Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI in Rat Hepatocytes*

Received for publication, February 20, 2001
Published, JBC Papers in Press, February 28, 2001, DOI 10.1074/jbc.M101557200

Brennon L. O’Callaghan‡, Seung-Hoi Koo‡, Yue Wu§, Hedley C. Freakes§, and Howard C. Towlé‡‡

From the ‡Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, Minnesota 55455 and the §Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06269

The rat acetyl-CoA carboxylase (ACC) α gene is transcribed from two promoters, denoted PI and PII, that direct regulated expression in a tissue-specific manner. Induction of ACC, the rate-controlling enzyme of fatty acid biosynthesis, occurs in the liver in response to feeding of a high carbohydrate, low fat diet, conditions that favor enhanced lipogenesis. This induction is mainly due to increases in PI promoter activity. We have used primary cultured hepatocytes from the rat to investigate glucose regulation of ACC expression. Glucose and insulin synergistically activated expression of ACC mRNAs transcribed from the PI promoter with little or no effect on PII mRNAs. Glucose treatment stimulated PI promoter activity in transfection assays and a glucose-regulated element was identified (−126/−102), homologous to those previously described in other responsive genes, including i-type pyruvate kinase, S₄, and fatty acid synthase. Mutation of this element eliminated the response to glucose. This region of the ACC PI promoter was able to bind a liver nuclear factor designated ChoRF that interacts with other conserved glucose-regulated elements. This ACC PI element is also capable of conferring a strong response to glucose when linked to a heterologous promoter. We conclude that induction of ACC gene expression under lipogenic conditions in hepatocytes is mediated in part by the activation of a glucose-regulated transcription factor, ChoRF, which stimulates transcription from the PI promoter. Similar mechanisms operate on related genes permitting the coordinate induction of the lipogenic pathway.

Feeding a high carbohydrate, low fat diet to rodents increases the expression of genes encoding enzymes involved in lipogenesis, the conversion of carbohydrate to triglycerides, in the liver and adipose tissue (for review, see Refs. 1 and 2). These include the central enzymes of fatty acid biosynthesis: ATP-citrate lyase, acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). In addition, enzymes of glycolysis, such as pyruvate kinase (i-PK); of NADPH generation, such as malic enzyme; of fatty acid maturation, such as stearoyl-CoA desaturase and of triglyceride formation, such as glycerol-3-phosphate acyltransferase are induced in response to dietary carbohydrate. For most of the genes examined, transcription is the major site of control, although changes in stability play a role in controlling mRNA induction for certain gene products.

Both insulin and glucose play a role in the process by which high carbohydrate diet induces lipogenic gene expression. The actions of insulin are mediated at least in part by SREBP-1c (for review, see Refs. 3–5). This basic/helix-loop-helix/leucine zipper transcription factor is induced at the level of transcription in response to insulin (6–8). In turn, SREBP-1c binds to the promoters of a number of the lipogenic enzyme genes to enhance their expression (e.g. Refs. 9–11). Consequently, mice bearing a homozygous deletion of the SREBP-1 gene show an impaired ability to induce lipogenic enzymes in response to a high carbohydrate diet compared with normal littermates (12). Similarly, transgenic mice overexpressing the nuclear form of SREBP-1c in their liver have elevated rates of lipogenesis and increased mRNA levels for many of the lipogenic enzyme genes (13, 14).

In addition to insulin, glucose also provides an important signal for induction of the lipogenic enzyme genes (15). Primary hepatocytes cultured in the presence of constant insulin levels display a marked induction of most lipogenic enzyme genes when glucose concentrations in the media are elevated above fasting levels. This response requires increased glucose metabolism; however, the intracellular pathway responsible for mediating the actions of glucose remains unresolved. Sequences important for the transcriptional induction by glucose have been identified in three genes to date: FAS², i-PK, and S₁₄ (16–19). S₁₄ encodes a 17-kDa gene product postulated to play a regulatory role in the lipogenic process (20). In all three cases, the critical regulatory element, designated the ChoRE, consists of two E box half-sites related to the sequence 5’-CACG (19). These half-sites are found oriented in either direct or inverted fashion, but the spacing between them is critical for the ability to respond to glucose. Recently, we have detected by EMSA a novel nuclear protein that recognizes the ChoREs of these three genes and designated this factor as the carbohydrate responsive factor or ChoRF (19). The identity of ChoRF is currently unknown. Although ChoRF has been predicted to be a member of the basic/helix-loop-helix family based on its binding site, it is distinct from SREBP-1c by a number of criteria (21).

