Effects of Glucose and Insulin on Rat Apolipoprotein A-I Gene Expression*

Koji Murao‡§, Yoshinaru Wada‡, Takaaki Nakamura‡, Anthony H. Taylor‡, Arshag D. Mooradian†, and Norman C. W. Wong‡

From the ‡Endocrine Research Group, Departments of Medicine and Medical Biochemistry, the Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada and the ¶St. Louis Veterans Affairs Medical Center and Division of Endocrinology, Department of Internal Medicine, St. Louis University, St. Louis, Missouri, 63104.

We have examined the regulation of apolipoprotein A-I (apoA-I) gene expression in response to glucose and insulin. In Hep G2 cells, endogenous apoA-I mRNA was suppressed by one-half or induced 2-fold following 48 h of exposure to high concentrations of glucose (22.4 mM) or insulin (100 microunits/ml), respectively, compared with control. Transcriptional activity of the rat apoA-I promoter (−474 to −7) in Hep G2 cells paralleled endogenous mRNA expression, and this activity was dependent on the dose of glucose or insulin. Deletional analysis showed that a 50-base pair fragment spanning −425 to −376 of the promoter mediated the effects of both insulin and glucose. Within this DNA fragment there is a motif (−411 to −404) that is homologous to a previously identified insulin response core element (IRCE). Mutation of this motif abolished not only the induction of the promoter by insulin but also abrogated its suppression by glucose. Electrophoretic mobility shift assay analysis of nuclear extracts from Hep G2 cells revealed IRCE binding activity that formed a duplex with radiolabeled probe. The IRCE binding activity correlated with insulin induction of apoA-I expression. In summary, our data show that glucose decreases and insulin increases apoA-I promoter activity. This effect appears to be mediated by a single cis-acting element.

The serum protein, apolipoprotein A-I (apoA-I), has intrinsic antiatherogenic properties and is the major apoprotein component of the high density lipoprotein particles (1, 2). Increased abundance of apoA-I correlates inversely with the incidence of coronary arterial disease (3, 4). This beneficial feature of the protein arises from its pivotal role in a normal physiologic process called reverse cholesterol transport (5). ApoA-I mediates the transfer of cholesterol from extra-hepatic tissues to the liver for excretion from the body in the form of bile salts or free cholesterol (6). Enhanced reverse cholesterol transport lowers total body cholesterol.

Previous epidemiologic studies have shown a clear inverse correlation between levels of apoA-I and the incidence of coronary artery disease in both normal and diabetic individuals (7, 8). This protective feature of apoA-I is of primary importance in the clinical setting of diabetes mellitus (DM) because these patients have a 2–3-fold higher risk of developing premature arterial atherosclerosis compared with the general population (8). The increased risk of atherosclerosis often leads to premature death when the disease results in occlusion of the coronary or cerebral vessels.

One potential explanation for the higher risk of atherosclerosis in diabetics may be attributed to lower levels of apoA-I protein in these patients (9). Since hyperglycemia and deficient insulin action are pathognomonic features of DM, we wondered how these abnormalities affected expression of apoA-I. Regardless of whether the patient has insulin-dependent or non-insulin-dependent DM arising from hypo- or hyperinsulinemia, respectively, both diseases are associated with the enhanced risk of arteriosclerosis (10, 11).

To further understand the mechanisms by which glucose and insulin regulate levels of apoA-I, we have used both a cell culture and animal model to examine apoA-I expression in response to changes of these metabolic factors. The results of our studies show that glucose and insulin have opposite effects on activity of the apoA-I gene in vitro and in vivo. Furthermore, the repressive and stimulatory actions of glucose and insulin on apoA-I gene transcription appears to be mediated through a 50-bp fragment present in the apoA-I promoter.

MATERIALS AND METHODS

Plasmids—Construction of the pA1.474.CAT plasmid used in the transient transfection studies was described previously (12). Two constructs, pA1.425.CAT and pA1.375.CAT containing promoter segments −425 and −375 to −7, respectively, were synthesized using the parent pA1.474.CAT as a template in separate polymerase chain reactions. These reactions were primed with a unique 5′ oligonucleotide spanning −425 to −413 or −375 to −363, respectively, plus a common 3′ oligomer from −19 to −7. The mutant template was created in a similar fashion with the 5′ primer containing transverse mutations of all eight nucleotides in the putative insulin responsive core element (IRCE). Human apoA-I cDNA used in the Northern blots was a generous gift from J. L. Breslow, Columbia University, New York (13).

