Effect of Glucosamine on Apolipoprotein AI mRNA Stabilization and Expression in HepG2 Cells

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Previously published studies suggest that an alteration in hexosamine flux induces a state of insulin resistance in muscle, liver, and other cell types. Glucosamine also alters the expression of several genes through an effect on transcription factors such as Sp1. Since the anti-atherogenic protein apolipoprotein AI (apoAI) is positively regulated by insulin, at least partly through its effect on Sp1, we investigated the effect of glucosamine on apoAI gene expression in the hepatocyte cell line, HepG2. By 24 hours of treatment with 0.1, 1, or 3 mmol/L glucosamine, the amount of apoAI protein secreted into the culture media increased 1.8-fold, 5.5-fold, and 2.3-fold, respectively. The decline in apoAI secretion at the highest glucosamine levels may be due to toxicity since the percentage of cells able to exclude trypan blue was lower in this group than in control cells (98.5% ± 1.5% in control cells *v* 89.2% ± 2.1% in cells treated with 3 mmol/L glucosamine, *P* < .01). ApoAI mRNA levels increased 2.4-fold in hepatocytes treated with 1 mmol/L glucosamine for 24 hours (1,158.1 ± 78.8 *v* 482.2 ± 24.3 arbitrary integrator units [AIU], *P* < .02), suggesting that the increase in apoAI protein secretion was due, at least partly, to an increase in apoAI mRNA levels. However, glucosamine had no effect on apoAI gene transcription rate as measured by nuclear runoff analysis (3,155 ± 46.0 in control cells *v* 3,181 ± 30.0 AIU in glucosamine-treated cells). Similarly, apoAI promoter activity measured in HepG2 cell transfected with an apoAI reporter plasmid containing the full-length apoAI promoter including an insulin-responsive Sp1 binding site did not change with glucosamine addition. In this assay, the chloramphenicol acetyltransferase (CAT) activity was 12.4% ± 3.1%, 10.1% ± 2.4%, 9.8% ± 2.0%, 9.7% ± 2.2%, and 11.9% ± 2.9% in cells treated with 0, 0.03, 0.1, 0.3, and 1 mmol/L glucosamine, respectively. The apoAI mRNA turnover studies showed that 1 mmol/L glucosamine treatment of HepG2 cells was associated with increased apoAI mRNA half-life, from 7.6 to 16.6 hours. These findings suggest that increases in apoAI gene expression by glucosamine occur primarily through stabilizing apoAI mRNA.

HIGH PLASMA LEVELS of apolipoprotein AI (apoAI), the primary protein component of the high-density lipoprotein (HDL) particle, are associated with a reduction in the risk of developing atherosclerosis.[1-3] However, in certain pathological conditions, such as obesity and type 2 diabetes, apoAI and HDL levels are suppressed.[4-7] Insulin resistance is common to both of these conditions and may play an important role in regulating apoAI gene expression since the apoAI gene is responsive to insulin.[8-10] The main pathophysiologic mechanism of reduced apo AI levels in diabetic and insulin resistant subjects is increased fractional clearance rate of apo AI.[7] However, additional mechanisms suppressing the expression of apo AI gene may play a role. The precise cause of apoAI gene downregulation in insulin resistance is not known, although several mechanisms have been hypothesized.

A potential mediator of insulin resistance is the metabolite glucosamine. Elevated glucosamine flux in muscle cells and hepatocytes, both in vitro and in vivo, is associated with changes in gene expression characteristic of insulin resistance, including a reduction in glucose transport activity.[11,12] The reduction in glucose transporter 4 translocation brought about by an elevation in glutamine: fructose-6-phosphate amidotransferase (GFAT) activity in transgenic mice can be reversed by treatment with insulin sensitizers like thiazolidinediones.[13] On the other hand, the Sp1 transcriptional activity and half-life are regulated by glycosylation on serine and threonine residues by N-acetylglucosamine, the primary end product of elevated glucosamine levels due to GFAT overactivity.[14,15] Activation of the insulin-responsive transcription factor Sp1 as a result of increased glucosamine levels accounts for changes in transforming growth factor-α[16] and plasminogen activator-1[17] gene expression. Since Sp1 is also implicated in the transcriptional modulation of apoAI gene by insulin,[10] and Sp1 activity can be altered by glucosamine,[14,15] we examined the effect of glucosamine, a putative mediator of insulin resistance on apoAI gene expression in HepG2 cells.