ACC catalyzes the first step in fatty acid synthesis and is under complex control (for review, see Ref. 22). Two genes for ACC, designated α and β, exist. The β form is expressed predominantly in the heart and skeletal muscle and may play a role in regulating fatty acid oxidation (23). The α gene is expressed ubiquitously and its expression is induced by nutritional and hormonal signals that promote increased lipogenesis (24–26). In chickens, diet-induced changes in ACCα abundance

* This work was supported by National Institutes of Health Grant DK26919 (to H. C. T.) and United States Department of Agriculture Hatch Grant CONS00665 (to H. C. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Biochemistry, Molecular Biology & Biophysics, 6-155 Jackson Hall, 321 Church St. SE., Minneapolis, MN 55455. Tel.: 612-625-3662; Fax: 612-625-5476; E-mail: towle@mail.ahr.umn.edu.

‡ To whom correspondence should be addressed: Dept. of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, Minnesota 55455.

§ To whom correspondence should be addressed: Dept. of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06269.

¶ The abbreviations used are: ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; i-PK, i-type pyruvate kinase; SREBP, sterol regulatory element-binding protein; ChoRE, carbohydrate response element; ChoRF, carbohydrate responsive factor; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pairs(s).
Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI

have been shown to be mediated by alterations in the rate of transcription (25). The ACCα gene is transcribed from two promoters, designated PI and PII (27). Studies on the physiological regulation of these two promoters indicate that the PII promoter is fairly uniformly expressed in all tissues and does not respond to dietary changes (28). The PI promoter is expressed predominantly in adipose and liver and its utilization is markedly elevated in response to diets inducing lipogenesis (24, 28). Hence, the 20-fold increase in ACC mRNA in livers of fasted rats that have been shifted to a high carbohydrate diet (26) is largely a consequence of PI promoter induction.

Studies on the control of the ACCα promoter function have largely focused on the PI promoter to date. Although it is not strongly influenced by dietary status in animals, the PII promoter shows a modest ability (∼3-fold) to respond to glucose/insulin in the 30A5 adipocyte cell line (29). This regulation has been mapped to a region of the PII promoter between −340 and −249. Within this region, binding sites for both SREBP-1 and Sp1 have been identified as being important (29, 30). It has been proposed that Sp1 binding to the ACCα PII promoter is controlled by glucose through a mechanism involving dephosphorylation by protein phosphatase 1 (31).

Despite its apparent physiological significance, little is known about the regulation of the ACCα PI promoter. Although an intrinsic repressor element has been described (22), the role of this element in regulation of the PI promoter is unknown. In this study, we examined the control of PI promoter function in primary rat hepatocytes and identified gluco- cose as a major signal controlling its expression. We also identify a glucose responsive sequence in the PI promoter with similarity to the previously characterized ChoRE and demonstrate that this element can bind to ChoRF. Thus, the PI promoter of the ACCα gene is regulated in a manner similar to other lipogenic enzyme genes in response to glucose.

EXPERIMENTAL PROCEDURES

Primary Hepatocyte Culture and Transfections—Primary hepatocytes were isolated from male Harlan Sprague-Dawley rats (180–280 g) maintained on a 12-h light/dark cycle with free access to normal rat chow as described previously (32). After a 3-h attachment period, cells were transfected with P1 reagent (Targeting Systems, San Diego, CA) in modified Williams’ E media containing 23 mM HEPES, 0.01 μM dexamethasone, 0.1 unit/ml insulin, 50 μM streptomycin, and 5.5 mM glucose for 12–14 h. Cells were then cultured in the same media containing either 5.5 or 27.5 mM glucose with or without insulin with an overlay of 0.53 mg/ml Matrigel (Collaborative Biomedical Products, Bedford, MA). In some experiments, 0.5 μM 35,3′-triiodo-t-thyronine was added as indicated in figure legends. After 27 h, cells were harvested for assay of luciferase activity. Luciferase activities are expressed as relative light units measured per microgram of protein. All experiments were repeated three times with duplicate samples for each point and showed identical response patterns. Variation within duplicates is generally less than 20%.