Cell Culture and Measurement of CAT Activity—The Hep G2, human hepatoma cells chosen for the studies express both a glucose transporter and the insulin receptor (14). These cells were maintained in RPMI-ISE supplemented with 1% fetal calf serum, as described previously (15). Cells were transfected with the plasmid of interest using the calcium phosphate precipitation method (16). Two μg of Rous sarcoma virus-β-galactosidase was added to all transfections to monitor the efficiency of DNA uptake by Hep G2 cells. Transfected cells were maintained in control media containing 5.5 mM for 24 h before treatment with con-
Insulin Increases ApoA-I Gene Expression

centrations of glucose up to 40 mM, 2-deoxyglucose (Sigma) up to 22.4 mM, or insulin (Humulin R, Eli Lily) ranging from 0 to 1000 micromolars/ml for 24 or 48 h. The only exception being the glucose dose response studies in which one set of cells was treated without glucose. Harvested cells were lysed by several cycles of freeze-thawing in a dry ice/ethanol slurry, and cellular debris was collected at 13,000 rpm for 5 min. An aliquot of the supernatant was taken for the measurement of β-galactosidase activity (17). The cytoplasmic fraction was heated at 60°C for 10 min to destroy endogenous deactylases, and the chloramphenicol acetyltransferase (CAT) activity was measured as described previously (18).

Animals—Male Sprague-Dawley rats weighing between 150–175 grams were purchased from Harlan Laboratories, Indianapolis, IN. In the fructose studies, the rats were fed a standard chow (caloric content was 62% complex carbohydrates, 23% protein, and 14.9% fat) or a high fructose diet (caloric content was 65.7% fructose, 21.9% protein, and 12.3% fat) purchased from Teklad Laboratories, Madison, WI as described previously (19). Serum for apoA-I protein determinations by Western analysis was collected by cardiac puncture prior to the induction of DM or inferior vena cava puncture at the time they were killed. Blood glucose and serum insulin levels were measured as described previously (21). The livers were excised and snap-frozen in liquid nitrogen for RNA extraction.

Northern Blot Analysis—Total RNA was isolated from Hep G2 cells or rat liver using a single step acid guanidinium phenol/chloroform extraction procedure (22). Aliquots of total RNA (10 μg) were separated electrophoretically on a denaturing 1% agarose gel containing 0.4 M formaldehyde (23). The separated RNA was transferred to a nylon membrane (Zeta Bind, Cuno AMF) and probed with a 32P-labeled human apoA-I cDNA for 18–24 h at 62°C as described previously (24). The 18 and 28 S ribosomal RNA bands were visualized by ethidium bromide staining to ensure that equal amounts of total RNA were applied to each lane.

Western Blot Analysis—Serum from experimental animals was separated by polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membrane (Millipore, Waters Corp.). The blot was probed with a polyclonal antibody the characteristics of which were described previously (25).

Electrophoretic Shift Mobility Assay—Nuclear extracts from Hep G2 cells were prepared by a technique described previously (26). Synthetic DNA duplexes spanning −419 to −388 (AATGCAAGCGAAGTTGACGCGGGTGTAAAGAAG, synthesized by Life Technologies, Inc.) and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) IRCE (AACTTTCCCGCCTCTCAGCCTTTGAAAG) used in these studies were radiolabeled at the 5' ends by incubating each strand separately with [γ-32P]ATP and polynucleotide kinase prior to the annealing. Each binding reaction of 20 μl contained 25 mM Hepes, 50 mM KCl, 1 mM EDTA, 0.5 mM spermidine, 0.6 mM dithiothreitol, 12% glycerol, 5 μg of poly(d-dC), 1 fmol of radiolabeled probe, and 20 μg of nuclear extract. In competition analysis, 50- or 300-fold molar excess of unlabeled homologous or non-homologous competitor DNA was added to the reaction prior to the addition of nuclear extracts. A sequence homologous to the IRCE from the glucagon (TAGTTTTTCACGCCTCTCAGC) gene served as a competitor. All reactions were incubated at room temperature for 20 min and then separated on a 6% polyacrylamide nondenaturing gel (27). Electrophoresis was performed at 10 volts/cm² for 3 h at 4°C. The gel was then dried and exposed to Kodak XAR-5 film at −80°C in the presence of intensifying screens.

