Effects of Diabetes Mellitus on Hepatocyte Nuclear Factor 1 Decrease Albumin Gene Transcription*

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We have previously reported that albumin gene transcription is reduced in diabetes mellitus (DM). The present study explored the mechanism by which albumin gene transcription is down-regulated in DM. Deletional studies and displacement of factors binding to site B of the albumin promoter indicated that the repressive effects of DM are mediated by nuclear factors binding to this site. Since hepatocyte nuclear factor 1 (HNF1) activates albumin promoter activity and is the predominant factor binding to site B, we examined HNF1. The abundance and binding activity of HNF1 were reduced in hepatonuclear extracts from diabetic compared to control rats. However, HNF1 mRNA levels were unchanged, suggesting that the effect of DM on HNF1 is at the post-transcriptional level. Extracts from diabetic animals also contained another protein, distinct from HNF1 and vHNF1, which bound to site B in gel retardation studies. In summary, our studies demonstrate that the reduced abundance and binding activity of HNF1 correlates with decreased albumin gene transcription in DM.

Diabetes mellitus (DM) alters the transcription of numerous genes in a variety of tissues (1–6). Although DM increases or decreases gene transcription, the mechanism underlying these changes is largely unknown. We have shown previously that transcription of the albumin gene is reduced in DM (7). Therefore, we are using the albumin gene as a model to understand how DM down-regulates gene transcription.

Extensive studies have shown that control of albumin gene transcription resides primarily within the proximal 170 bp of the albumin promoter (8, 9). At least six important cis-acting elements (A–F) have been defined within this promoter sequence (10). Of these elements, site B plays a pivotal role in mediating liver-specific transcription of the gene (11–13). Furthermore, mutations of this site decrease promoter activity to a greater extent than do changes to any of the other cis-acting elements (11). Multimerization of site B also yields a strong artificial promoter active only in liver (11). In addition, of these six elements, only site B is conserved from Xenopus to human (12, 14).

Hepatocyte nuclear factor 1 (HNF1) binds to site B and trans-activates albumin gene transcription (14–17). Albumin gene expression correlates closely with the presence of HNF1. The association between HNF1 and albumin is exemplified by the appearance of HNF1 in the transition from a-fetoprotein to albumin expression during hepatic ontogeny (18). Similarly, HNF1 concentrations are high in differentiated hepatocyte cell lines (FAO and H4I1) that secrete albumin, and low in dedifferentiated hepatic cell lines (C2 and H5) that do not produce appreciable levels of albumin (19, 20). In addition, there is a tight correlation between the tissue-specific expression of albumin and the expression of HNF1 (21–23). A variant form of HNF1 termed vHNF1 also binds to site B of the albumin promoter (16, 17, 24–27). vHNF1 is, however, much less effective than HNF1 in transactivating the albumin gene, and the presence of vHNF1 does not correlate well with albumin expression. In fact, vHNF1 is abundant in cell lines (H5 and C2) and kidney, where albumin expression is low (16, 17, 24, 25, 27). Both HNF1 and vHNF1 bind DNA as a hetero-tetrameric complex comprising two molecules each of HNF1 or vHNF1 and a dimerization cofactor, DCoH, which enhances the transcriptional activity of its partner (28).

The critical role of HNF1 and site B in regulating albumin gene expression prompted us to postulate that decreased albumin gene transcription in DM is mediated through site B. Not only do our results support this hypothesis, they also show that HNF1 protein levels are reduced in the diabetic state. To our knowledge, this represents the first description in DM of a reduction in a nuclear factor essential for the transcription of an eukaryotic gene, albumin.

MATERIALS AND METHODS

Animals—Male Sprague-Dawley rats (175–200 g; Charles River Canada Inc., Quebec, Canada) were rendered diabetic with intraperitoneal injections of streptozotocin (Ujohn, 60 mg/kg). The presence of DM (defined by blood glucose > 15 mm) was determined by measuring glucose in whole blood (ExaTech, Medisense) and urine (Ketodiastix, Ames). Animals were fed ad libitum (Purina rat chow) and sacrificed between 9:00 and 10:00 a.m. 3–5 days after the onset of DM.

Expression of HNF1 and vHNF1—COS-7 cells were transfected by the calcium phosphate method with HNF1-A/pBJ5 or vHNF1-A/pBJ5 expression vectors (gifts from G. R. Crabtree). Cells were cultured in ISE-RPMI containing 5% fetal calf serum (29), and whole cell extracts were prepared from transfected or untransfected COS-7 cells, as described previously (30).