Plasmid Constructs—A rat genomic P1 library was screened by Genome Systems (St. Louis, MO) using primers recognizing ACCα exon 1. A single positive 85-kilobase clone was obtained. Digestion with BamHI yielded a 12-kilobase subclone including exon 1 and the PI promoter region. PCR was used to generate products of this subclone corresponding to −1049 to +21 or −220 to +21 containing additional BamHI and XhoI restriction sites on their 5′- and 3′-ends, respectively. These fragments were cleaved with these restriction enzymes and inserted into the corresponding sites of a modified pGL3 luciferase reporter vector described previously (21). The ChoRE mutant of ACC PI promoter (−220/+21) was obtained by inverse PCR as described previously (19). This mutation introduced a 6-bp sequence that changed nucleotides −109 to −104 from CGTGGG to ATGGCAT. Oligonucleotides containing the ACC PI promoter sequence from −126 to −102 were synthesized with additional BamHI and BglII sites at their 5′- and 3′-ends, respectively. Oligonucleotides were ligated and a fragment with two copies oriented 5′-GCACCTCGGGCCAGGGGCG-3′ and 3′-CTGCAGCCTCCAGGTCCTG-5′ were added together with radiolabeled probe into the sample prior to electrophoretic mobility shift assay (EMSA). EMSA was carried out under conditions previously described (33). A typical reaction contained 100,000 cpm (approximately 6 fmol) of 32P-labeled oligonucleotide with 15 μg of nuclear protein. Nonspecific competitors were 0.1 μg of poly(dI-dC) and 1.9 μg of poly(dI-dC) in binding experiments. EMSA was acquired as a function of cycle number at a temperature of 82 °C. Variation within duplicates is generally less than 20%.

RESULTS

Effects of Glucose and Insulin on ACC Transcript Levels from PI and PII Promoters—Previous work in one of our laboratories (H. C. F.) used an RT-PCR assay to monitor transcripts from PI and PII promoters in a variety of tissues. These studies demonstrated PI-generated transcripts were elevated in response to feeding a high carbohydrate, low fat diet in the liver, while transcripts from the PI promoter did not respond (28). We wished to verify that this pattern of control can be recapitulated in primary hepatocytes and to examine the contribution of insulin and glucose to the process. Consequently, primary hepatocytes were isolated from rats and cultured under conditions of low glucose (5.5 mM) minus insulin, low glucose plus insulin, high glucose (27.5 mM) minus insulin or high glucose plus insulin for 27 h. RNA was isolated from these hepatocytes and used in an RT-PCR assay with primers that would specifically detect PI or PII transcripts, as described previously (28). As shown in Fig. 1, transcripts from the PI promoter are barely detectable in hepatocytes maintained in low glucose media in the absence of insulin. Treatment with insulin in the presence of low glucose caused a significant increase above the basal level. PII-generated transcripts was observed. Using a real time RT-PCR system to quantify PI transcripts, increases of 9-, 6.6-, and 48-fold were found in cells treated with insulin alone, high glucose alone, or the combined treatment, respectively, compared with control, the following ribosomal protein L32-specific primers were used: 5′-AACACTCGGGCCAGGGGCG-3′ and 3′-CTGCAGCCTCCAGGTCCTG-5′. Primers were annealed at a temperature of 58 °C and 24 cycles were used for the PCR. Reaction products were subjected to electrophoresis in 2% low melting point agarose gels and band intensities were compared by imaging of ethidium bromide-stained gels.

To quantify PI transcripts, the LightCyder System (Roche Molecular Biochemicals, Indianapolis, IN) was used. This system allows amplification and detection by fluorescence of PCR products in the same tube using a kinetic approach. RT-PCR reactions were run using SYBR Green I master mix and the same primers as indicated above. Data was acquired as a function of cycle number at a temperature of 82 °C below the melting point for the ACC1 PCR product. LightCycler software was used to compare amplification in the experimental samples during the log-linear phase to a standard curve established from a dilution series of control RNA.