RESULTS

Glucose and/or Insulin Effects on ApoA-I mRNA in Hep G2 Cells—To analyze the effects of glucose and insulin on apoA-I expression, we measured the levels of endogenous apoA-I mRNA in the human hepatoma cell line, Hep G2. Exposure of these cells to 22.4 mM glucose for 24 or 48 h decreased the abundance of endogenous apoA-I mRNA to one-half as compared with that in cells maintained in control media (Fig. 1). Cells exposed to 100 micromolars/ml of insulin for 48 h showed a 2-fold increase in apoA-I mRNA (Fig. 1). Treatment of the cells for 48 h with both factors at the concentrations quoted elicited an induction (1.3-fold) that was intermediate between that of glucose and insulin. Ethidium bromide staining of the 18 and 28 S rRNA bands showed no significant differences in the quantity of total RNA loaded into each of the lanes (data not shown). These results show clearly that glucose and insulin have opposite effects on the expression of apoA-I mRNA in Hep G2 cells.

Glucose and/or Insulin Effects on Rat Apo A1 Promoter in Hep G2—The preceding observations prompted us to measure transcriptional activity of the apoA-I promoter in the Hep G2 cells. For these studies, we have used the rat apoA-I promoter because it has been studied extensively and its similarity to the human counterpart (28, 29). The response of pAI.474.CAT to both 22.4 mM of glucose and/or 100 micromolars/ml of insulin in the Hep G2 cells is shown in Fig. 2A. Consistent with the observed changes in the level of endogenous apoA-I mRNA, 22.4 mM glucose inhibited promoter activity. Conversely, 100 micromolars/ml insulin stimulated CAT activity. In the presence of both factors, there was a small but not significant increase in CAT activity. The inhibitory actions of glucose were not due to an osmotic effect because exposure of the cells to 2-deoxyglucose (22.4 mM) did not inhibit activity of the promoter (results not shown). These findings indicate that activity of the rat apoA-I promoter in Hep G2 cells following exposure to glucose and insulin matched endogenous expression of apoA-I mRNA.

The optimal concentration of glucose or insulin leading to maximal suppression or induction, respectively, of apoA-I promoter activity was determined by assaying the activity of pAI.474.CAT in the presence of increasing doses of either factor (Fig. 2B). Activity of the template was inhibited in a dose-dependent manner by glucose with a maximal 50 ± 7% reduction in the presence of 22.4 mM glucose (Fig. 1). Insulin, on the other hand, increased CAT-activity in a dose-dependent manner, with a maximal 2 ± 0.2-fold induction at 100 micromolars/ml (Fig. 2). Results revealed that activity of pAI.474.CAT was maximally inhibited in the presence of 22.4 mM glucose, and the highest induction was observed following exposure to 100 micromolars/ml of insulin. Additionally, response of the promoter to either factor was dose-dependent. In the remainder of the transfection studies, we used the optimal doses of glucose or insulin as defined above.

DNA Motif That Mediated Effects of Glucose and Insulin—To locate cis-acting site(s) in the apoA-I promoter that mediated the effects of glucose and/or insulin, deletional templates were constructed by removing segments of the promoter contained in pAI.474.CAT. Constructs arising from the removal of 47 and 97 base pairs from pAI.474.CAT yielded templates, pAI.425.CAT and pAI.375.CAT, respectively, as shown schematically in Fig. 3A.