MATERIALS AND METHODS

Materials

RapidHyb, Redi-Prime labeling kits, Hybond nitrocellulose, and nylon membrane were from Amersham Pharmacia Biotech (Arlington Heights, IL). Acetyl-coenzyme A and glucosamine were from Sigma Chemical Co (St Louis, MO). Lipofectamine and Dulbecco's modified Essential medium (DMEM) were purchased from Life Technologies (Gaithersburg, MD). Radionuclides ([α-^{32}P]-dCTP and ^{14}C-chloramphenicol) were from New England Nuclear (Boston, MA). Fetal calf serum and antibiotics were purchased from BioWittaker (Walkersville, MD). All other reagents were from Sigma (St Louis, MO) or Fisher (Pittsburgh, PA).

Cell Culture

HepG2 cells were maintained in DMEM containing 5% fetal bovine serum and penicillin and streptomycin (100 U/mL and 100 μg/mL, respectively). Cells were maintained in a humidified environment at 37°C and 5% CO_2. To study the effect of glucosamine, HepG2 cells were cultured in glucose-free DMEM for 24 hours, followed by treatment with various concentrations of glucosamine for 24 hours. It is necessary to have glucose-free media when the effect of glucosamine

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treatment is studied because glucose will competitively inhibit glucosamine uptake by the cells.[18] To assure that the changes observed are not the result of a refeeding phenomenon, additional control experiments were carried out where HepG2 cells were incubated in serum- and glucose-free DMEM for 24 hours, at which time the media was changed and glucose was added to final concentrations of 0, 1.4, 2.8, 4.1, and 5.5 mmol/L. After 24 hours, the conditioned media was collected and apoAI protein levels were measured by Western blot analysis.

Western Blot Analysis

Conditioned cell culture media was collected and protein content determined using the Bradford assay[19] with bovine serum albumin as the standard. Five micrograms of secreted protein was fractionated by electrophoresis on a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel,[20] and transferred to nitrocellulose as previously described.[21] The membrane was blocked in 5% ovalbumin in phosphate-buffered saline (PBS) for 2-hours, and incubated with the anti-apoAI primary antibody (1:750) (Calbiochem, San Diego, CA) overnight, followed by the goat–anti-mouse IgG–horseradish peroxidase secondary (1:5,000) (Sigma). The membrane was then immersed in ECL (Amersham Pharmacia Biotech) and exposed to film. The amount of signal was quantified with a scanning laser densitometer (Molecular Dynamics, Sunnyvale, CA).

Northern Blot Analysis

Total RNA was isolated from HepG2 cells using the method described by Chomczynski and Sacchi.[22] Fifteen micrograms of RNA was fractionated by electrophoresis through a 1% agarose gel containing 2.2 mol/L formaldehyde, and transferred to a nylon hybridization membrane.[23] The apoAI cDNA probe was labeled with ^{32}P by oligolabeling,[24] hybridized to the RNA on the membrane, and washed under high-stringency conditions (0.1x standard saline citrate [SSC, 1x SSC is 0.15 mol/L NaCl, 15 mmol/L sodium citrate, pH 7.0], 0.1% SDS, 2 washes at 65°C, 30 minutes each). The membranes were exposed to film and the amount of signal quantified by densitometry. The membrane was stripped and hybridized to a ^{32}P-labeled probe for G3PDH in order to normalize mRNA levels. The total number for each group was 6.