Electrophoretic Mobility Shift Assay—Nuclear proteins were extracted from livers of rats using the procedure described by Koo and Towle (19). The fraction that precipitated between 0 and 10% polyethyleneglycol 8000 (Hampton Research, Boston, MA) was used for DNA binding experiments. EMSA was carried out under conditions previously described (33). A typical reaction contained 100,000 cpm (approximately 6 fmol) of 32P-labeled oligonucleotide with 15 μg of nuclear protein. Nonspecific competitors were 0.1 μg of poly(dI-dC) and 1.9 μg of poly(dI-dC). Following incubation at room temperature for 30 min, samples were subjected to electrophoresis on a 4.5% nondenaturing polyacrylamide gel and subjected to PhosphorImager analysis. For competition EMSA, the indicated molar excess of unlabeled oligonucleotide was added together with radiolabeled probe into the sample prior to incubation.
Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI

Identification of Possible ChoRE in the ACC PI Promoter—
The ACC PI promoter from −1049 to +21 was examined for sequences that might function as a ChoRE. One site was found that matched the previously identified ChoREs from the S14, 1-PK, and FAS genes. This sequence contained a direct repeat of the E box half-site 5′-CACG separated by 7 bp (Fig. 3). Identical sequence and spacing has recently been shown to support a glucose response of the FAS promoter.2 To test the possible significance of this element from the ACC PI promoter, we asked whether ChoRF recognized an oligonucleotide containing this sequence. ChoRF was detected as a gel shift band that formed between rat liver nuclear proteins and various ChoREs (19). In Fig. 4A, an oligonucleotide containing the putative ChoRE of the ACC PI promoter (−126/−102) was found to form two major bands on EMSA with rat liver nuclear proteins. The slower migrating and predominant band comigrated with the previously identified ChoRF band observed with 1-PK and S14 ChoREs. To confirm that this band is due to binding of the same complex, a competition experiment was performed. An oligonucleotide containing the ChoRE of the 1-PK gene was radiolabeled and used as a probe in EMSA. The ability of the putative ACC PI ChoRE to compete for ChoRF binding was assessed. As shown in Fig. 4B, the ACC oligonucleotide was a strong competitor for ChoRF binding and appeared to compete at least as effectively as the 1-PK oligonucleotide itself. ChoRE-containing oligonucleotides from the rat S14 and FAS genes also competed for ChoRF binding, whereas oligonucleotides containing binding sites for SREBP or HNF-4 were ineffective. Hence, the putative ChoRE of the ACC PI promoter is indeed an effective binding site for the ChoRF.

Functional Role for the ACC PI Promoter ChoRE—To test the functional significance of the putative ChoRE in the ACC PI promoter, we examined the effects of mutating this site. For this experiment, an ACC PI promoter fragment from −220 to +21 was used. As can be seen in Fig. 5, this segment supported a robust response of the ACC PI promoter to glucose, indicating that sequences between −1049 and −220 are not critical for glucose regulation. It should be noted that this deletion removes a DNA sequence reported by others to possess intrinsic repressor activity (22). Introduction of a 6-bp mutation that disrupted the putative ChoRE site of the ACC PI promoter resulted in a complete loss of the glucose response of this promoter. Consequently, this site is necessary to support ACC PI promoter activation by glucose.

To test also whether this element can function in an independent manner to confer a glucose response, an oligonucleotide containing the putative ChoRE of the ACC PI promoter was linked in two copies to a minimal 1-PK promoter containing sequences from −40 to +12. This construct was compared with a series of similarly organized plasmids containing oligonucleotides from other genes. As seen in Fig. 6A, a construct containing the ACC oligonucleotide linked to the minimal 1-PK promoter supported a strong response to glucose. Two previously characterized ChoREs from the rat S14 and 1-PK genes also supported a glucose response. However, this effect was not observed when a consensus SREBP-binding site was fused to glucose-responsive (17), supported a 5-fold increase in the presence of high glucose, whereas a basal 1-PK promoter from −96 to +12 lacking the ChoRE was unresponsive. These experiments indicated that the ACC PI promoter was indeed responsive to glucose in primary hepatocytes and that at least in part the induction of PI-generated mRNA is due to increased transcription.