The activity of pAI.425.CAT in Hep G2 cells following exposure to glucose and/or insulin was identical to that of the full-length promoter construct, pAI.474.CAT (compare Fig. 3, B with C). Glucose inhibited the activities of pAI.474.CAT and pAI.425.CAT by a similar degree, 50 ± 7 and 55 ± 5%, respec-
tively. As expected, insulin stimulated the activity of pAI.474.CAT and pAI.425.CAT by the same amount, 2.6 ± 0.2- and 1.9 ± 0.1-fold, respectively. The combination of both glucose and insulin had an intermediate effect resulting in a small induction of pAI.474.CAT (1.4 ± 0.2-fold) and pAI.425.CAT (1.5 ± 0.1-fold). However, the pAI.375.CAT construct (Fig. 3D) containing the -325 to -376 fragment of the promoter was no longer responsive to either glucose or insulin. Together these observations show that glucose and insulin, respectively, represses and stimulates transcription of the apoA-I gene, and these effects require the -425 to -376 segment of the promoter.

**Insulin Response Element in Rat Apo A1 Promoter**—To further define cis-acting motif(s) in the rat apoA-I promoter that mediated the effects of glucose and insulin, we searched the promoter for known glucose or insulin response elements within the -425 to -376 fragment. Our search revealed an octanucleotide motif (CCCGCCTC, -411 to -404, Fig. 4) that is identical to the insulin core response element (IRCE) of the GAPDH gene (30) and shares an 88% homology with a similar IRCE found in domain A of the glucagon gene (31). No similarities to previously described carbohydrate response elements found in the L-pyruvate kinase and S14 genes were identified in the -425 to -376 fragment (32).

To show that the -411 to -404 motif mediated the effects of insulin, we created a construct, pmAI.425.CAT (Fig. 4A) that contained a mutated putative IRCE. The wild-type pAI.425.CAT construct produced CAT activities similar to pAI.474.CAT in HepG2 cells following exposure to 22.4 mM glucose, 100 microunits/ml insulin, or both factors (Fig. 4B, open bars). In contrast, the activity of the pmAI.425.CAT construct was no longer induced by 100 microunits/ml insulin (Fig. 4B, shaded bars). These data suggest that the CCCGCCTC motif in the wild-type promoter mediates the stimulatory effect of insulin. Unexpectedly, activity of the mutant template was not inhibited by glucose. These observations suggest the IRCE in
apoA-I DNA mediates the stimulatory actions of insulin and the same site may also mediate the inhibitory effects of glucose.

Effects of Glucose and/or Insulin on IRCE Binding Activity—Next we examined whether the actions of insulin or glucose affected the binding activity of nuclear proteins that interacted with the rat apoA-I IRCE. Nuclear proteins extracted from HepG2 cells treated with glucose or insulin were tested using an electrophoretic mobility shift assay (Fig. 5). IRCE binding activity in control Hep G2 cells bound to the radiolabeled probe to form complexes that migrated as a doublet (Fig. 5A). The intensity of the lower band in the doublet was not affected by treatment with glucose or insulin (Fig. 5A). In contrast, intensity of the upper complex was increased in cells treated either with insulin alone or in combination with glucose (Fig. 5A).

To ensure that the observed pattern of binding activity from Hep G2 cells was a specific interaction between putative nucleoproteins and the radiolabeled probe, electrophoretic mobility shift assays were performed in the presence of a 50- or 300-fold molar excess of unlabeled competitor DNA. Addition of the glucagon IRCE, which differed from the apoA-I IRCE by one nucleotide, did not decrease binding activity (Fig. 5B, lanes 2 and 3). In contrast, a 50-fold molar excess of the IRCE from the apoA-I or GADPH genes competed for binding to the radiolabeled apoA-I probe (Fig. 5C, lanes 2 and 4). Additional confirmation of these observations comes from studies of radiolabeled GAPDH IRCE tested in binding reactions. Results showed the formation of protein-DNA complexes identical to that of the apoA-I probe (Fig. 5C, lane 5). Furthermore, the binding activity to the GAPDH probe is abolished in the presence of 50-fold molar excess of GAPDH or apoA-I IRCE (Fig. 5C, lanes 6 and 8). Together these observations showed that in Hep G2 cells, the DNA binding activity that recognizes rat apoA-I IRCE is specific, and this activity is enhanced by the actions of insulin but not glucose. Furthermore, the protein(s) responsible for the binding activity recognizes the IRCEs from both apoA-I and GAPDH genes.

