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Protein or Amino Acid Deprivation Differentially Regulates the Hepatic Forkhead Box Protein A (FOXA) Genes Through an Activating Transcription Factor-4–Independent Pathway

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Abstract

The FOXA (forkhead box A) proteins (FOXA1, FOXA2, and FOXA3) play a critical role in the development of the liver, and they also regulate metabolism in adult hepatic tissue. The liver responds to changes in nutrient availability by initiating a number of stress signaling pathways. The present studies demonstrated that in mouse dams fed a low-protein diet hepatic expression of FOXA2 and FOXA3 messenger RNA, but not FOXA1, was induced. Conversely, fetal liver did not exhibit this regulation. Amino acid deprivation of HepG2 hepatoma cells also enhanced transcription from the FOXA2 and FOXA3 genes. In contrast, endoplasmic reticulum stress inhibited the expression of FOXA1, only slightly induced FOXA2, and had no effect on FOXA3. The FOXA2 and FOXA3 messenger RNA induction by amino acid deprivation did not require activating transcription factor 4, a critical component of the conventional amino acid response (AAR) pathway, but their induction was partially dependent on CCAAT/enhancer-binding protein β. Simultaneous knockdown of both FOXA2 and FOXA3 by small interfering RNA did not affect the activation of other amino acid responsive genes, suggesting that the FOXA proteins are not required for the known AAR pathway. Collectively, the results document that the hepatic FOXA family of genes are differentially regulated by amino acid availability.

A complex gene network functions to maintain whole body homeostasis of metabolites such as glucose and amino acids. One group of such genes is the FOXA (forkhead box A) family, also known as hepatic nuclear factor 3 (HNF3).[1] The mammalian FOXA family consists of three members, FOXA1, FOXA2, and FOXA3, which contribute to embryonic development[2, 3] and to hormonal control of gene expression. [4] FOXA proteins bind to similar recognition sequences and the proteins are proposed to have redundant function in regulating some of their target genes.[4–7] However, each Foxa protein also has distinct actions. Foxa1 plays a pivotal role in pancreatic cell function[4] and Foxa1 knockout mice develop severe hypoglycemia and die shortly after birth.[8] Foxa2 knockout mice have severe defects in liver and pancreas development, and die during early embryogenesis.[9] Interestingly, inhibition of hepatic Foxa2 expression during later stages of fetal development has little or no effect on metabolism.[10] Foxa3 knockout mice show no gross phenotypic changes, although the expression of several Foxa target genes are reduced.[7] This lack of

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phenotype may be partly explained by a compensatory up-regulation of Foxa1 and Foxa2 in the Foxa3 knockout animals.[7]

Although the FOXA proteins are known for their role in metabolic control, information on their own regulation is limited, including by metabolites. Foxa3 expression is induced in the protein-deprived mice,[11] but there are no such data available for human cells, nor have FOXA1 and FOXA2 been studied. In mammals, limitation of dietary protein or amino acids *in vivo* or amino acid deprivation of cultured cells triggers the amino acid response (AAR) pathway.[12] The AAR, triggered by a decline in transfer (t)RNA charging, activates eukaryotic initiation factor 2alpha (eIF2α) kinase activity of GCN2.[13] In contrast to amino acid deprivation, limitation of glucose causes accumulation of misfolded glycoproteins and, subsequently, endoplasmic reticulum (ER) stress, which in turn initiates the unfolded protein response (UPR) pathway. Other causes of ER stress that initiate UPR include perturbation of calcium $(Ca)^{++}$ stores, for example, by the Ca^{++}-ATPase inhibitor thapsigargin (Tg).[14] One component of the UPR involves activation of an eIF2α kinase called PERK.[15] Both GCN2 and PERK phosphorylate eIF2α at serine 51, which suppresses global protein synthesis, but paradoxically increases translation of selected messenger (m)RNAs including activating transcription factor 4 (ATF4),[16, 17] which induces the transcription of a number of genes.[12]

In the present study the hepatic expression pattern of all three FOXA family members was investigated during protein limitation *in vivo* and amino acid deprivation or ER stress of HepG2 human hepatoma cells in culture.

Materials and Methods

Animal Treatments

C57BL/6J pregnant mice were ordered from the Jackson Laboratory (Bar Harbor, ME). At day 5 of pregnancy, weight-matched control and experimental mice were pair-fed to ensure equal food consumption between weight-matched pairs. The control group was fed a normal protein diet (19.4% protein) and the experimental group was fed a low-protein diet (8% protein) (Purina Mills, St. Louis, MO). At day 18.5 of gestation, liver tissue was collected from both the fetuses and dams and immediately frozen in liquid nitrogen and stored at −80°C. The studies were approved by the University of Florida Institutional Animal Care Committee.

Cell Culture

HepG2 human hepatoma cells were cultured in minimal essential medium (MEM, pH 7.4, Mediatech, Herndon, VA), as described.[18] The amino acid response was activated by culture medium containing 2 mM histidinol (HisOH), which blocks charging of histidine onto the corresponding tRNA, and thus mimics histidine deprivation and triggers activation of the AAR cascade.[19] ER stress was induced by culture medium containing 300 nM Tg.[14]

Quantitative RT-PCR

RNA was isolated from HepG2 cells using the Qiagen RNeasy kit (Qiagen, Valencia, CA). To measure mRNA, quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analysis was performed using an Opticon 3 system (MJ Research, Reno, NV) and detection with SYBR Green. The primers used were: for FOXA1, sense, 5′-CAGCAAACAAAACCACACAAACC-3′ and antisense, 5′-ACACTTGTGGATCATTAAACTTCGC-3′; for FOXA2, sense, 5′-GTTGTTGTTGTTCTCCTCCATTGC-3′ and antisense, 5′-AACTACATGGTTTTACACCGAGTCAC-3′; for FOXA3, sense, 5′-GGTCTATTACTTACTGTGATGACTGCTG-3′ and antisense, 5′-

