foam cells (37). This finding further suggests that abnormal regulation of macrophage PPARs in the arterial wall by high glucose contributes to the accelerated atherosclerosis associated with type 2 diabetes. More studies are needed to investigate whether high glucose may directly affect the expression of PPAR at the transcriptional level, rather than indirectly influence the corresponding ligands that activate PPAR nuclear proteins.

PPARs bind DNA as a heterodimer with retinoid X-activated receptor (RXR) (43), which represents a typical DNA-binding mode in the nuclear hormone receptor superfamily that is involved in the transcriptional regulation of HDL-mediated reverse cholesterol transport. For example, liver X-activated receptor (LXR)/RXR is involved in sterol-dependent transcriptional regulation of ABCA1 and CETP genes (25,44), and FXR/RXR is involved in the regulation of PLTP gene by bile acid (23,24). LXR/RXR may activate expression of ABCA1 gene in the cholesterol-loaded peripheral cell, leading to enhanced efflux of free cholesterol onto apo A-I and resulting in the formation of mature HDL particles. FXR/RXR–mediated increased expression of PLTP gene may lead to the production of prebeta HDL and facilitate lipid efflux from cholesterol-loaded cells (13). Furthermore, both human SR-BI and ABCA1 genes were recently found to be regulated by PPAR activators in macrophage-form cells (45,46), although the PPAR elements have yet to be identified at their promoter regions. Similarly, human apo A-I gene promoter may interact with PPAR-α nuclear protein for its transcriptional regulation by fibrates (47). These findings, taken together, support the concept that these nuclear hormone receptors are functionally associated with the same transcriptional regulatory process and can be further regulated by the same physiological regulators.

As two PPAR-binding sites do not completely contribute to the increased PLTP gene transcription (Fig. 2), we cannot rule out the possibility that some elements other than PPAR elements are also involved in this glucose-mediated regulation. In DNA analysis of human PLTP promoter region (–759 to –377), we have identified other potential glucose-responsive elements. For example, the binding motif for Sp1 (GGCAGG) that is involved in the activation of the plasminogen activator inhibitor-1 promoter by glucose (48) is also located at –448 to –442 in the human PLTP gene, while the DNA elements (GAGGGCGG) for the glucose-mediated transcriptional regulation of rat apo A-I (49) are also found in the promoter region (–754 to –747) of the human PLTP gene. Furthermore, the binding sequences (ATCGGGCCAC) for sterol regulatory element–binding protein-1 (SREBP-1) that is involved in the regulation of lipid metabolism in diabetic mice (50) are located in the PLTP promoter (–729 to –720). It is interesting that the SREBP-1 gene was found to be regulated by glucose at the transcriptional level (51). We are currently investigating whether these potential glucose elements play an additional role, in association with PPAR elements, in the transcriptional regulation of human PLTP by glucose.

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REFERENCES


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**TABLE 1**

<table>
<thead>
<tr>
<th>Glucose (mmol/l)</th>
<th>ABCA1 (−1.013/+31)</th>
<th>SR-BI (−913/+23)</th>
<th>HL (−908/+19)</th>
<th>Apo A-I (−1.096/+15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>1.54 ± 0.10</td>
<td>0.10 ± 0.23*</td>
<td>0.815 ± 0.067*</td>
<td>0.313 ± 0.019*</td>
</tr>
<tr>
<td>25</td>
<td>2.09 ± 0.23*</td>
<td>0.170 ± 0.22</td>
<td>0.042 ± 0.010</td>
<td>0.173 ± 0.008</td>
</tr>
<tr>
<td>5.5</td>
<td>0.233 ± 0.036*</td>
<td>0.114 ± 0.013</td>
<td>0.063 ± 0.008*</td>
<td>0.196 ± 0.006*</td>
</tr>
<tr>
<td>25</td>
<td>0.162 ± 0.022*</td>
<td>0.018 ± 0.003</td>
<td>0.028 ± 0.002</td>
<td>0.028 ± 0.002</td>
</tr>
<tr>
<td>5.5</td>
<td>22.5 ± 1.3</td>
<td>0.012 ± 0.002</td>
<td>0.240 ± 0.020</td>
<td>0.240 ± 0.020</td>
</tr>
<tr>
<td>25</td>
<td>40.7 ± 0.4†</td>
<td>0.022 ± 0.004*</td>
<td>0.291 ± 0.029*</td>
<td>0.291 ± 0.029*</td>
</tr>
</tbody>
</table>

Data are means ± SE of at least three independent experiments. *P values were determined by Student’s t test for paired data (*P < 0.05, †P < 0.01).
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