Effect of Hyperglycemia and Hyperinsulinemia on ApoA-I Expression in Vivo—Although in vitro experiments in cultured cells above have helped to elucidate the actions of glucose and insulin, whether the same effects of these factors extend to an in vivo model remains unanswered. The lack of animal models with features of either hyperglycemia or hyperinsulinemia alone makes it difficult to study these parameters in isolation. However, a model characterized by both hyperglycemia and hyperinsulinemia may be obtained by feeding fructose to rats (19). To ensure that fructose-fed rats did indeed have elevated levels of both insulin and glucose, the levels of these parameters were measured in the treated animals. In fructose-fed rats, blood glucose increased 30% from 7.4 ± 0.7 mM to 9.6 ± 0.8 mM, and insulin was elevated by 42% from 2.4 ± 0.5 to 3.4 ± 0.5 microunits/ml (Fig. 6, right panel).
The higher levels of glucose and insulin in the fructose-fed animals should increase apoA-I mRNA and protein, according to the in vitro model (Figs. 1 and 2). In agreement with this prediction, apoA-I mRNA was increased by 1.5 ± 0.3-fold, and apoA-I protein was increased by 2.2 ± 0.4-fold (Fig. 6, left panel). These findings show that glucose and insulin effects on apoA-I gene expression in rats is similar to that in cultured Hep G2 cells.

**DISCUSSION**

In this report, we have examined the regulation of apoA-I gene expression in response to glucose and insulin. Interest in this topic stems from the clinical observation showing that hyperglycemia arising from hypo/hyperinsulinemic states such as diabetes mellitus decreases the levels of apoA-I (33). Reduced abundance of this protein is believed to underlie the higher rates of coronary arterial disease (7, 8). Although previous clinical observations clearly document that hyperglycemia is associated with lower levels of apoA-I in diabetics, a suitable in vitro model for studying the mechanisms responsible for this effect is not available. Therefore, Hep G2 cells were chosen for the studies because they express apoA-I, insulin receptor, and glucose transporter (14).

When Hep G2 cells were exposed to high (22.4 mM) glucose concentrations, it reduced endogenous human apoA-I mRNA to one-half that in cells maintained in control media (5.5 mM). The importance of this finding is that the apoA-I lowering effect of hyperglycemia in vivo is also observed in the in vitro cell culture model. Additionally, the inhibitory effects of hyperglycemia on apoA-I is significant because eukaryotic genes repressed by glucose, excluding those found in yeast, are rare and include the Drosophila α-amylase, rat albumin, and the glucose transporter, GLUT-4 (20, 21, 34–36).

Insulin has the opposite effect to that of glucose and stimulates apoA-I expression in Hep G2 cells. Cells treated with insulin had 2-fold higher levels of the mRNA compared with controls. To our knowledge this is the first report showing that insulin increases apoA-I mRNA in liver-derived cells. Insulin has previously been shown to enhance the activity of several genes, such as (37), GAPDH (38), growth hormone (39), prolactin (40), and pancreatic amylase (41). Since response of endogenous apoA-I in Hep G2 cells to glucose and insulin parallels the behavior of this gene in vivo, these features make the cultured cells a useful model for dissecting the mechanisms of glucose and insulin action.

Both insulin and to a lesser extent, glucose are known to have direct effects on the transcription of many genes (42–45), but the nuclear mechanisms involved are incompletely understood. This study shows that the repression and induction by insulin and glucose, respectively occur at the transcriptional level. The CAT-activity of transfected Hep G2 cells decreased to 50% or increased by 2-fold relative to that in control cells following exposure to 22.4 mM glucose or 100 microunits/ml of insulin, respectively. Furthermore, the magnitude of the opposing effects of the two metabolic parameters was dose-dependent (Figs. 2 and 3). Deletional analysis of the promoter allowed the identification of a 50-bp DNA fragment in the promoter-spanning nucleotides –425 to –376 that is required for the effects of both glucose and insulin.