In Vitro Transcription Assay—Hepatocellular extracts were prepared according to the method of Gorski et al. (8). The in vitro transcription assay was performed essentially as described previously (7), except that 1.5 μg of pAlb-320 and 0.3 μg of pAd·MLP-200 were used. pAlb-320 is the mouse albumin promoter containing nucleotides −650 to −22 of the gene attached to a G-free cassette (GFC) of 320 nucleotides (8), and pAd·MLP-200, a plasmid containing the adenovirus major late
promoter (−404 to +10) fused to a 200-nucleotide GFC, served as the internal control (plasmids were gifts from U. Schibler). Deletional constructs of the albumin promoter were obtained by linearizing pAlb-400 (albumin promoter construct fused to a 400-bp GFC) at its 5′ end and digesting with Exonuclease III for varying periods of time. The resultant products were circularized and clones verified by dideoxy sequencing. Competition experiments were performed with 40 pmol of DNA homologous to site B (AATTTAGCTGGTTAATGCTCAG) of the mouse albumin promoter or to site C (AATTGACCTTAAGTTCCATCGGCCA) of the rat apolipoprotein A1 promoter. The labeled transcripts were detected by autoradiography and quantitated by video-assisted densitometry, as described previously (7).

HNF1 Partial Purification and Gel Retardation Assay—HNF1 was partially purified by a heparin-agarose and wheat germ agglutinin procedure, essentially as described by Lichtsteiner and Schibler (14). These preparations were used in gel retardation and Western blot analyses. The site B oligonucleotide duplex used in the gel retardation assay was based on the HNF1 binding site of the mouse albumin promoter (31). Partially purified HNF1 was incubated with −25,000 cpm (−20 fmol) of 32P-labeled blunt-ended probe in a reaction mixture containing 10% sucrose, 3.75 mM HEPES, pH 7.6, 2.75 mM MgCl2, 1.5 mM spermidine, 200 ng of poly(dI-dC), 100 ng of bovine serum albumin, and 75 ng of sonicated salmon sperm DNA at room temperature for at least 30 min, then separated by 5% nondenaturing polyacrylamide gel electrophoresis (PAGE) in 0.5 × TBE buffer at 100 V for at least 2.5 h. Where indicated, 1 μl of antisera was added to the gel retardation reaction and incubated for 1 h at room temperature prior to addition of the probe. HNF1 and VNF1 antisera were gifts from A. Nicosia and G. R. Crabtree, respectively.

UV-Cross-linking—Covalent attachment of radiolabeled DNA to protein was performed essentially as described by Cereghini et al. (15). Briefly, a standard gel retardation reaction was performed with bromodeoxyuridine-substituted labeled probe. Samples were irradiated with UV light for 20 min at 254 nm. One third of each reaction was analyzed by nondenaturing PAGE as described in the previous section. The wet gel was irradiated with UV light for 10 min and then exposed to x-ray film for approximately 18 h at 4°C with intensifying screens. Bands of interest were excised from the gel and analyzed by 10% SDS-PAGE (32). The molecular mass of the double-stranded oligomer probe was subtracted from the estimated molecular mass of the protein-DNA complexes (15, 27).

Western Blot—Nuclear proteins were separated by 7.5% SDS-PAGE (32) and then electroblotted onto polyvinylidene difluoride (Millipore) for at least 2 h at 4°C. Membranes were blocked in 10% dry skim milk powder and washed in blotting buffer at room temperature, according to standard procedures (33). A 1/1000 dilution of HNF1 antiserum was used as the primary antibody. Blots were developed using the methods described in the ECL kit (Amersham Corp.).

Northern Blot—Total RNA was extracted from rat liver using RNAzol B (Cinna/Biotex Laboratories, Inc., Houston, TX) by the guanidinium thiocyanate method. RNA samples were subjected to formaldehyde-electrophoresis and transferred onto nitrocellulose (NitroPlus, Micron Separations Inc.). Hybridization and washes were performed as described previously (24), except that 2.5 × 106 cpm/ml of a 32P-labeled HNF1 polymerase chain reaction generated cDNA fragment of 565 bp (+199 to +764 bp) (34) was used as a probe, and 10% dextran sulfate was added to the hybridization mixture. Membranes were exposed to Kodak XAR-5 film and quantitated by video-assisted densitometry (7). Ethidium bromide staining was used to assess RNA integrity and loading.