**Glucose Stimulates Activity of the ACC PI Promoter—**

To determine whether the regulation of PI-generated ACC mRNA is mediated at the transcriptional level, transfection experiments were performed. A segment of the ACC PI gene from −1049 to +21 was initially tested. This segment was fused to a luciferase reporter gene and the resulting construct was introduced into primary hepatocytes. Cells were subsequently maintained in varying glucose and insulin conditions as described above for 27 h, prior to measurement of luciferase activities. The level of reporter gene activity was minimal in cells maintained in low glucose, regardless of whether insulin was present or not (Fig. 2). In contrast, a strong elevation in promoter activity was observed in cells treated with high glucose. Insulin did not increase this level and may, in fact, have caused a modest decrease. By comparison, a fragment from the 1-PK promoter from −183 to +12, which was previously shown to be

---

2 Rufo, C., Teran-Garcia, M., Nakamura, M., Koo, S.-H., Towle, H. C., and Clarke, S. D. (2001) *J. Biol. Chem.* 276, in press.
Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI

**DISCUSSION**

ACC is generally recognized as the rate-limiting step for fatty acid biosynthesis. Consequently, the enzymatic activity of ACC is tightly controlled by a variety of mechanisms including covalent modification, allosteric control, and alterations in its polymeric state (22, 34). In addition, diet and hormonal factors influence the level of ACC production (22). In this regard, ACC is a member of a family of lipogenic enzymes that are induced under conditions favoring energy storage and repressed under conditions of energy demand.

The ACCα gene is expressed from two promoters and variably spliced to include or exclude alternative exons within the 5'-untranslated region (27). Transcripts from the PII promoter are expressed in all tissues examined in the rat and do not respond to fasting or refeeding a high carbohydrate diet (28). Consistent with these previous observations, we did not detect any major changes in the levels of PII-generated transcripts in response to alterations in glucose or insulin in the cultured rat hepatocytes. Consequently, the PII promoter likely provides enzyme to serve basic cellular needs, such as synthesis of membrane phospholipids. In contrast, PI-generated transcripts are expressed predominantly in adipose and liver, two major sites of triglyceride formation in mammals, and are induced under conditions favoring lipogenesis (24, 28). Thus, it is reasonable to speculate that the PI promoter provides enzyme for meeting the enhanced needs of tissues capable of lipogenesis under favorable conditions. Consistent with this notion, we have found that transcripts from the PI promoter are increased dramatically by glucose and insulin in the primary hepatocyte. Given that an overall 20-fold increase is observed in total ACC mRNA in the fasted response in rats (12, 26), the PI promoter is likely the predominant one used under lipogenic conditions in the liver.

The control of ACCα promoter usage in chickens appears to be somewhat different than that observed in rats. Yin et al. (35) recently demonstrated that transcripts from both PI and PII promoters are elevated in liver when 12-day-old chicks are refed a high carbohydrate diet after a 24-h fast. PI-generated transcripts were induced 10.5-fold, whereas PI-generated transcripts were induced 6.4-fold. Similarly, thyroid hormone regulation of ACC promoters shows a species-specific variation. In rats, changes in thyroid hormone were found to affect predominantly PI-generated transcripts (36). However, in chickens, the activity of the PII promoter, as well as the PI promoter, was influenced (35). Whether these differences solely reflect species-specific variation or might also be due to developmental differences between 12-day-old chicks and adult rats remains to be determined.