Nuclear Runoff Transcription Assay

HepG2 cells in 75-cm^2 flasks were exposed in triplicate either to glucosamine (1 mmol/L) or an equivalent volume of dH_2O solvent for 24 hours. The culture medium was then removed and the cells were washed twice with PBS, removed by scraping, and collected by centrifugation at 500 × *g* for 5 minutes at 4°C. While vortexing, 4 mL of NP-40 lysis buffer (10 mmol/L *tris*-[hydroxymethyl]aminomethane hydrochloride [Tris-Cl], pH 7.4, 10 mmol/L NaCl, 3 mmol/L $MgCl_2$, 0.5% Nonidet P-40) was added, and vortexing continued for another 10 seconds. After a 5-minute incubation on ice, the nuclei were collected by centrifugation at 500 × *g* for 5 minutes at 4°C. The nuclear pellet was suspended in another 4 mL of NP-40 lysis buffer while vortexing as described above, and the nuclei collected by centrifugation at 500 × *g* for 5 minutes at 4°C. The pellet was then suspended in 200 μL of glycerol storage buffer (50 mmol/L Tris-Cl, pH 8.3, 40% glycerol, 5 mmol/L $MgCl_2$, and 0.1 mmol/L ethylenediaminetetraacetic acid [EDTA]) and stored in liquid nitrogen.

To perform the runoff reaction, 200 μL of nuclei were thawed and incubated with 200 μL of 2x reaction buffer (10 mmol/L Tris-Cl, pH 8.0, 2 mmol/L $MgCl_2$, 0.3 mol/L KCl, 1 mmol/L adenosine triphosphate, 1 mmol/L guanosine triphosphate, 1 mmol/L cytidine triphosphate, 5 mmol/L dithiothreitol) and 10 μL of [α-^{32}P]-uridine triphosphate (760 Ci/mmol, 10 mCi/mL), and incubated at 30°C for 30 minutes. Each reaction was then diluted in 0.6 mL of 10 mmol/L Tris-Cl, pH 7.4, 0.5 mol/L NaCl, 50 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$ containing RNase-free DNaseI (40 μL of a 1-mg/mL solution), and incubated at 30°C for another 5 minutes before the addition of 200 μL of 0.5% SDS, 0.5 mol/L Tris-Cl, pH 7.4, 0.125 mol/L EDTA, and 10 μL of 20 mg/mL proteinase K. The samples were incubated for 30 minutes at 42°C, and then extracted with phenol/chloroform/isoamylalcohol (25:24:1). To the samples, 2 mL of dH_2O and 10 μL of 10 mg/mL *Escherichia coli* tRNA were added prior to the addition of 3 mL of 10% trichloroacetic acid (TCA) containing 60 mmol/L pyrophosphate. Samples were incubated on ice for 30 minutes, the precipitate collected onto Whatman GF/A glass fiber filters (Clifton, NJ), and washed 3 times with 10 mL of 5% TCA, 30 mmol/L pyrophosphate. The filters were transferred to glass scintillation vials and incubated in RNase-free DNase I (37.5 μL of a 1-mg/mL solution per sample) in 20 mmol/L N-2 hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.5, 5 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, for 30 minutes at 37°C. The reaction was stopped by addition of 45 μL of 0.5 mol/L EDTA and 68 μL of 20% SDS, and heated to 65°C for 10 minutes. The RNA was transferred to a new tube while the filter was soaked in 1.5 mL elution buffer (1% SDS, 10 mmol/L Tris-Cl, pH 7.5, 5 mmol/L EDTA), for 10 minutes at 65°C, and combined with the previous tube. The samples were then digested with proteinase K (4.5 μL of a 20-mg/mL solution) for 30 minutes at 37°C, extracted with phenol/chloroform, isoamyl alcohol, and transferred to a 30 mL Corex tube (Corning Inc, Corning, NY). To the RNA, 0.75 mL of 1 mol/L NaOH was added, incubated on ice 10 minutes, then the pH adjusted by the addition of 1.5 mL of 1 mol/L HEPES (free acid). The RNA was precipitated by addition of 0.53 mL of 3 mol/L sodium acetate and 14.5 mL absolute ethanol, and then placed −20°C overnight, and collected by centrifugation at 10,000 × *g* for 30 minutes at 4°C. One milliliter of TES solution (10 mmol/L N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid [TES], pH 7.4, 10 mmol/L EDTA, 0.2% SDS) was added to the RNA, and when dissolved, 5 μL was counted in a scintillation counter. Equivalent CPM were prepared in a total volume of 1 mL TES solution, to which 1 mL of TES/NaCl (10 mmol/L TES, pH 7.4, 10 mmol/L EDTA, 0.2% SDS, 0.6 M NaCl) was added. The labeled RNA was hybridized to linearized plasmid probes immobilized on nylon filters (Hybond, Amersham) for 24 hours at 65°C. The membranes were then washed 2 times in 2x SSC for 1 hour each at 65°C. They were then incubated in 8 mL of 2x SSC containing 8 μL of 10 mg/mL RNase A at 37°C for 30 minutes, washed again in 2x SSC for 1 hour at 37°C, and exposed to film for autoradiography. The amount of signal in each spot was quantified by densitometry.