A motif identical to the IRCE found in the GAPDH promoter (30), and differs by 1 bp from that in the glucagon gene (31), is present in the –425 to –376 fragment of the rat apoA-I promoter. Site-directed mutagenesis of this motif abolished the response of the promoter to hormone and thus, confirmed that it mediated the actions of insulin (Fig. 4). Both the rat apoA-I and the GAPDH genes are induced by insulin, whereas the glucagon gene is repressed by insulin (30, 31, 46, 47). Whether the difference between the apoA-I and glucagon IRCE can switch it from an inducible to an inhibitory element is uncertain. Despite insulin-mediating properties and sequence similarities among the IRCE from the rat apoA-I, GAPDH and glucagon genes, one marked difference is that the apoA-I promoter is inhibited by glucose. Additionally, although the apoA-I and GAPDH IRCEs are identical, insulin induction of GAPDH appears to be more complex requiring the cumulative actions of at least three cis-acting elements: gTRE, IRE-A, and IRE-B (48). In contrast, the rat apoA-I IRCE alone appears to be the critical region that mediates the actions of the hormone.

In the preceding section, examples of IRCE with marked similarities were mentioned. However, the IRCE is not the only motif that mediates the actions of insulin because insulin responsive cis-acting elements that differ from the IRCE have been identified in several genes. For example, insulin stimulation of the promoters that regulate prolactin, thyroxine kinase, and somatostatin expression appear to be mediated by one or more CGGA motifs (49). The transcription factor(s) that bind to the CGGA motif in these genes are related to the Ets family of nuclear proteins, such as Sap and Elk-1 (49). The observed nucleotide differences between the apoA-I IRCE and insulin responsive element from other genes suggests that there exists more than one class of cis-acting elements which mediate the
effects of insulin. Additional support for this idea arises from the lack of homology between the IRCE and the negative insulin response element from the PEPCk gene (50). The existence of more than one type of insulin-regulated motif shows the complexities of gene control similar to the glucocorticoid response element and units found for example in the tyrosine aminotransferase and PEPCk genes (51, 52).

The rat apoA-I IRCE also appears to mediate the inhibitory effects of glucose. This possibility is supported by the absence of a recognizable carbohydrate response element within the −245 to −376 fragment and the unexpected finding that mutation of the IRCE abolishes the inhibitory effects of glucose. A similar but not identical scenario applies to l-pyruvate kinase (l-PK) gene. Previous studies suggest that the stimulatory actions of both glucose and insulin co-localize to a single motif (−168 to −144) present in the l-PK promoter (53, 54). More recent data are in keeping with the idea of a response unit comprised of several cis-acting elements that cooperate to mediate the actions of glucose and insulin (55). Although l-PK promoter response requires the presence of insulin, it does not appear to be the primary signal (56). Glucose seems to be more important, and its action is dependent on the binding of two transcription factors, LF-A1 and a c-myc related protein, to the carbohydrate response element located between −171 to −124 (57). An additional hepatic enriched factor, HNF-4, is also required as an accessory factor that functions with the carbohydrate response element (58).

Although it is tempting to speculate based on our data that a single motif mediates the actions of both glucose and insulin, this conclusion is likely premature. If so, then what are the potential explanations for our observation? Perhaps, the IRCE has a permissive effect on an adjacent carbohydrate responsive element. This means that the IRCE must be present to unmask the inhibitory actions of the adjacent element. Another possibility is that the IRCE may overlap with an element that mediates the effects of glucose. This possibility may explain why mutation of the IRCE also abolishes the activities of both glucose and insulin.