The induction of lipogenic enzyme genes in response to feeding of a high carbohydrate diet can be attributed to the actions of insulin and glucose. Stimulation of most lipogenic enzyme
FIG. 4. Putative ChoRE of ACC PI promoter binds to ChoRF. A, EMSA was performed with the 0–10% polyethylene glycol 8000-precipitated fraction of rat liver nuclear proteins (15 μg) and various radiolabeled probes, as indicated. The arrow indicates the position of the previously described ChoRF complex. rS14(m3–5) is a variant form of the rat S14 ChoRE that retains activity, but has reduced affinity for binding USF (44). B, EMSA was performed as described in A using a radiolabeled probe containing the L-PK ChoRE. Various oligonucleotides were added in increasing amounts as indicated to each binding reaction. Position of the ChoRF complex is indicated. The FAS ChoRE oligonucleotides contained the sequence from −7218 to −7194 of the rat FAS gene. L-PK and S14 ChoREs represented sequences from −171 to −142 and −1448 to −1422 of these genes, respectively. The HNF-4 oligonucleotide was derived from the L-PK gene (−1467–124). The consensus SREBP-binding site, SRE, was defined previously (21).
in primary adipocytes (37). We have recently presented evidence that two transcription factors are involved in the induction of lipogenic enzyme genes by insulin and glucose (21). One of these factors is SREBP-1c. The expression of SREBP-1c is elevated in hepatocytes in response to insulin (8, 38). In turn, SREBP-1c binds to the promoters of several lipogenic enzyme genes, including fatty acid synthase (6, 9), stearoyl-CoA desaturase (11), and glycerol-3-phosphate acyltransferase (10). In each of these cases, co-transfection of an SREBP-1c expression vector together with promoter/reporter plasmids containing the SREBP-binding site led to induction of reporter gene activity. Based on this evidence, SREBP-1c is responsible, at least in part, for mediating effects of insulin on lipogenic enzyme induction.

In addition to SREBP-1c, an independent transcription factor, ChoRF, appears to be critical for regulation of lipogenic enzyme genes by glucose. ChoRF was identified by its ability to bind to the ChoRE of l-PK and S14 promoters (19). This element is responsible for conferring a response of these genes to glucose in hepatocytes and is not recognized by SREBP-1c. We further postulated that many of the lipogenic enzyme genes would require both SREBP-1c and ChoRF during induction by high carbohydrate diet. This has been shown to be the case for two genes to date: the S14 gene (21) and the FAS gene.2 In both of these cases, SREBP-1c- and ChoRF-binding sites are present and can function independently to mediate modest effects of insulin and glucose, respectively. However, together the two sites give a strong synergy to the combined treatment. It is noteworthy that the ChoRF- and SREBP-1c-binding sites of the S14 and FAS genes are located at considerable distances from each other (>1000 bp). Hence, the role of ChoRF in synergizing with SREBP appears distinct from the synergy reported earlier for SREBP and other factors such as Sp1 or NF-Y (39–42). In these cases, auxiliary factors binding to sites adjacent to the SREBP-1c-binding site functionally cooperate to give effective SREBP action.

For ACC, the data of this study strongly implicate ChoRF as a transcription factor regulating ACC PI promoter activity in response to glucose. ACC thus adds a fourth gene to the list of previously characterized ChoRE-containing genes. Interestingly, two of these, ACC and FAS, are the central enzymes of fatty acid biosynthesis. In these two genes, the ChoREs are identical in their core motifs and the arrangement of these motifs. However, the location of these sites is markedly different; in the FAS gene the element is located several thousand base pairs upstream from the transcription start site, whereas the ACC element is located in the proximal promoter region.

A role for SREBP-1c in the control of ACC gene expression has been suggested by several observations. First, in studies on fasted-refed mice, the amount of nuclear SREBP-1c was found to increase in parallel with the amounts of mRNA encoding lipogenic genes, including ACC (7). Second, transgenic mice that express the nuclear form of SREBP-1c in their liver show increased levels (2.1–2.4-fold) of ACC mRNA and elevated (4-fold) levels of hepatic triglycerides (13, 14). Furthermore, in two mouse models of diabetes, the ob/ob mouse and the transgenic aP2-SREBP-1c mouse (which overexpress SREBP-1c only in adipose), nuclear levels of SREBP-1c were elevated in liver. In both models, increased ACC mRNA levels (2.5- and 4.7-fold, respectively) were found and correlated with increased rates of fatty acid synthesis and triglyceride accumulation in the liver. It should be noted that only total ACC mRNA was measured in these studies. Thus, extreme changes in SREBP-1c may have provoked alterations in PI1 promoter activity, which responds to this transcription factor in adipocytes. Finally, mice bearing a homozygous deletion of the SREBP-1 gene show a blunted
response to feeding of a high carbohydrate diet (12). The normal 20-fold induction of ACC seen in mice upon a fast-refeed is reduced to −6-fold in the mutant mice. We predict that this remaining response observed in the SREBP-1 knockout mice is likely to be mediated by the actions of ChoRF binding to the ACC PI promoter.