Plasmids and Transient Transfection Analysis

The effect of glucosamine on apoAI promoter activity was also examined by transient transfection analysis with the reporter gene plasmid pAI.474.CAT. This plasmid contains the apoAI gene promoter spanning nucleotides −474 to +7 bp (relative to the transcriptional start site). Construction of this plasmid has been described previously[25] and it has been used extensively to study apoAI promoter activity.[26-29] HepG2 cells were transfected with 1 μg of apoAI reporter plasmid and 1 μg of the plasmid pCMV.SPORT-β-gal (to normalize transfection efficiency) using Lipofectamine as described by the manufacturer. The plasmid pCMV.SPORT-β-gal (Life Technologies) expresses β-galactosidase under the control of the cytomegalovirus immediate-early promoter. After 24 hours, the culture media was replaced with glucose-free DMEM, with or without the indicated amount of glucosamine. After another 24 hours, the cells were collected and assayed for chloramphenicol acetyltransferase (CAT)[30] and β-galactosidase[31] activity. Cells treated with glucose containing DMEM were also used as additional controls to examine the effect of glucose-feeding on apoAI gene expression.

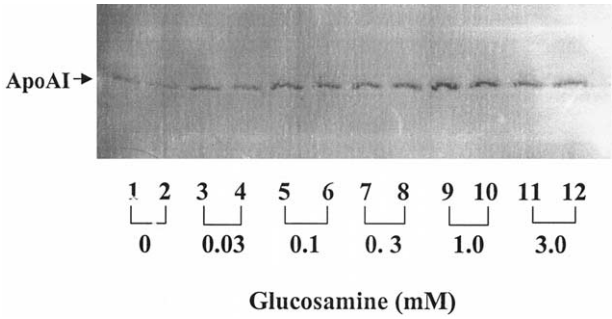

Fig 1. Effect of glucosamine on apoAI protein expression. HepG2 cells in glucose-free DMEM were treated for 24 hours with 0, 0.03, 0.1, 0.3, 1.0, and 3.0 mmol/L glucosamine. Steady-state apoAI protein levels were measured by Western blot analysis. The apoAI band is indicated with an arrow. Glucosamine increased apoAI protein in the culture medium in a dose-dependent fashion. (See Table 1 for quantitative data.)

Measurements of apoAI mRNA Turnover

ApoAI mRNA half-life was determined using actinomycin D as previously described.[32,33] Briefly, HepG2 cells were treated with 1 μg/mL actinomycin D in glucose-free DMEM, and 1 mmol/L glucosamine, and the cells were collected at 0, 4, 12, and 24 hours of treatment. Total RNA was isolated as described above and subjected to Northern blot analysis with the apoAI cDNA probe. ApoAI mRNA levels as percent of baseline at time zero were plotted against time on a semilogarithmic scale and curve fitting was performed using Statistica software (StatSoft, Tulsa, OK).