RESULTS

Site B Mediates Suppressive Effect of DM—To delineate the region of the albumin gene that mediates the inhibitory effect of DM, we measured the activity of templates containing 5′ deletions of the albumin promoter using a cell-free in vitro transcription assay. We confirmed our previous findings (7) that the transcriptional activity of a −650 to +22 albumin promoter construct was decreased in hepatonuclear extracts from diabetic relative to euglycemic control animals (data not shown). A schematic representation of the −130 and −70 to +22 deletional constructs attached to a GFC reporter gene is shown in Fig. 1A. Both of these constructs retained the ability to mediate the suppressive effects of DM (Fig. 1, B and C). Furthermore, the percent decrease in transcription of diabetic extracts compared to their respective controls was not significantly different in any of the constructs. These results indicate that a minimal promoter fragment (−70 to +22), which includes the binding sites A and B and the TATA box, retains the ability to mediate the inhibitory effect of DM on albumin transcription. Promoter fragments lacking site B supported only minimal levels of albumin transcription in either extract (data not shown) and were therefore not useful in deletional studies (8, 11). Consequently, a different approach was used to determine which particular cis-element(s) within the −70 to +22 promoter fragment was responsible for mediating the inhibitory effect of DM.

Since site B (−72 to −58) is critical for albumin promoter activity (11–13, 35), we asked whether decreased albumin gene transcription in DM was mediated through this site. To eliminate the effect mediated through site B, we displaced the binding of transcription factors to this site. Accordingly, we measured the transcriptional activity of an albumin promoter construct (−650 to +22) in the presence or absence of oligonucleotide homologous to site B (Fig. 2). The addition of site B oligomer to the reactions reduced transcriptional activity of the promoter in extracts from both euglycemic control (compare lanes 1 and 3) and diabetic (compare lanes 4 and 6) animals. Moreover, in the presence of site B oligomer, the level of albumin transcription supported by extracts from diabetic animals was the same as that from controls (compare lanes 3 and 6).
Residual albumin transcription (11) is due to the effect of transcription factors other than HNF1, which interact with the promoter at other binding sites. In contrast, transcription of the construct in either extract was not significantly affected by adding nonspecific oligomer, and lane 3, with DNA homologous to site B. The activity of the promoter with extract from a diabetic rat appears as follows: lane 4, by itself; lane 5, with a nonspecific oligomer; and lane 6, with DNA homologous to site B. Panel B, graph of the relative albumin promoter activity in the two types of extracts. Each bar represents the mean ± S.D. (control n = 5; diabetic, n = 4; *, p < 0.005; **, p < 0.001, by Student’s t test).

Displacement of factors binding to site B of the albumin promoter allowed us to examine whether the suppressive effects of DM arise from a reduction in the abundance or activity of an activator of site B, the actions of an inhibitor(s), or a combination of both. If the effect of DM is due mainly to the action of an inhibitor, we would expect displacement of this factor to increase albumin gene transcription. Alternatively, if the effect of DM is due to reduced abundance or activity of a site B activator, we would anticipate displacement of factors binding to this site to further decrease albumin gene transcription.

Our results are consistent with the latter possibility, suggesting that in DM the reduced abundance or activity of an activator of transcription is the principal mechanism responsible for decreasing albumin gene activity through site B.

Hepatic HNF1 Is Reduced in Diabetic Animals— Since HNF1 is the predominant factor activating albumin transcription through site B, we examined whether down-regulation of albumin in DM was associated with reduced HNF1 protein levels. We compared the relative abundance of HNF1 in hepatocellular extracts by Western blot analysis. HNF1 protein levels were reduced in crude and partially purified extracts from diabetic animals when compared to the corresponding euglycemic control rats (Fig. 3A). HNF1 expressed in COS-7 cells, with an estimated molecular mass of ~88 kDa, served as the positive control (Fig. 3B).