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CCAAAGAAGATGTCACTGAAATGCT-3′ for asparagine synthetase (ASNS), sense, 5′-GCAGCTGAAAGAAGCCCAAGT-3′ and antisense, 5′-TGTCTTCCATGCCAATTGCA-3′; for sodium-coupled neutral amino acid transporter (SNAT2), sense, 5′-GTGTCCTGTGGAAGCTGCTTTGA-3′ and antisense, 5′-CAGGTACAAGAGCTGTTGGCTGTGT-3′; for C/EBP-homology protein; (CHOP), sense, 5′-CATCACCACACCTGAAAGCA-3′ and antisense, 5′-TCAGCTGCCATCTCTGCA-3′; for vascular endothelial growth factor (VEGF), sense, 5′-AGCTCCAGAGAGAAGTCGAGGAAGA-3′ and antisense, 5′-TCACTTTGCCCCTGTCGCTTT-3′; for tribbles homolog 3 (TRB3), sense, 5′-TGGTACCAGCTCCTCTACG-3′ and antisense, 5′-GACAAAGCGACACAGCTTGA-3′; for cationic amino acid transporter 1 (CAT1), sense, 5′-TGCCATTGTCATCTCCTTCCTG-3′ and antisense, 5′-AGCTCTCCAACGGTGACATAGC-3′ and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense, 5′-TTGGTATCGTGGAAGGACTC-3′ and antisense, 5′-ACAGTCTTCTGGGTGGCAGT-3′. The PCR reactions were performed as described.[20] PCR for each of three samples was done in duplicate and the means ± the standard error of the means (SEM) between conditions were compared by Student's *t* test.

Immunoblotting

Total cell protein extract (30 μg/lane) was probed by immunoblotting,[20] using rabbit polyclonal antibodies against ATF4, ATF5, CCAAT/enhancer-binding protein β (C/EBP β), FOXA1, FOXA2, or FOXA3 (0.4 μg/mL). Bound secondary antibody was detected using an enhanced chemiluminescence kit (Amersham Biosciences, Piscataway, NJ).

Short-Interfering RNA (siRNA) Transfection

The indicated human siRNA reagents and DharmaFECT 4 transfection reagent were purchased from Dharmacon (Lafayette, CO). HepG2 cells were seeded in 12-well plates (2.5×10^5 cells/well) in MEM and grown for 16 hours. Transfection was performed according to Dharmacon using 3 μL of DharmaFECT-4 and 100–200 nM siRNA per well. At 24 hours following transfection, cells were rinsed with phosphate-buffered saline (PBS), given fresh MEM, and cultured for another 12 hours. The medium was then removed and replaced with control MEM, MEM containing 2 mM HisOH, or MEM containing 300 nM Tg. After 8 hours incubation, total RNA was analyzed by qRT-PCR.

mRNA Stability

HepG2 cells were used to assay mRNA turnover as described.[21] Quantitative RT-PCR analysis was used to measure the steady-state mRNA content of FOXA2, FOXA3, p21, or GAPDH.

Results

Regulation of FOXA3 Genes by Amino Acid Limitation and ER Stress

Hepatic FOXA3 mRNA levels are increased by a low-protein diet (LPD).[11] We tested the maternal and fetal hepatic response to an LPD during pregnancy and also included analysis of the *FOXA1* and *FOXA2* genes. As positive controls, increased mRNA content for the *ASNS* and *SNAT2* genes, which are activated by amino acid limitation,[20, 22] was documented in the dams on the LPD compared to the normal protein diet (NPD) (Fig. 1A). For the maternal liver tissue, FOXA1 mRNA expression was not responsive to the LPD, but both FOXA2 and FOXA3 mRNA was increased by 1.5–2.0 times the NPD control (Fig. 1A). For the fetal liver, the mRNA content for FOXA1 and FOXA3 were reduced and FOXA2 was unchanged in response to the LPD (Fig. 1B).

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HepG2 hepatoma cells were cultured for 8 hours in MEM alone, MEM containing 2 mMHisOH to activate the AAR pathway, or MEM containing 300 nM Tg to activate the UPR. The FOXA1 mRNA content was decreased following HisOH treatment and Tg (Fig. 2A). In contrast, the FOXA2 and FOXA3 mRNA levels were induced by HisOH treatment, but were only modestly increased (FOXA2) or unchanged (FOXA3) in Tg-treated cells. The data of Fig. 2B document that the response was the same regardless of whether the cells were deprived of histidine or treated with HisOH to trigger the AAR pathway. To monitor the time course of FOXA expression, HepG2 cells were subjected to HisOH or Tg treatment for 0–24 hours (Fig. 2C). The FOXA1 mRNA level slowly declined in control MEM medium over the 24-hour period, but was reduced more strongly in both HisOH and Tg treatment from 0–4 hours. Tg caused a greater decrease than HisOH thereafter. Reasons for the decline in FOXA1 in MEM may be related to the medium change at the start of experiment or to cell density. The stronger decline after Tg suggests that a lower expression of FOXA1 is associated with ER stress. After a 4-hour lag period, HisOH treatment induced the FOXA2 mRNA by almost 5-fold over the remainder of the 24 hours studied (Fig. 2C). Activation of the UPR caused a modest increase in FOXA2 expression, whereas FOXA3 mRNA content was unaffected by UPR activation, but was induced within 2 hours following HisOH treatment (Fig. 2C).

Mechanism of FOXA2 and FOXA3 mRNA Regulation

To determine if the increase in FOXA2 or FOXA3 mRNA was due to increased stability, HepG2 cells were incubated in HisOH for 12 hours and then transferred to fresh medium with or without HisOH in the presence of actinomycin D (ActD) to block further *de novo* synthesis (Fig. 3A). As a control,[21] p21 mRNA was shown to be significantly stabilized in the presence of HisOH (Fig. 3A). The decay rate of both FOXA2 and FOXA3 mRNA was slightly reduced by amino acid limitation, but not to a degree that could account for the increase in steady-state mRNA content. To determine if increased transcription was also a factor, HepG2 cells were treated with HisOH in the presence or absence of ActD (Fig. 3B). Inhibition of transcription blocked the HisOH-induced mRNA expression for both FOXA2 and FOXA3, but this effect could be indirect by blocking the synthesis of a factor required for FOXA gene expression. To determine if the FOXA3 promoter region was responsible for the amino acid responsiveness, a genomic fragment covering nucleotide −1000/+151 was inserted upstream of a Firefly luciferase reporter. HepG2 cells were transfected with this reporter plasmid and cultured in MEM or MEM containing 2 mM HisOH for 12 hours. The FoxA3 fragment exhibited strong promoter activity, but transcription was not increased by HisOH (data not shown).

Previous studies have documented that protein content may not correlate with increased mRNA following amino acid limitation.[23] To investigate the expression pattern of FOXA proteins, cells were cultured in MEM alone or MEM containing 2 mM HisOH for 0–24 hours (Fig. 4). The protein content of FOXA1 and FOXA2 was largely unchanged, whereas FOXA3 was increased at 8–24 hours following HisOH treatment.