Statistics

All results are expressed as mean ± SEM. Analysis of variance (ANOVA) followed by the Neuman-Keuls procedure for subgroup analysis was performed using the statistical package Statistica for Windows (StatSoft). Significance was defined as a 2-tailed *P* value less than .05.

RESULTS

Effect of Glucosamine on apoAI Protein Secretion

A representative Western blot of the conditioned media of HepG2 cell cultures is shown in Fig 1. The amount of apoAI protein secreted into the culture medium increased in a dose-dependent fashion, to a maximum of 5.5-fold at 1.0 mmol/L glucosamine (Table 1). At 3.0 mmol/L glucosamine, apoAI levels were 2.3-fold higher than control cells. This decline in apoAI secretion at the highest glucosamine level may be due to glucosamine-related cell toxicity, since the percentage of cells able to exclude trypan blue was lower in this group than in control cells (98.5% ± 1.5% in control cells *v* 89.2% ± 2.1% in cells treated with 3.0 mmol/L glucosamine, $P < .01$).

In another set of experiments, the effect of glucose feeding on apoAI protein secretion was examined. HepG2 cells were incubated in serum- and glucose-free DMEM for 24 hours, at which time the media was changed and glucose was added to final concentrations of 0, 1.4, 2.8, 4.1, and 5.5 mmol/L. After 24 hours, the conditioned media was collected and apoAI protein levels were measured by Western blot analysis. ApoAI protein levels did not change with glucose addition (Table 1). These results suggest that the effect of glucosamine on apoAI protein levels is not due to a feeding effect.

Effect of Glucosamine on apoAI mRNA Levels

In order to determine if the increase in apoAI protein levels is due to an increase in apoAI mRNA, HepG2 cells were treated with 1.0 mmol/L glucosamine for 24 hours. Northern blot analysis with the apoAI cDNA (Fig 2A) shows that glucosamine treatment increases apoAI mRNA levels 2.4-fold (1,158.1 ± 78.8 *v* 482.2 ± 24.3 arbitrary integrator units [AIU], $P < .02$). No change in G3PDH mRNA levels was observed with glucosamine treatment (1,606 ± 32.1 *v* 1,561 ± 26.0 AIU, in glucosamine-treated cells and controls, respectively; Fig 2B). These results suggest that the increase in apoAI protein secretion observed in the Western blot is due, at least in part, to an increase in apoAI mRNA.

Effect of Glucosamine on apoAI Transcription Rate

Glucosamine treatment had no effect on either apoAI transcription or G3PDH transcription rate (Fig 3). In control cells, newly transcribed apoAI mRNA levels were 3,168 ± 75.2 AIU versus 3,106 ± 61.2 AIU in glucosamine-treated cells. The newly transcribed G3PDH mRNA levels were 5,624 ± 253.8 AIU versus 5,318 ± 282.3 AIU in control and glucosamine-treated cells, respectively. These experiments were repeated 3 times. These results indicate that glucosamine does not increase apoAI gene transcription.

Table 1. Effect of Glucosamine and Glucose on apoAI Protein Levels and Promoter Activity

Glucose (mmol/L)	apoAI Protein (AIU)	CAT Activity (%Acetylation)	Glucosamine (mmol/L)	apoAI Protein (AIU)	CAT Activity (%Acetylation)
0	1,054 ± 18.0	25.5 ± 1.5	0	423 ± 10.2	23.4 ± 1.4
1.4	1,047 ± 4.0	28.5 ± 0.4	0.03	763 ± 30.2*	24.4 ± 0.8
2.8	1,042 ± 14.0	28.8 ± 1.5	0.1	1,395 ± 28.6†	21.8 ± 1.0
4.1	1,030 ± 9.0	27.2 ± 0.9	0.3	1,480 ± 26.4†	22.1 ± 1.6
5.5	1,043 ± 20.0	25.5 ± 0.3	1.0	2,326 ± 62.4‡	23.7 ± 1.8
			3.0	973 ± 25.6*	ND

NOTE. HepG2 cells were exposed to the indicated concentrations of glucosamine or glucose for 24 hours prior to measurement of apoAI protein level by Western blot analysis and promoter activity by transient transfection analysis with the apoAI reporter gene construct. The results are expressed as mean ± SEM arbitrary integrator units (AIU) for the Western blot, and mean ± SEM % acetylation for CAT activity. N = 6.