Different Site B Binding Activities in Extracts from Diabetic and Euglycemic Rats— Next, we determined whether the decrease of HNF1 in DM was reflected in the protein binding activity to site B. We measured site B binding activity of partially purified extracts from control and diabetic rats by gel retardation assay. Extracts from euglycemic rats incubated with radiolabeled site B yielded complexes (upper band) (Fig. 3A, lane 1 and 3) comparable to those reported in the literature for HNF1 (22, 24, 25, 27). This protein-site B complex (upper band) was less abundant in reactions with extracts from diabetic animals (Fig. 3A, lanes 2 and 4). This finding was consistent with results from Western blot studies. Unexpectedly, we observed another complex in extracts from diabetic animals (lower band) that migrated faster than the protein-site B complex (upper band) observed in extracts from control rats (Fig. 4A).

Site B binds not only HNF1 (80–93 kDa) (16, 17), but also vHNF1 (68–72 kDa) (15, 17, 36), a nuclear protein that correlates with decreased albumin gene expression (24, 37). We examined whether the faster migrating complex in extracts from diabetic animals might be due to the binding of vHNF1 to site B by adding specific antisera raised against HNF1 or vHNF1 to the gel retardation reaction (Fig. 4B). Rat Apo A1 antiserum (36) served as a negative control. HNF1 antiserum retarded the mobility of complexes formed using extracts from both control (lane 2) and diabetic (lane 6) rats. In contrast, vHNF1 antiserum had no effect on the mobility of these complexes (Fig. 4B, lanes 3 and 7). The functional integrity of the antiserum against vHNF1 and Apo A1 was confirmed using control antigens (data not shown). In addition, the mobility of COS-7-expressed vHNF1 (see Fig. 6) was clearly different from that of the protein-site B complex in extracts from diabetic animals. These results suggest that the more rapidly migrating complex observed with extracts from diabetic animals arises from the binding of a protein to site B which is not vHNF1. This site B-binding protein is, however, recognized by antiserum against HNF1.

The Faster Electrophoretic Mobility Complex Was Not Due to Proteolytic Degradation—Although all protein extracts were...
prepared on ice and in the presence of protease inhibitors, we wondered whether the faster mobility complex observed in extracts from diabetic animals was due to proteolytic degradation of the samples. To examine this possibility, we performed a series of experiments. Denaturing gel analysis of extracts from both groups of rats produced the same banding patterns following Coomassie Blue (data not shown) or silver staining (Fig. 5A), with no evidence of degradation. We also encouraged proteolysis of partially purified extracts by overnight incubation at room temperature before gel retardation studies (Fig. 5B). Similarly, we tested the integrity of two unrelated nuclear proteins, thyroid hormone receptor-α, and retinoid X receptor-α, by gel retardation and Western blot assays, respectively (data not shown). No sign of proteolysis was detected in any of these experiments. In addition, extracts from control and diabetic animals were mixed, incubated, and then analyzed by gel retardation assay, with no evidence of degradation (data not shown). Together, these results indicate that neither the reduced HNF1 protein levels nor the faster mobility of protein-site B complex in extracts from diabetic animals appears to arise from proteolysis.

Lack of DCoH Could Not Account for Faster Mobility of Protein-Site B Complexes in Extracts from Diabetic Animals—Since HNF1 binds to site B in the form of a hetero-tetramer comprising two molecules each of itself and DCoH, we investigated whether absence of DCoH could account for the faster mobility of the protein-site B complex in extracts from diabetic animals. COS-7 cells lack endogenous DCoH (28), and therefore HNF1 expressed in these cells is not associated with this cofactor. We compared the electrophoretic mobility of liver-derived HNF1, which contains DCoH, with that of COS-7 expressed HNF1 (Fig. 7). The molecular masses of HNF1 and vHNF1 expressed in COS-7 cells, determined by UV-cross-linking, were clearly different from that of the complex present in extracts from diabetic animals (data not shown). These findings indicate that the protein binding to site B in extracts from diabetic animals is distinct from HNF1 and vHNF1. We have named this site B-binding protein DM-X.

Since the molecular mass of DM-X (∼59 kDa) is different from that of HNF1 and it is recognized by specific antisera against HNF1, one would expect to detect DM-X as a distinct band by Western blot analysis. Although DM-X was recognized by anti-HNF1 antisera in the gel retardation assay, a ∼59-kDa band was not detected by Western blot. This discrepancy could arise from the different conditions used in the two experiments. There is documented evidence of differential behavior of a single antibody in gel retardation and Western blot analysis (39).