Induction of FOXA2 and FOXA3 Is ATF4-Independent

Although there is evidence for ATF4-independent activation mechanisms following amino acid deprivation,[24] most transcriptionally induced genes are ATF4-responsive.[12] In addition, ATF5 is translationally controlled in a manner similar to ATF4,[25] and ATF5 overexpression can activate ATF4-target genes such as *ASNS*.[26] The induction of FOXA2 by amino acid deprivation was partially inhibited by ATF4 siRNA knockdown, whereas the induction of FOXA3 was largely unaffected by knockdown of either ATF4 or ATF5; a modest reduction by the combination was not statistically significant (Fig. 5A). These results suggested that amino acid limitation induces FOXA2 and FOXA3 expression through an ATF4-

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independent pathway. Analysis of ATF4 and ATF5 mRNA confirmed that the siRNA treatment successfully knocked down expression of both (Fig. 5C). It is worth noting that the ATF5 expression was also inhibited by ATF4 knockdown, consistent with a previous study.[25] Computer analysis of the FOXA3 gene locus revealed four sites similar in sequence to known ATF4-binding amino acid response elements (AARE), but in agreement with the siRNA results, chromatin immunoprecipitation assays revealed that none of the regions showed increased binding of ATF4 or C/EBPβ after amino acid deprivation (data not shown). As controls, the mRNA levels for ASNS and SNAT2, known to be ATF4-dependent, were also documented (Fig. 5B). Induction of the ASNS gene was completely blocked by ATF4 knockdown, but was largely unaffected by ATF5 knockdown (Fig. 5B). Given that ATF5 expression was also suppressed by the ATF4 knockdown, it cannot be established whether or not ATF5 alone is sufficient to activate the *ASNS* gene in the absence of ATF4. Induction of the *SNAT2* transporter gene by amino acid deprivation can occur in the absence of ATF4,[24] and consistent with that study, SNAT2 was only partially repressed by ATF4 knockdown and was not affected by ATF5 knockdown (Fig. 5B). The data of Fig. 5D complement the mRNA data (Fig. 5C) and document the decrease in ATF4 and ATF5 protein content after siRNA treatment.

C/EBPβ Is Important for the Induction of FOXA3

Friedman et al.[27] reported that FOXA3 expression is decreased in C/EBPβ knockout mice. To investigate whether or not the induction of FOXA2 and FOXA3 by amino acid deprivation was dependent on C/EBPβ, a C/EBPβ siRNA knockdown approach was used (Fig. 6A). The induction of C/EBPβ expression was strongly inhibited by the siRNA treatment (Fig. 6B). The induction of both FOXA2 and FOXA3 mRNA was statistically reduced by suppression of C/EBPβ, but the decline in FOXA2 and FOXA3 was only 30% and 50%, respectively (Fig. 6A). Hiemisch et al.[28] demonstrated that the FOXA3 gene contains a tissue-specific enhancer at about +16 kb. Linking that enhancer element to a luciferase reporter driven by the FOXA3 promoter did result in C/EBPβ-induced expression, but the element did not respond to amino acid limitation (data not shown).

FOXA2 and FOXA3 Are Not Required for the Induction of AARE-Containing Genes

Given that FOXA3 is a transcriptional activator, it was of interest to determine if it played a role in the regulation of known amino acid responsive genes. Genomic fragments containing the AARE sites from either the *ASNS* or the *SNAT2* genes were placed in front of the Firefly luciferase reporter gene.[20, 22] Overexpression of FOXA3 had little effect on either the basal or the amino acid-responsive enhanced transcription of either *SNAT2* or *ASNS* (data not shown).

As an alternative test for FOXA2 or FOXA3 action on other AARE-containing genes, siRNA was used to simultaneously knockdown both factors to avoid possible functional redundancy. The mRNA abundance of several amino acid-responsive genes was measured (Fig. 7A). Although the siRNA against FOXA2 or FOXA3 was effective (Fig. 7C), the induction of ASNS, TRB3, VEGF, and CAT1 mRNA levels by the AAR pathway was not affected (Fig. 7A). However, the induction of the SNAT2 transporter mRNA was inhibited by about 50% in the basal state (MEM medium) as well as during activation by the AAR pathway (Fig. 7A). When the experiments were repeated with siRNA for FOXA2 or FOXA3 individually, the results indicated that FOXA2 appears to contribute to the basal and the induced components of SNAT2 expression (Fig. 7B). Collectively, the data indicate that the FOXA family does not play a major role in the global AAR pathway, but that FOXA2 may impact specific genes, such as *SNAT2*.

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Discussion

The results described in the present study illustrate the differential regulation of the three FOXA genes by amino acid deprivation or ER stress and document the following novel observations. (1) The *in vivo* expression of FOXA2 and FOXA3 mRNA in maternal liver, but not fetal liver, is induced by feeding pregnant dams an LPD. (2) In HepG2 hepatoma cells, FOXA2 and FOXA3 mRNA levels increase during amino acid deprivation, largely due to elevated transcription. (3) ER stress, which triggers the UPR pathway, down-regulates the FOXA1 mRNA expression, has little or no effect on FOXA3 mRNA, and modestly increases the expression of FOXA2. (4) Although both FOXA2 and FOXA3 mRNA are induced by amino acid deprivation, only FOXA3 exhibits a significant increase in protein abundance. (5) The induction of FOXA2 and FOXA3 mRNA is not through the conventional ATF4-driven AAR pathway. Consistent with this latter observation, the FOXA3 gene does not appear to have a functional ATF4-responsive element within the gene locus. (6) The amino acid-dependent induction of FOXA2 and FOXA3 expression is partially dependent on C/EBPβ. (7) With the possible exception of FOXA2 action on the SNAT2 gene, FOXA2 and FOXA3 are not critical to the activation of most AARE-containing genes.

As an organ that contributes to nutrient homeostasis, the liver relies on a number of tissue-specific metabolic pathways controlled by liver-specific transcription factors, such as the FOXA proteins.[1] The present data extend the report by Imae et al.[11] showing that hepatic FOXA3 expression is up-regulated in mice deprived of protein. In our studies, a differential response to the AAR and the UPR pathways was observed for the FOXA genes and this selective expression under stress conditions may be indicative of their individual contributions to the regulation of specific target genes. The AAR data indicate that of the three members, FOXA3 responds to the greatest degree, especially at the level of protein expression. Given that the siRNA and overexpression studies indicate that FOXA3 is not a critical factor in the activation of currently recognized target genes of the AAR pathway, global expression studies will be necessary to establish the FOXA3 target genes during protein amino acid limitation.