Abbreviation: ND, not determined.

*$P < .05$, †$P < .01$, ‡$P < .001$.

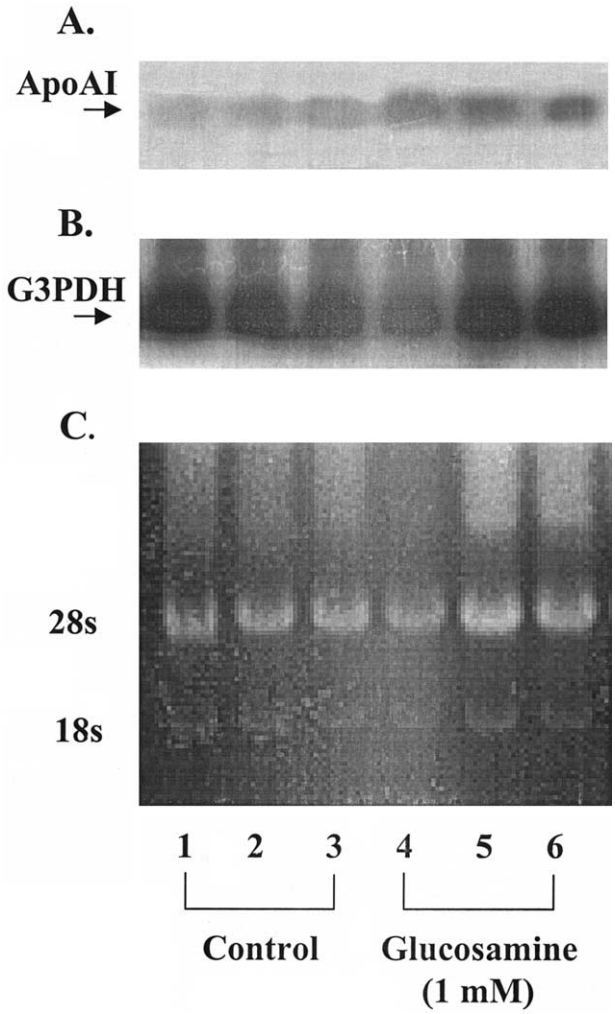

Fig 2. Effect of glucosamine on steady-state apoAI mRNA levels. HepG2 cells in glucose-free DMEM were treated with 1 mmol/L glucosamine in triplicate for 24 hours prior to RNA isolation and Northern blotting. The experiment was repeated twice. (A) Hybridization of the apoAI probe to its mRNA (indicated with an arrow), (B) that for the G3PDH probe. (C) The ethidium bromide stained gel with the corresponding 28 and 18s rRNA bands. Steady-state apoAI mRNA levels were increased in cells exposed to glucosamine; however, no changes were observed in G3PDH mRNA levels, indicating that the effect of glucosamine was specific.

Effect of Glucosamine on apoAI Promoter Activity

The effect of glucosamine on apoAI promoter activity was assessed using transient transfection analysis. As expected, mock-transfected cells (no plasmid DNA) had no CAT or β-galactosidse activity (Table 1), while CAT activity was present in control cells transfected with pAI.474.CAT. However, the amount of CAT activity did not change with glucosamine treatment, even at the highest doses (1.0 mmol/L) used (Table 1). Glucosamine also had no effect on β-galactosidase activity (data not shown).

To determine whether glucose feeding effects apoAI promoter activity, HepG2 cells were transfected with pAI.474.CAT and after 24 hours, treated with 0, 1.4, 2.8, 4.1, and 5.5 mmol/L glucose. CAT activity did not change with increasing concentrations of glucose (Table 1).