HNF1 mRNA Levels Remain Unchanged in Diabetic Animals—To determine whether the decrease in HNF1 protein levels in diabetic animals was due to a reduction in HNF1 mRNA levels, we performed Northern blot analysis of total RNA extracted from livers of control and diabetic animals.
abundance of HNF1 in DM represents, to our knowledge, the
transcriptional effects. Therefore, since the HNF1
binding activity present in protein extracts from diabetic animals (lane 4), control animals (lane 3), and COS-7 expressed HNF1 (lane 2) or vHNF1 (lane 1).

FIG. 6. Gel retardation analysis in the presence or absence of
dCoH. Electrophoretic mobility of the site B binding activity present in
protein extracts from diabetic animals (lane 4), control animals (lane 3), and COS-7 expressed HNF1 (lane 2) or vHNF1 (lane 1).

Fig. 7. Estimated molecular mass of proteins bound to site B.
Autoradiograph of protein-site B complexes formed by exposing gel
retardation reactions to UV irradiation, followed by denaturing SDS-
PAGE analysis. The migration of molecular mass markers (kDa) is
indicated on the left. The estimated molecular mass of proteins bound to
site B is indicated on the right. A discrepancy in size such as that obtained from UV-cross-linking and Western blot analysis of HNF1 has
been reported previously (67).

(Fig. 8). Consistent with previous reports, Northern blot analysis of RNA from euglycemic animals revealed two bands corresponding to the 3.6- and 3.2-kilobase HNF1 mRNA species (19–21, 24). No differences in the steady state levels of HNF1 mRNA were detected in RNA preparations from control and diabetic animals. This finding suggests that the differences in HNF1 protein levels between the two states are due to post-
transcriptional effects.

In addition, Northern blot analysis served to determine whether DM-X arises from translation of a mRNA that differs from that of HNF1, based on the following criteria. The anti-
HNF1 antibodies used in gel retardation studies recognized both HNF1 and DM-X (Fig. 4B). Therefore, since the HNF1 cDNA used as a probe in the Northern blot corresponds to the area of the molecule encoding the epitope recognized by the HNF1 antibodies, we might detect an extra band in RNA from diabetic animals if DM-X arises from translation of a mRNA distinct but similar to that of HNF1. The absence of any additional band in RNA from livers of diabetic animals (Fig. 8) suggests that DM-X does not arise from a distinct RNA.

DISCUSSION

In this study we examined whether the cis-acting element B
mediates the suppressive effect of DM on albumin gene transcription. We show that this suppressive effect is retained by promoter fragments containing site B, and that it is abolished by displacing factors binding to this motif. Our results reveal that DM reduces the abundance and binding activity of the major site B-binding protein, HNF1. The reduced abundance of HNF1 may account for decreased albumin gene transcription in DM.

Our results suggest that suppression of albumin gene transcription in DM is due to reduced transactivation through site B. This mechanism of albumin gene suppression is supported by our observation that DM decreases the abundance and binding activity of HNF1, the major site B activator. The reduced abundance of HNF1 in DM represents, to our knowledge, the first report of a transcription factor essential for albumin gene expression being altered in this disease. In contrast to HNF1, the mRNA levels of two other transcription factors, which also enhance albumin transcription, C/EBPα and C/EBPβ, are increased in DM (40, 41). If this increase in mRNA levels reflects an enhanced abundance of C/EBPα and C/EBPβ proteins, then it should increase albumin gene activity in DM. However, trans-
scription of the albumin gene actually falls in DM, emphasizing the functional importance of decreased HNF1 protein levels in this disease. Similar mechanisms may regulate the expression of other genes in DM. For example, the hormone binding activity of thyroid hormone receptor, a ligand-dependent transcrip-
tion factor, is decreased in DM and correlates with reduced expression of α2 globulin (42). The abundance of other transcription factors, such as c-jun and c-Fos (43, 44), is al-
tered by insulin, suggesting that their levels may also be a-
fected in DM. In addition, other disorders appear to affect HNF1 expression. HNF1 mRNA has been shown to decrease significantly in response to burns (45), although HNF1 protein levels were not determined in this study. Recently, oncotic pressure has been shown to reduce albumin gene transcription in hepatoma cells through decreased HNF1 binding activity (46).