Most of the genes that are induced by the AAR have an AARE, which is recognized by ATF4, in a self-limiting temporal program.[12, 20] The observations that the FOXA3 gene does not have an AARE sequence that binds ATF4 after amino acid limitation and that the induction of FOXA3 is not blocked by knockdown of ATF4, suggests that FOXA3 expression is increased by a nonconventional AAR pathway. Interestingly, the SNAT2 transporter[24] and insulin-like growth factor binding protein-1[29] genes can also be activated by amino acid limitation in an ATF4-independent manner. The signaling mechanism(s) by which these genes are activated are unknown, but it does not appear to require GCN2 detection of uncharged tRNA[29] or phosphorylation of elF2.[24] The partial suppression of FOXA2 and FOXA3 induction by siRNA against C/EBPβ, which is also induced by amino acid deprivation, [30] indicates that this factor may contribute to FOXA regulation, but the identification of the specific element will require further experimentation. The FOXA3 gene represents an excellent model through which to further investigate the ATF4-independent pathway.

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Abbreviations

AAR	amino acid response
AARE	amino acid response element
ActD	actinomycin D
ASNS	asparagine synthetase
ATF	activating transcription factor
CAT1	cationic amino acid transporter 1
C/EBP	CCAAT/enhancer-binding protein
CHOP	C/EBP-homology protein
eIF2α	eukaryotic initiation factor 2alpha
ER	endoplasmic reticulum
FOXA	forkhead box protein A
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
qPCR	quantitative real-time PCR
RT-PCR	reverse transcriptase polymerase chain reaction
SNAT2	sodium-coupled neutral amino acid transporter
TRB3	tribbles homolog 3
VEGF	vascular endothelial growth factor

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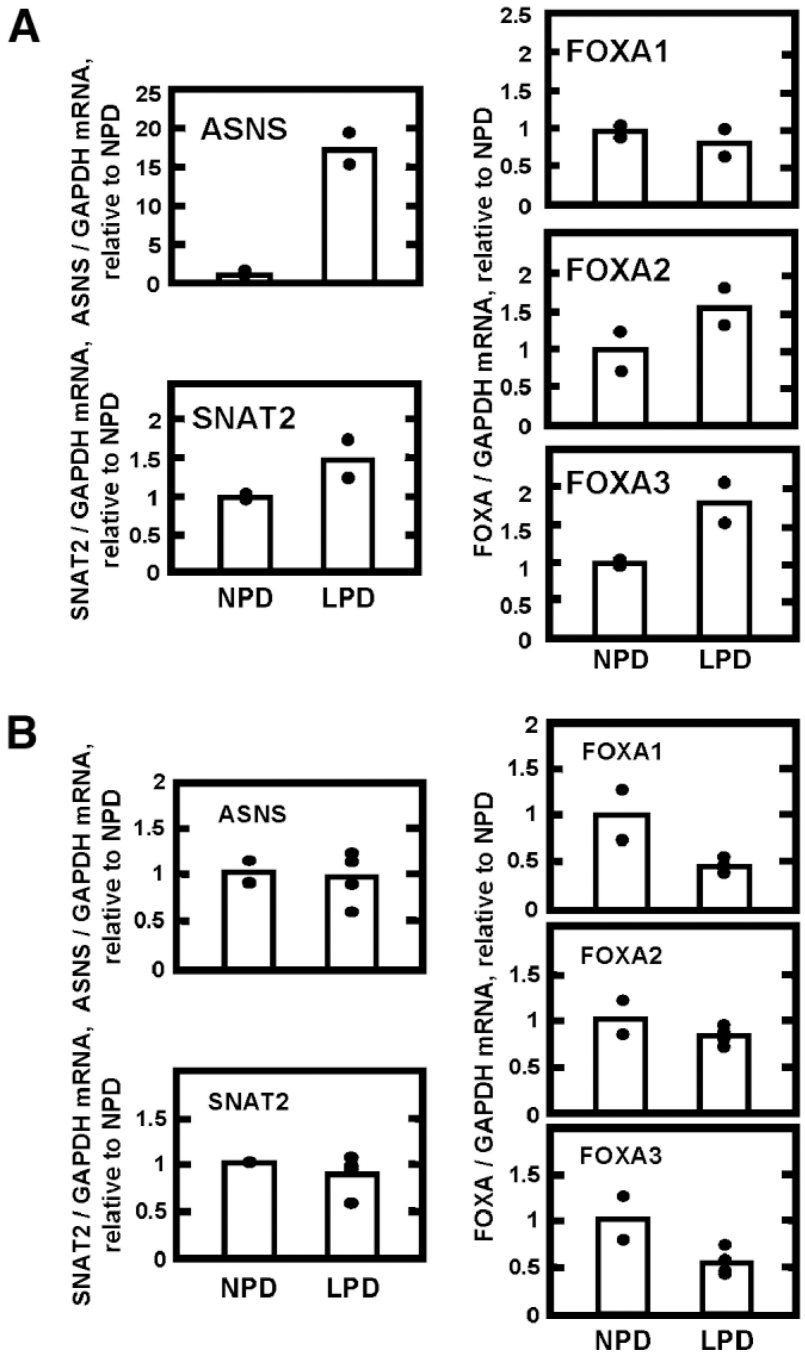

Fig. 1.

Regulation of the FOXA gene family by low-protein diet (LPD). Beginning at day 5 of pregnancy, dams were fed a normal protein diet (NPD) or pair-fed with an LPD and then the maternal (A) and fetal (B) livers were collected at day 18 of gestation. Total hepatic RNA was subjected to qRT-PCR analysis for mRNA content as indicated. The ratio of specific mRNA to the GAPDH control was calculated and the data are presented as the fold-induction relative to the NPD mice. The absolute values are illustrated as individual points and the bars illustrate the average of two to four mice in each group.