In the present experiments, insulin treatment causes a modest elevation of ACC PI-transcribed transcript levels when added in low glucose conditions and synergized with glucose to increase levels dramatically in the combined treatment. However, we did not observe an effect of insulin on ACC PI promoter activity for the −1049/+21 construct. Furthermore, co-transfection of a vector expressing nuclear SREBP-1c did not enhance activity of the ACC(−1049/+21) luciferase reporter. Two explanations may account for this discrepancy. The most likely is that an SREBP-binding site on the ACC PI promoter exists outside of the region that we have tested. This situation would be somewhat unusual for SREBP, since all previously characterized functional sites in both cholesterogenic and lipogenic genes lie within 300 bp upstream of the transcriptional start site. The second explanation is that ACC may not be directly acted upon by SREBP-1c. In this scenario, the effects of insulin on ACC PI transcription would be indirectly mediated by stimulation of another gene product or occur at the level of mRNA stability. For example, glucokinase gene expression is stimulated by insulin/SREBP-1c, it could contribute to the overall effect of carbohydrate diet on ACC PI activity by affecting the rate of glucose metabolism and the activation of ChoRF.

In summary, we have shown that the activity of ACCa promoter PI is regulated by glucose in primary rat hepatocytes. This regulation is mediated by a conserved DNA element, the ChoRE, found in several other glucose-regulated genes that binds to the hepatic factor ChoRF. Given the central role of ACC in mediating the lipogenic response of the liver, ChoRF, together with SREBP, are likely to be major regulators of the ACC in mediating the lipogenic response of the liver, ChoRF, found in several other glucose-regulated genes that

Acknowledgements—We thank Ross E. Newman for technical assistance, Angela Dutcher and Jenny Xanthos for help with the LightCycler system, and Yangha Moon for preparation of the initial ACC reporter construct.

REFERENCES
1. Girard, J., Ferre, P., and Foufelle, F. (1997) Annu. Rev. Nutr. 17, 325–352
2. Towle, H. C., Kaytor, E. N., and Shi, H.-M. (1997) Annu. Rev. Nutr. 17, 405–433
3. Brown, M. S., and Goldstein, J. L. (1997) Cell 89, 331–340
4. Horton, J. D., and Shimomura, I. (1999) Curr. Opin. Lipidol. 10, 143–150
5. Osborne, T. F. (2000) J. Biol. Chem. 275, 32379–32382