Although HNF1 protein levels are decreased in the diabetic state, the HNF1 mRNA levels are the same in control and diabetic animals. It therefore appears that the effect of DM on HNF1 expression is exerted at the translational or post-translational level. Control of HNF1 at the translational or post-translational level has been reported previously by others (25, 47). Diabetes could potentially lower the translational efficiency of HNF1, increase the protein turnover, or a combina-
tion of both. In addition, sequestration of the HNF1 mRNA in translationally inaccessible messenger ribonucleoprotein particles is a possibility (48–50). Effects of DM at the translational or post-translational level have been reported for other proteins. For example, the levels of apolipoprotein B and E drop in diabetic rats primarily as a result of slowed translation, with the levels of their respective mRNAs remaining unchanged (51). In addition, translational regulation has been reported for several transcription factors, such as LAP and Pit-1/GHF-1 (52, 53). Regulating translation allows a cell to respond more rapidly to environmental cues than does de novo transcription (48). This type of control is often seen for genes that play a role in development, as is the case for HNF1 (16–18).

In addition to reduced HNF1 protein levels, our results indi-
cate that the liver of diabetic animals contains a 59-kDa protein (DM-X) which binds to site B of the albumin promoter. Since displacement of factors binding to this site in DM mini-

mally decreases transcription of the albumin gene (Fig. 2), the effect of DM-X through site B could at most be that of a weak

FIG. 8. Northern blot analysis of HNF1. Total RNA extracted from
rat livers was analyzed by Northern blot, as described under "Materials
and Methods." Lanes 1 and 2 and lanes 3 and 4 contain 45 µg of total
RNA from control and diabetic animals, respectively. To the right is
indicated the migration position of the 3.6- and 3.2-kilobase HNF1
mRNA species. Means obtained by videodensitometry were analyzed by
Student's t test (p > 0.05).
activator. Therefore, in contrast to the dominant role of decreased HNF1 on albumin gene expression, our results suggest that DM-X plays a minor, if any, role in lowering albumin gene transcription in DM. The lack of a major function for this protein on albumin transcription is also supported by other studies showing that correction of HNF1 binding activity alone, without diminishing the binding of DM-X, is sufficient to normalize albumin mRNA levels in diabetic animals. These findings on the albumin gene, however, do not rule out transcriptional regulation of other HNF1-regulated genes by this 59-kDa protein. Although the identity of this protein is unknown, the fact that it is recognized by anti-HNF1 antiserum (Fig. 4) suggests that it is somehow related to HNF1. It is possible that DM-X arises from post-translational modification of HNF1, or alternatively, from the same message as HNF1 by mRNA editing, as has been described for apolipoprotein B (54–55). In addition, we cannot exclude the possibility that DM-X might arise from in vivo post-translational processing of HNF1. Since endogenous proteolytic enzyme activity is altered in DM (56–57), this protein might be generated by truncation of the HNF1 protein.

Since levels of HNF1 are reduced in DM, one would expect this change to affect the expression of not only albumin, but also other hepatic genes that interact with this transcription factor. Two genes that contain HNF1 binding sites in their promoter sequences are those encoding α₁-antitrypsin and β-fibrinogen. As the hepatic expression of these genes is dependent on HNF1 (58–60), one would anticipate β-fibrinogen and α₂-antitrypsin protein levels to parallel those of albumin. Although the reduction of α₂-antitrypsin protein levels in DM (61) correlates with the reduced abundance of HNF1, increased β-fibrinogen protein levels (62, 63) do not. The down-regulation of albumin gene expression in DM is regulated mainly at the level of transcription. Whether the same is true for the β-fibrinogen gene remains unknown. It is conceivable that transcription of the β-fibrinogen gene is low in DM, even though its protein levels are increased. Changes in the rate of gene transcription are not always accompanied by similar changes in protein levels. For example, growth hormone up-regulates transcription of albumin, even though protein levels remain unchanged due to a compensatory increase in albumin mRNA degradation (64). The expression of many hepatic genes other than albumin is decreased in DM. Since some of these genes do not contain HNF1 binding sites in their promoters, reduced levels of HNF1 cannot function as a general mechanism to inhibit hepatic gene expression in DM. In addition, other genes that are affected in DM contain HNF1 binding sites but are not primarily regulated by this factor in the liver (e.g. PEPCK; Refs. 65 and 66). However, changes in the abundance of HNF1 in DM appear to affect the expression of a subset of hepatic genes whose expression is predominantly regulated by this factor, such as albumin and α₁-antitrypsin.

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