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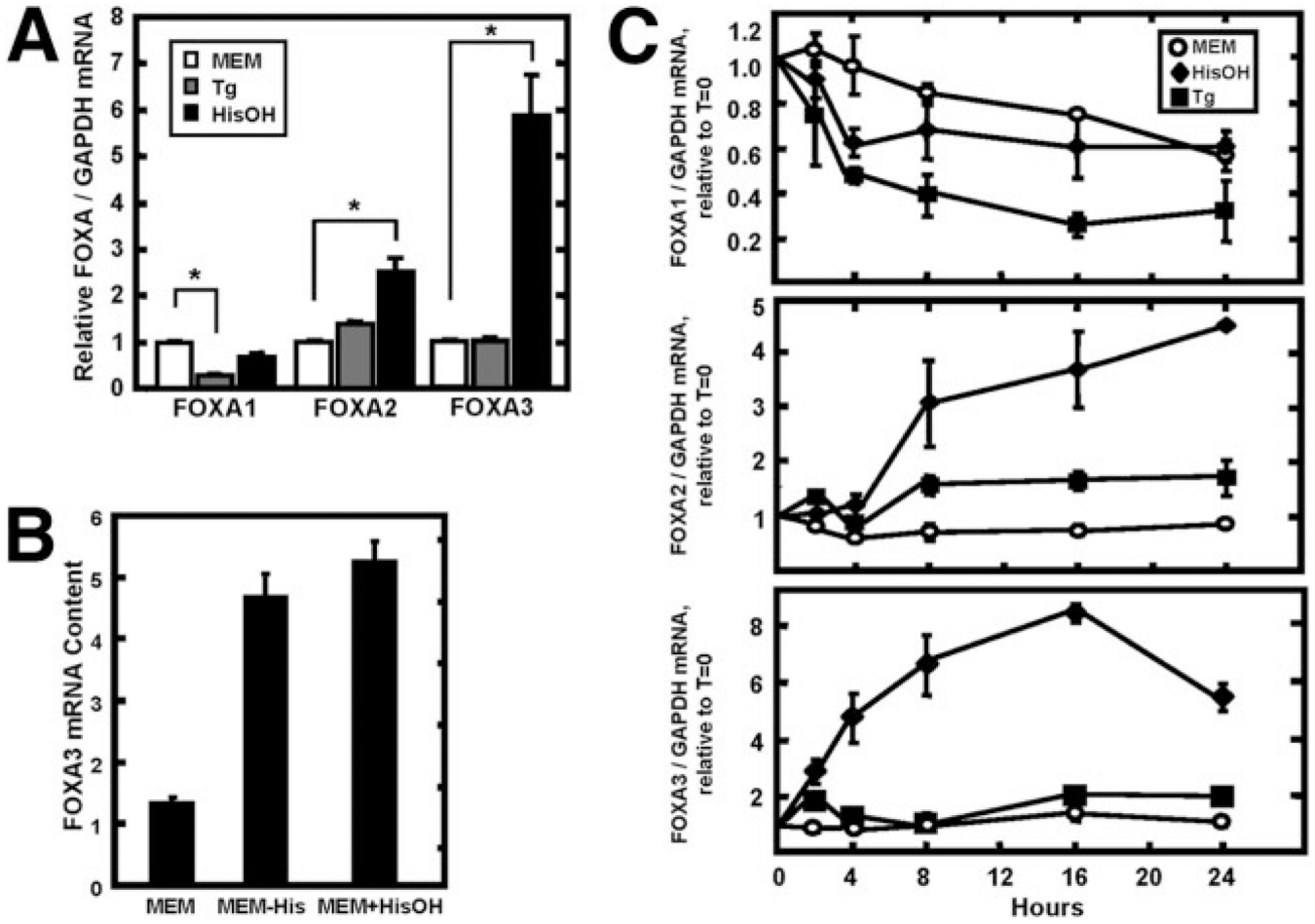

Fig. 2.

FOXA mRNA expression during amino acid deprivation and ER stress. (A) HepG2 cells were incubated in MEM, MEM containing 2 mM HisOH, or MEM containing 300 nM Tg for 8 hours. RNA was subjected to qRT-PCR analysis for mRNA content and the ratios of FOXA to GAPDH mRNA are presented as the fold-induction relative to the MEM control. The graph illustrates the means ± SEM for three independent experiments. Those values that are significantly different ($P < 0.05$) from the corresponding MEM control are indicated with asterisks. (B) HepG2 cells were incubated in MEM, MEM lacking histidine, or MEM containing 2 mM HisOH for 8 hours. RNA was subjected to qRT-PCR analysis for FOXA3 mRNA content. (C) HepG2 cells were incubated as described for Panel A, but for the times indicated, and then RNA was analyzed by qRT-PCR. Data are presented as the fold-induction relative to the zero time for each condition. The graph illustrates the means ± SEM for three independent experiments. Where not shown, the SEM bars are within the symbol.

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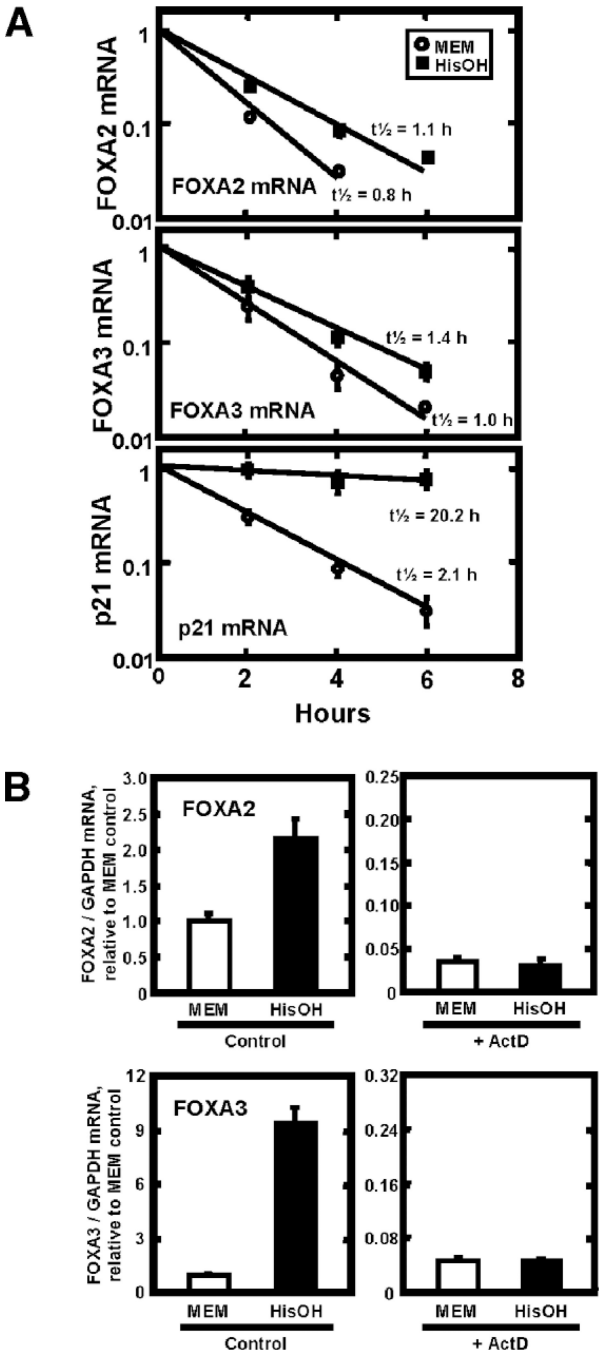

Fig. 3.