Effect of Glucosamine on apoAI mRNA Half-life

Since glucosamine increases steady-state apoAI mRNA levels without affecting its transcription rate, the effect of glucosamine on apoAI mRNA half-life was examined. The amount of apoAI mRNA was quantified by densitometry, and the mRNA levels as percent of baseline were plotted against time on a semilogarithmic scale (Fig 4). The estimated mean half-life of apoAI mRNA in glucosamine-treated cells was twice that measured in control cells (16.6 hours *v* 7.6 hours in control cells). No significant toxicity (assessed by trypan blue exclusion) was observed during the course of the experiment (data not shown). These results suggest that glucosamine increases apoAI mRNA levels in HepG2 cells by increasing its apparent half-life.

DISCUSSION

In order to determine the potential role of insulin resistance in modulating apoAI gene expression, we examined the effect of exogenous glucosamine, an intracellular mediator of insulin resistance, on apoAI gene expression in HepG2 cells. We had expected that apoAI gene expression would decrease in cells treated with glucosamine due to attenuation of insulin signaling. Alternatively, it is also possible that glucosamine, through glycosylation of Sp1,[14,15] increases apoAI gene transcription. However, the results of these studies show that treatment of cells with glucosamine neither increased or decreased apoAI gene transcription. Nevertheless, glucosamine increased apoAI protein expression in a dose-dependent fashion to a maximum of 5.5-fold at a glucosamine concentration of 1 mmol/L (Fig 1). This increase in apoAI protein expression was at least in part, due to an increase in apoAI mRNA levels (Fig 2). The increase in apoAI mRNA could not be attributed to an increase in

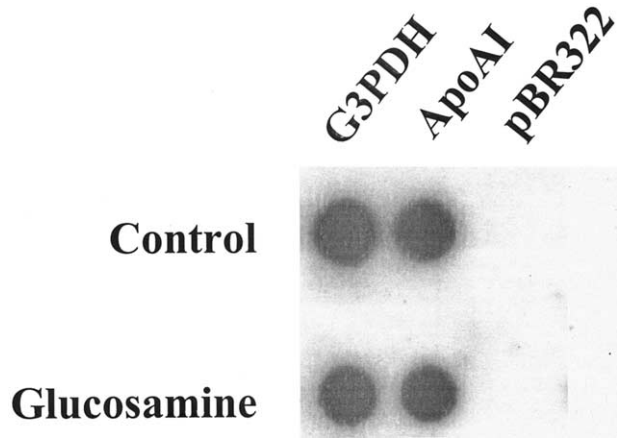

Fig 3. Effect of glucosamine on apoAI gene transcription rate. ApoAI mRNA was isolated from the nuclei of HepG2 cells either left untreated (control) or treated with glucosamine (1 mmol/L) for 24 hours. The labeled RNA was hybridized to immobilized cDNA probes for G3PDH, apoAI, and pBR322. Glucosamine treatment had no effect on apoAI transcription rate in HepG2 cells. These experiments were repeated 3 times.

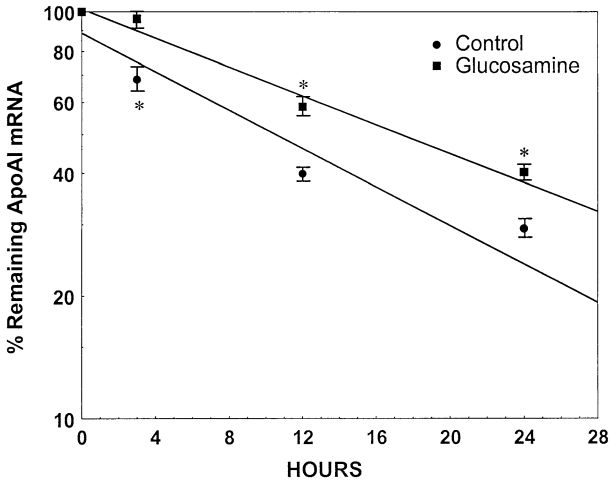

Fig 4. Effect of glucosamine on apoAI mRNA stability. HepG2 cells in glucose-free DMEM were treated with glucosamine (1 mmol/L) and 1 μg/mL actinomycin D and RNA isolated at the indicated times. ApoAI mRNA levels were measured by Northern blot analysis and followed by scanning densitometry. ApoAI mRNA levels as percent of baseline at time zero were plotted against time on a semilogarithmic scale. Mean ± SEM of n = 3 at each time point. ApoAI mRNA half-life was twice as long in glucosamine-treated cells compared to control cells (16.6 *v* 7.6 hours).