Gel retardation analysis of nuclear extract from Hep G2 cells treated with high glucose or insulin revealed IRCE binding activity that formed a doublet following electrophoretic separation. Nucleoproteins binding to the IRCE from hyperglycemic cells were no different from those in control cells, suggesting that glucose has no effect on this activity. However, insulin alone or in combination with hyperglycemia increased the abundance of the upper band in the doublet. These findings suggest that insulin alone increases IRCE binding activity. The observation that glucose does not modulate IRCE binding activity supports the idea that the IRCE may serve in a permissive role for the inhibitory effects of glucose or the possibility of overlapping elements. That insulin alone increases the activity of nucleoproteins which bind to the apoA-I IRCE is not unexpected. Previous studies of insulin-treated NIH-3T3 cells or an animal model of raised insulin levels produced by fasting followed by re-feeding showed increased binding activity to the GADPH IRCE (30). The rise in binding activity correlated with expression of GAPDH. The nuclear factor, IRF-ABP, believed to bind the IRCE from GAPDH has been cloned and is known to be related to mouse SRY (59, 60). The possibility that the apoA-I IRCE binding activity may be related to SRY is further supported by the finding that the GAPDH IRCE motif inhibits complex formation (Fig. 5C) and that the pattern of binding to both radio-labeled apoA-I and GAPDH IRCE are identical (Fig. 5C).

Although in vitro experiments in cultured cells above allow us to study the actions of glucose and insulin, whether the same effects of these metabolic factors extend to an in vivo model is difficult to test. The reason being that in animal models of DM, some insulin must be present or the animal will die from ketoacidosis. Conversely, hyperinsulinemic animals must have some glucose or they will die from hypoglycemia. Thus an animal model of either hyperglycemia or hyperinsulinemia alone is not available. However, an opportunity to extend the Hep G2 cell culture conditions to an in vivo model of both hyperglycemia and hyperinsulinemia, is possible. The fructose fed rat reflects a model of combined hyperglycemia and hyperinsulinemia. Increased levels of hepatic apoA-I protein and mRNA in these animals parallel closely the observed effects of glucose and insulin in vitro on Hep G2 cells. These results demonstrate clearly that the combined effects of glucose and insulin in both in vivo and in vitro are the same.

In summary, the results in this study show that glucose inhibits and insulin stimulates the expression of the endogenous apoA-I mRNA in human Hep G2 cells. The opposite effects of these metabolic parameters on mRNA levels arises from their actions on transcriptional activity of the apoA-I promoter in these cells. Although the actions of glucose and insulin are mediated by the same DNA fragment, we only identified one motif that matches a previously described IRCE and none that resembles any of the known carbohydrate response elements. The nuclear proteins from Hep G2 cells that bind to the cis-acting site which mediate the effects of glucose and insulin increased following exposure to insulin but not glucose. The ability of insulin to enhance apoA-I gene expression reinforces the recommendation for tight glycemic control to reduce the risk of atherosclerotic disease in diabetic patients.