Role of mRNA stabilization and *de novo* transcription on the induction of FOXA2 and FOXA3 by amino acid deprivation. (A) HepG2 cells were incubated in MEM containing 2 mM HisOH for 12 hours to induce mRNA expression, then transferred to MEM or MEM containing 2 mM HisOH, each supplemented with 10 μg/mL of ActD. At the times indicated after the transfer, RNA was analyzed by qRT-PCR for FOXA2, FOXA3, or p21 mRNA. The ratio to GAPDH is given as the fold-induction relative to the zero time after transfer to the ActD media. The graphs illustrate the means ± SEM for three independent experiments. (B) HepG2 cells were incubated for 30 minutes in MEM without (control) or with ActD, and then transferred to MEM or MEM with 2 mM HisOH for 8 hours with or without ActD. The

ratio of FOXA mRNA to the GAPDH control is presented as the fold-induction relative to the MEM value in the control group (no ActD). Note the difference in the values in the presence or absence of ActD. The graph illustrates the means ± SEM for three independent experiments.

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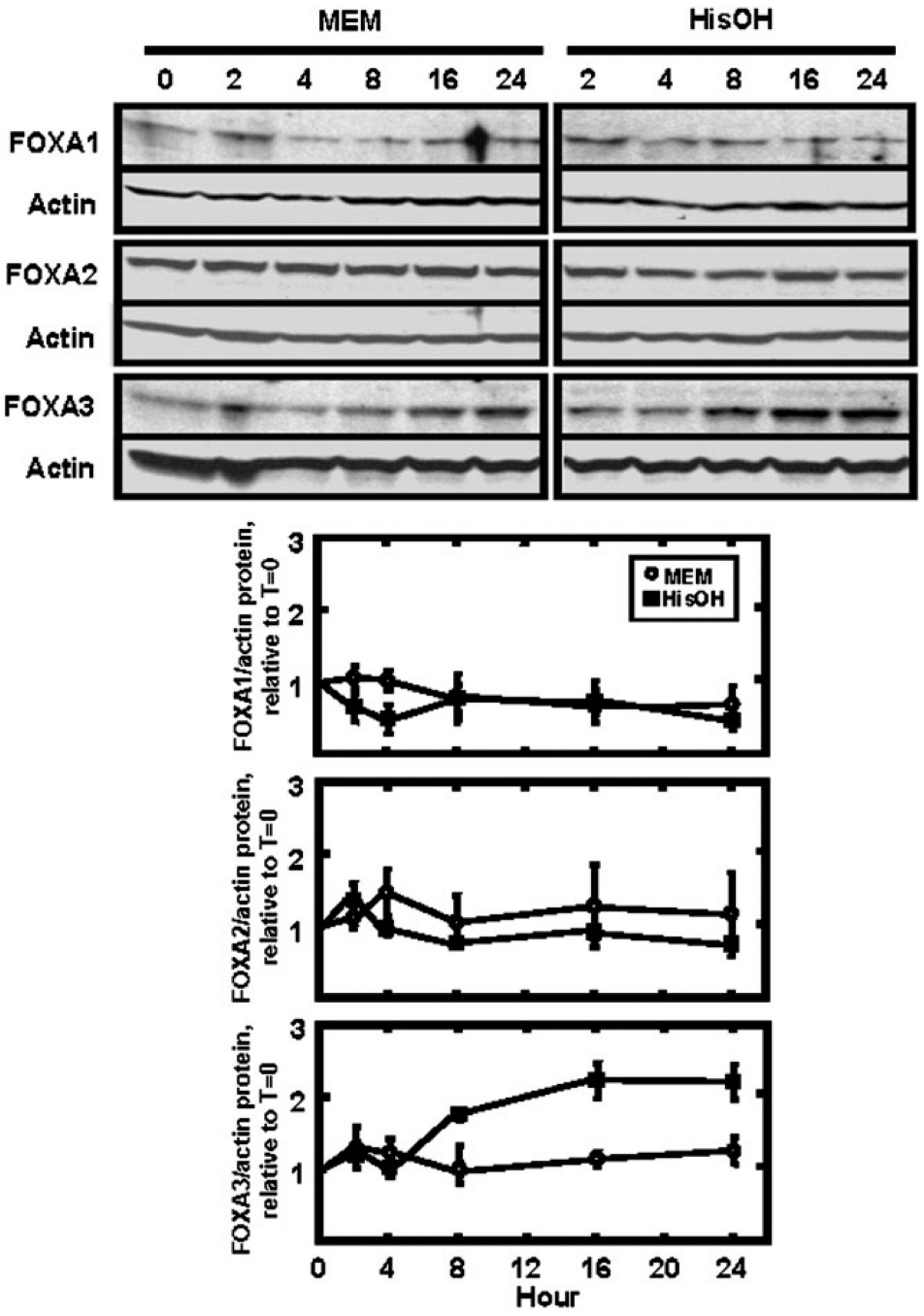

Fig. 4.

Time course of FOXA protein expression during amino acid deprivation. HepG2 cells were incubated in MEM, or MEM containing 2 mM HisOH and then protein extracts were subjected to immunoblot analysis. Quantified immunoblot data are plotted relative to the zero time value. The graphs illustrate the means ± SEM for three independent experiments.