3 B. L. O'Callaghan and H. C. Towle, unpublished results.

6. Kim, J. B., Sarraf, P., Wright, M., Yao, K. M., Mueller, E., Solanes, G., Lowell, B. B., and Spiegelman, B. M. (1998) J. Clin. Invest. 101, 1–9
7. Horton, J. D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5587–5592
8. Foretz, M., Guichard, C., Ferre, P., and Foufelle, F. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12737–12742
9. Magana, M. M., and Osborne, T. F. (1996) J. Biol. Chem. 271, 32688–32694
10. Ericsson, J., Jackson, S. M., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1997) J. Biol. Chem. 272, 7298–7305
11. Tabor, D. E., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1998) J. Biol. Chem. 273, 22052–22058
12. Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A. H., Osuga, J.-I., Tamura, Y., Shinohara, F., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S., and Yamada, N. (1999) J. Biol. Chem. 274, 35832–35839
13. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997) J. Clin. Invest. 99, 846–854
14. Shimomura, I., Shimano, H., Korn, B. S., Bashmakov, Y., and Horton, J. D. (1998) J. Biol. Chem. 273, 35299–35306
15. Vaulont, S., Vasseur-Cognet, M., and Kahn, A. (2000) J. Biol. Chem. 275, 31553–31558
16. Bergot, M.-O., Diaz-Guerra, M.-J. M., Puzenat, N., Raymondjean, M., and Kahn, A. (1992) Nucleic Acids Res. 20, 1871–1878
17. Liu, Z., Thompson, K. S., and Towle, H. C. (1993) J. Biol. Chem. 268, 12787–12795
18. Shi, H.-M., Liu, Z., and Towle, H. C. (1995) J. Biol. Chem. 270, 21991–21997
19. Koo, S.-H., and Towle, H. C. (2000) J. Biol. Chem. 275, 5200–5207
20. Cunningham, B. A., Moncur, J. T., Huntington, J. T., and Kinlaw, W. B. (1998) Thyroid 8, 815–825
21. Koo, S.-H., Dutcher, A. K., and Towle, H. C. (2001) J. Biol. Chem. 276, 9437–9445
22. Kim, K.-H. (1997) Annu. Rev. Nutr. 17, 77–99
23. Ruderman, N. B., Saha, A. K., Vavvas, D., and Witters, L. A. (1999) Am. J. Physiol. 276, E1–E18
24. Lopez-Casellas, F., Ponce-Castaneda, M. V., and Kim, K.-H. (1991) Endocrinology 120, 1049–1058
25. Hillgartner, F. B., Charbon, T., and Chenuet, K. A. (1996) Biochem. J. 318, 263–268
26. Kim, T.-S., and Freake, H. C. (1996) J. Biol. Chem. 271, 611–617
27. Luo, X., and Kim, K.-H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4042–4046
28. Kim, T.-S., Leahy, P., and Freake, H. C. (1996) Biochem. Biophys. Res. Commun. 225, 647–653
29. Daniel, S., and Kim, K.-H. (1996) J. Biol. Chem. 271, 1385–92
30. Lopaschuk, G. D., Himeda, K., and Towle, H. C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1049–1053
31. Shih, H.-M., and Towle, H. C. (1999) J. Biol. Chem. 271, 14992–14997
32. Shih, H.-M., and Towle, H. C. (2002) J. Biol. Chem. 277, 13222–13228
33. Shih, H.-M., and Towle, H. C. (1994) J. Biol. Chem. 269, 9380–9387
34. Hardie, D. G. (1989) Prog. Lipid Res. 28, 117–146
35. Yin, L., Zhang, Y., Charron, T., and Hillgartner, F. B. (2000) Biochim. Biophys. Acta 1517, 91–99
36. Huang, C., and Freake, H. C. (1998) Biochem. Biophys. Res. Commun. 249, 704–708
37. Foufelle, F., Gouh, B., Perdereau, D., Girard, J., and Freere, P. (1994) Eur. J. Biochem. 223, 893–900
38. Shimomura, I., Bashmakov, Y., Remoto, S., Horton, J. D., Brown, M. S., and Goldstein, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13656–13661
39. Sanchez, H. B., Yieh, L., and Osborne, T. F. (1995) J. Biol. Chem. 270, 1161–1169
40. Ericsson, J., Jackson, S. M., and Edwards, P. A. (1996) J. Biol. Chem. 271, 24359–24364
41. Dooley, K. A., Millinder, S., and Osborne, T. F. (1998) J. Biol. Chem. 273, 13549–13556
42. Magana, M. M., Koo, S.-H., Towle, H. C., and Osborne, T. F. (2000) J. Biol. Chem. 275, 4726–4733
43. Iynedjian, P. B., Jotterand, D., Nouspikel, T., Asfari, M., and Pilot, P. R. (1989) J. Biol. Chem. 264, 21824–21829
44. Kayter, E. N., Shi, H.-M., and Towle, H. C. (1997) J. Biol. Chem. 272, 7525–7531
Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI in Rat Hepatocytes
Brennon L. O’Callaghan, Seung-Hoi Koo, Yue Wu, Hedley C. Freake and Howard C. Towle

J. Biol. Chem. 2001, 276:16033-16039.
doi: 10.1074/jbc.M101557200 originally published online February 28, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101557200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 44 references, 28 of which can be accessed free at
http://www.jbc.org/content/276/19/16033.full.html#ref-list-1