transcriptional rate of apoAI gene (Fig 3) or to alterations in apoAI promoter activity (Table 1), but appeared to be the result of prolongation of mRNA half-life from 7.6 to 16.6 hours (Fig 4). These effects were not due to simple availability of cellular substrate since apoAI protein levels and promoter activity did not change with exposure to up to 5.5 mmol/L glucose (Table 1). However, the increase in protein content (5.5-fold) was out of proportion to the increase in apoAI mRNA (2.4-fold). This suggests that there may be additional changes in either translational efficiency of the mRNA or protein-turnover rate that may contribute to the increase in apoAI expression.

The fact that glucosamine did not alter apoAI gene transcription was also unexpected since there are at least two Sp1 sites present in its promoter. One Sp1 site, located between nucleotides −390 to −410, is insulin-responsive and has been shown to bind phosphorylated Sp1 after exposure to insulin.[8-10] Another Sp1 site located immediately 5′ of site A, binds Sp1,[34] but is not insulin-responsive like the aforementioned Sp1 site. Both of these Sp1 sites are present in the full-length CAT reporter gene construct used in the studies described here. Changes in Sp1 phosphorylation and presumably glycosylation may alter apoAI gene promoter activity.[10] It is therefore surprising that treatment of cells with glucosamine, which can potentially alter Sp1 glycosylation, was not associated with any significant changes in transcription rate.

The human apoAI gene is located adjacent but convergent to the apolipoprotein CIII (apoCIII) gene[35] and both genes may be regulated in a coordinate manner by transcription factor sharing.[36,37] Furthermore, several Sp1 sites are located 5′ of the apoCIII gene and 3′ to the apoAI gene that may be important for regulating both genes.[38] These Sp1 sites are not present in the reporter gene construct used in our experiments. However, it is unlikely that these elements play a significant role in glucosamine induction of apoAI expression since the transcriptional rate of apoAI gene was not significantly altered (Fig 4).

Previously published studies have suggested that expression of the apoAI gene is regulated at multiple levels, including regulation at the translational level.[39-42] Conversely, several studies have demonstrated apoAI mRNA induction in the absence of changes in apoAI protein secretion.[37-41] However, few studies have reported changes in apoAI mRNA half-life as an important regulator of apoAI mRNA levels. In one such study, phenobarbital was shown to prolong apoAI mRNA half-life from 2.0 to 3.6 hours, as well as enhance transcription rate 2-fold in the hepatocyte cell line Hep3B.[43] The additive nature of these changes was suggested to account for the 4-fold change in apoAI mRNA observed by Northern blot analysis.[43] In another study the half-life of apoAI mRNA in zinc-depleted or -repleted HepG2 cells was estimated to be 44 hours.[44] The wide range of apoAI mRNA half-life reported in the literature may be due to differences in culture conditions and choice of immortalized hepatocyte cell lines (Hep3B *v* HepG2) used in different studies. It remains to be seen if different hepatocyte cell lines may have different responses to glucosamine treatment. In addition, future experiments with cells transfected with GFAT to increase endogenous glucosamine would be of interest.

In conclusion, glucosamine induces apoAI mRNA and protein levels by prolonging its mRNA half-life, without any effect on its transcription. It appears that increased intracellular content of glucosamine, a known mediator of insulin resistance, cannot account for the reduced expression of apoAI in clinical states of insulin resistance. The decline of apoAI levels in obese or diabetic insulin-resistant states could be secondary to other known mediators of insulin resistance such as tumor necrosis factor-α.[45]

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