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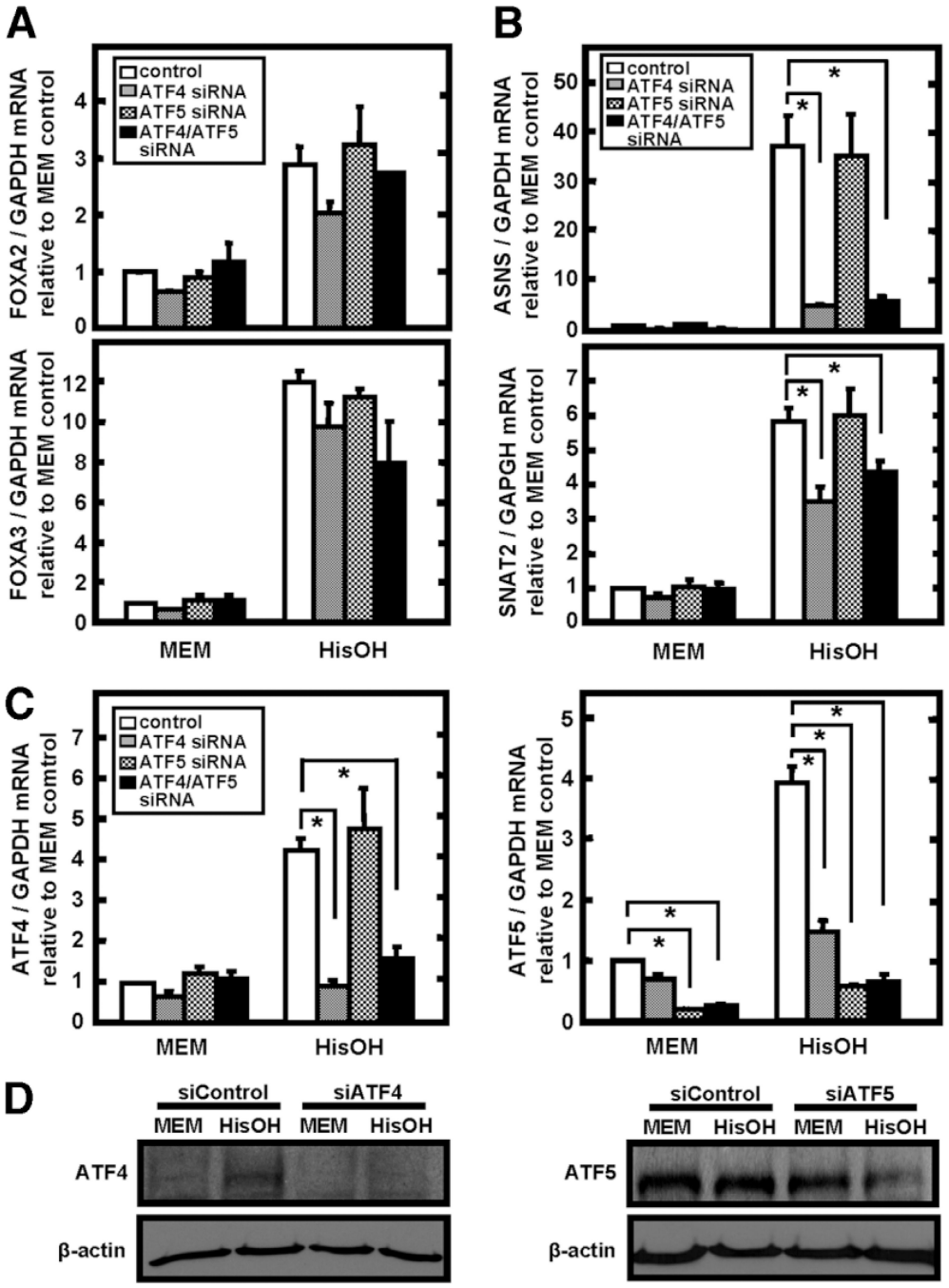

Fig. 5.

Effect of ATF4 and/or ATF5 knockdown on the expression of FOXA2 and FOXA3. HepG2 cells were transfected with either control siRNA or ATF4 and/or ATF5 siRNA. At 36 hours posttransfection, cells were incubated in MEM or MEM containing 2 mM HisOH for 8 hours. Total RNA was subjected to qRT-PCR for FOXA2, FOXA3 (A), ASNS, SNAT2 (B), ATF4, ATF5 (C), and GAPDH mRNA content. The data are presented by plotting the fold-induction relative to control (MEM, control siRNA). The graph illustrates the means ± SEM for three independent experiments. Those values that are significantly different ($P < 0.05$) from the corresponding control are indicated with asterisks. Immunoblotting for ATF4 and ATF5 protein was performed to illustrate the effectiveness of the siRNA treatment (D),

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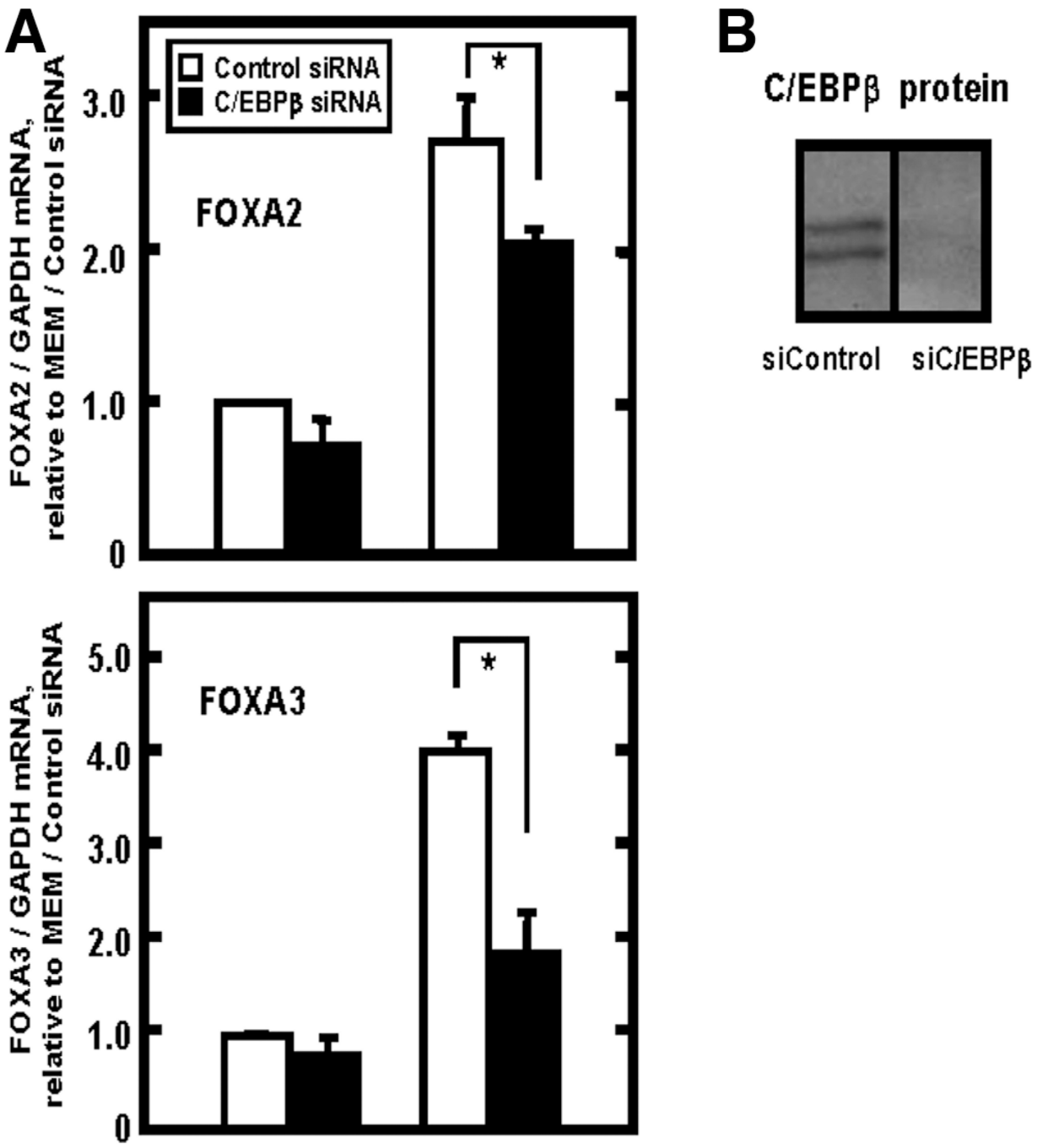