REFERENCES

1. Forte, T. M., and McColl, M. R. (1994) Curr. Opin. Lipidol. 5, 354–364
2. Barber, P. J., and Ryek, A. (1996) Atheroscler. 121, 1–12
3. Barr, D. P., Russ, E. M., and Eder, H. A. (1951) Am. J. Med. 11, 480–493
4. Miller, G. J., and Miller, N. E. (1975) Lancet 1, 16–19
5. Miller, N. E., Laville, A., and Creek, D. (1985) Nature 314, 109–111
6. Guyton, A. C., and Hall, J. E. (1996) Textbook of Medical Physiology, 9th Ed., W. B. Saunders Co., Philadelphia, PA.
7. Sniderman, A. R., Michel, C., Racine, N., (1992) J. Clin. Epidemiol. 45, 1357–1370
8. Stamler, J., Vacecko, O., Neaton, J. D., and Wentworth, D. (1993) Diabetes Care 16, 434–444
9. Lakoski, M., Voutilainen, E., Pivoralu, K., and Sarlund, H. (1985) Arteriosclerosis 5, 655–658
10. Despres, J. P., Lamarche, B., Mauriege, P., Cantin, B., Dagenais, G. R., Morelock, S., and Lusis, A. (1996) J. Biol. Chem. 271, 2745–2752
11. Solyomos, B. C., Marcil, M., Chaur, M., Gilf, B. M., Poitras, A. M., and Canpeau, L. (1995) Am. J. of Cardiol. 76, 1152–1156
12. Romero, J. S., Chan, J., Carr, F. E., Mooradian, A. D., and Wong, N. C. W. (1992) Mol. Endocrinol. 6, 1124–1132
13. Breslow, J. L., Ross, D., McPherson, J., Williams, H., Kurnit, D., Nussbaum, A. L., Karathanasa, S. K., and Zannis, V. I. (1982) Proc. Natl. Acad. Sci. U. S. A. 7992, 6691–6695
14. Hatada, E. M., McClain, D. A., Potter, E., Ullrich, A., Olefsky, J. M. (1989) J. Biol. Chem. 264, 6741–6747
15. Nakabayashi, H., Hashimoto, T., Miyao, Y., Tjong, K. K., Chan, J., and Kurokawa, H. (1995) Mol. Cell. Biol. 171 to 174
16. Watmuff, J., Herbst, K., and Kurokawa, H. (1993) J. Biol. Chem. 434–444
17. Garman, C. M., Moffat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1044–1051
18. Reaven, G. M. (1991) Diabetes Care 14, 195–202
19. Kintz, A. W., and Wong, N. C. W. (1996) J. Biol. Chem. 271, 9890–9975
20. Gross, J. M., Przybylez, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
21. Wong, N. C. W., and Oppenheimer, J. H. (1986) J. Biol. Chem. 261, 10387–10393
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Taylor, A. H., Raymond, J., Dione, J. M., Romony, J. S., Chan, J., Lawless, D. E., Wanke, I. E., and Wong, N. C. W. (1996) J. Lipid Res. 37, 2232–2243
24. Dyer, R. B., and Heroz, N. K. (1995) BioTechniques 19, 192–195
25. Wong, N. C. W., Perez-Castillo, A., Sanders, M. M., Schwartz, H. L., and Oppenheimer, J. H. (1999) J. Biol. Chem. 267, 4465–4770
26. Haddad, A., Ordovas, J. M., Fitzpatrick, T., and Karathanasa, S. K. (1996) J. Biol. Chem. 261, 13268–13277
27. Higuichi, K., Law, S. W., Hoge, J. M., Schumacher, U. K., Meglin, N., and
Insulin Increases ApoA-I Gene Expression

30. Nasrin, N., Ercolani, L., Denaro, M., Kong, X. F., Kang, I., and Alexander, M. (1990) Proc. Natl. Acad. Sci. 87, 5273–5277.
31. Philip, J. (1991) Proc. Natl. Acad. Sci. 88, 7224–7227.
32. Towle, H. C. (1995) J. Biol. Chem. 270, 23235–23258.
33. Barrett-Connor, E., Philip, T., and Khaw, K. T. (1987) Am. J. Prev. Med. 3, 206–210.
34. Flores-Riveros, J. R., Kaestner, K. H., Thompson, K. S., and Lane, M. D. (1993) Biochem. Biophys. Res. Commun. 194, 1148–1154.
35. Magoulas, C., Bally-Cuif, L., Loverre-Chyurlia, A., Benkel, B., and Hickey, D. (1993) Genetics 134, 507–515.
36. Hickey, D. A., Benkel, K. I., Fong, Y., Benkel, B. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11109–11112.
37. Meisler, M. H., and Howard, G. (1989) Annu. Rev. Physiol. 51, 701–714.
38. Alexander-Bridges, M., Ercolani, L., Kong, X. F., Gierse, L., Nasrin, N. (1992) Adv. Enzyme Regul. 32, 149–159.
39. Jacob, K. K., Ouyang, L., Stanley, P. M. (1995) J. Biol. Chem. 270, 27775–27779.
40. O'Brien, R. M., Lucas, P. C., Forest, C. D., Magnuson, M. A., and Granner, D. K. (1999) Science 249, 533–537.
41. Jantzen, H. M., Strahie, U., Forrest, C. D., Schmid, W., Boshart, M., Mikiieck, R., and Schutze, G. (1987) Cell 49, 29–38.
42. Noguchi, T., Inoue, H., and Tanaka, T. (1985) J. Biol. Chem. 260, 14393–14397.
43. Liu, Z., Thompson, K. S., and Towle, H. C. (1993) J. Biol. Chem. 268, 12787–12795.
44. Towle, H. C. (1995) J. Biol. Chem. 270, 23235–23238.
45. Nasrin, N., Ercolani, L., Kong, X. F., Gierse, L., Nasrin, N. (1992) J. Cell. Biochem. 48, 129–135.