Fig. 6.

Effect of C/EBPβ knockdown on the expression of FOXA2 and FOXA3. HepG2 cells were transfected with either control siRNA or C/EBPβ siRNA. (A) At 36 hours posttransfection, cells were incubated in MEM or MEM containing 2 mM HisOH for 8 hours. The mRNA content is presented as the fold-induction relative to value obtained for the MEM and control siRNA. The graph illustrates the means ± SEM for three independent experiments. Those values that are significantly different ($P < 0.05$) from the control are indicated with asterisks. (B) Whole-cell extracts from control and siC/EBPβ treated cells were subjected to immunoblotting for C/EBPβ content.

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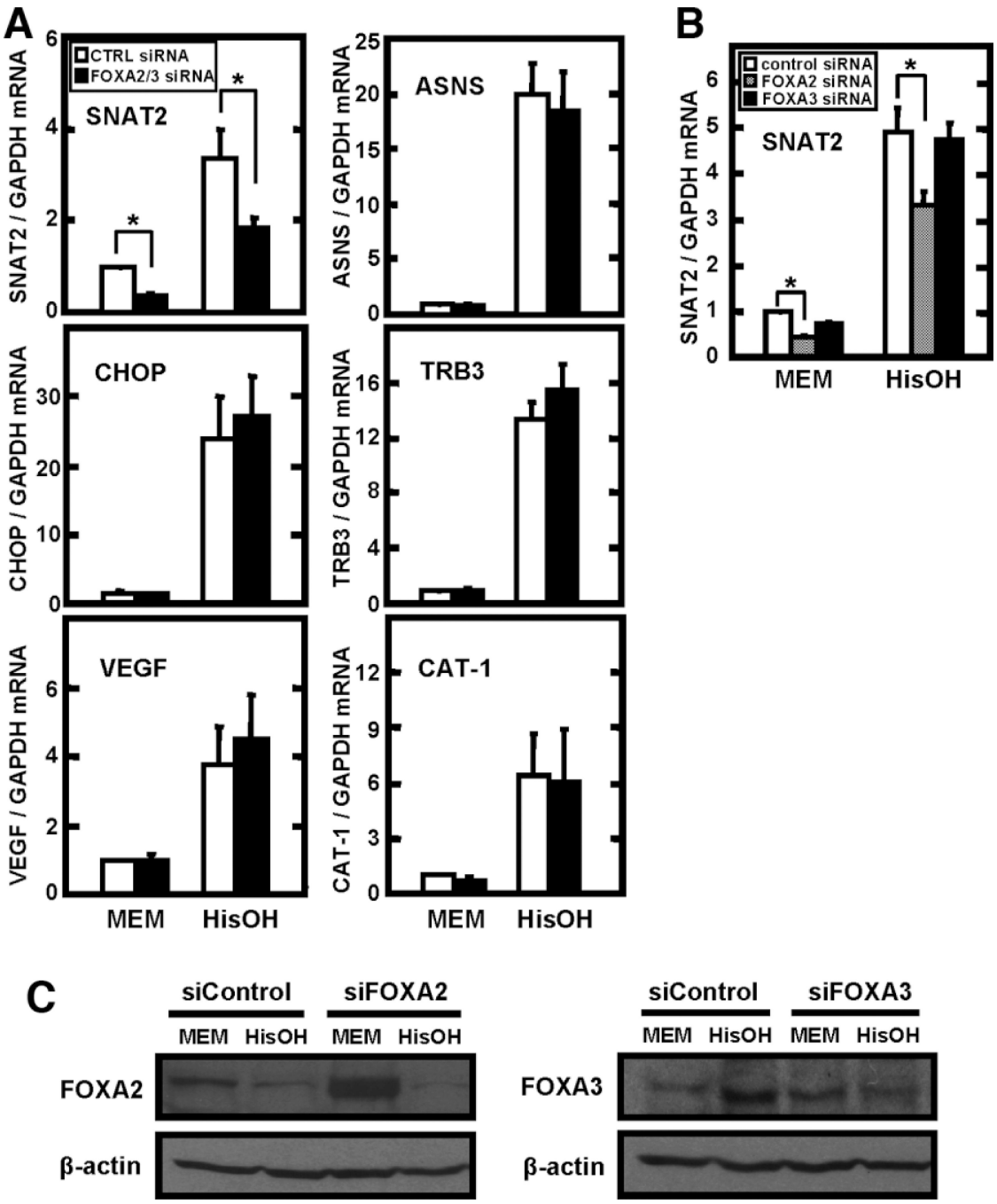

Fig. 7.

Effect of FOXA2 and FOXA3 knockdown on the expression of AARE-containing genes. HepG2 cells were transfected with either control siRNA or siRNA for FOXA2 and FOXA3 together (A) or separately (B). At 36 hours posttransfection, cells were incubated for 8 hours in MEM or MEM containing 2 mM HisOH. The mRNA content for ASNS, SNAT2, CHOP, TRB3, VEGF, and CAT1 (A) and GAPDH was analyzed. The data are presented as the fold-induction relative to the value obtained for MEM and control siRNA. The graph illustrates the means ± SEM for two (B) or three (A) independent experiments. Those values that are significantly different ($P < 0.05$) from the corresponding control are indicated with asterisks.

Immunoblotting for FOXA2 and FOXA3 protein was performed to illustrate the effectiveness of the siRNA treatment (C).

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