Refeeding carbohydrate to fasted rats induces the transcription of genes encoding enzymes of fatty acid biosynthesis, e.g. fatty-acid synthase (FAS). Part of this transcriptional induction is mediated by insulin. An insulin response element has been described for the fatty-acid synthase gene region of ~600 to +65, but the 2–3-fold increase in fatty-acid synthase promoter activity attributable to this region is small compared with the 20–30-fold induction in fatty-acid synthase gene transcription observed in fasted rats refeed carbohydrate. We have previously reported that the fatty-acid synthase gene region between −7382 and −6970 was essential for achieving high in vivo rates of gene transcription. The studies of the current report demonstrate that the region of −7382 to −6970 of the fatty-acid synthase gene contains a carbohydrate response element (CHO-RE<sub>FAS</sub>) with a palindromic sequence (CATG(TG)_2(GCGT)G) that is nearly identical to the CHO-RE of the L-type pyruvate kinase and S14 genes. The glucose responsiveness imparted by CHO-RE<sub>FAS</sub> was independent of insulin. Moreover, CHO-RE<sub>FAS</sub> conferred glucose responsiveness to a heterologous promoter (i.e. t-type pyruvate kinase). Electrophoretic mobility shift assays demonstrated that CHO-RE<sub>FAS</sub> readily bound a unique hepatic ChoRF and that CHO-RE<sub>FAS</sub> competed with the CHO-RE of the t-type pyruvate kinase and S14 genes for ChoRF binding. In vivo footprinting revealed that fasting reduced and refeeding increased ChoRF binding to CHO-RE<sub>FAS</sub>. Thus, carbohydrate responsiveness of rat liver fatty-acid synthase appears to require both insulin and glucose signaling pathways. More importantly, a unique hepatic ChoRF has now been shown to recognize glucose responsive sequences that are common to three different genes: fatty-acid synthase, L-type pyruvate kinase, and S14.

The consumption of a high carbohydrate diet increases the hepatic production of malonyl-CoA and its subsequent utilization for de novo fatty acid biosynthesis (1). In addition to being the substrate for fatty acid biosynthesis, malonyl-CoA governs the rate of fatty acid oxidation by virtue of its ability to function as a negative metabolite effector of carnitine palmitoyltransferase (2). The concentration of malonyl-CoA is determined by its rate of synthesis by acetyl-CoA carboxylase and its rate of utilization by fatty-acid synthase. Carbohydrate ingestion causes a coordinate induction in the expression of both hepatic enzymes (3–10). In the case of fatty-acid synthase the amount of protein is largely determined by mRNA abundance for fatty-acid synthase, and this in turn is determined by the rate of fatty-acid synthase gene transcription (5–10). The carbohydrate induction of hepatic fatty-acid synthase gene transcription requires both insulin and glucocorticoids (5, 8, 10–12). On the other hand, fatty-acid synthase expression is suppressed by polyunsaturated fatty acids and sterols (9, 12–14) and by the administration of glucagon and growth hormone (15, 16). The transcriptional response of the hepatic fatty-acid synthase gene to carbohydrate reportedly involves a glucose response region located within the first intron (17) as well as insulin and cAMP response sequences located between −444/−278, −278/−131, and −120/−50 (15, 18–20). Functional studies have revealed that the sequence between −70 and −57 imparts insulin responsiveness to the fatty-acid synthase promoter (19), while the region of −99 to −92 may contain the cAMP target (15). Transcription factors that interact with the insulin response region include USF-1,1 USF-2, SREBP-1, NF-Y, and Sp1 (19–26). Moreover, the hepatic content of SREBP-1 and the DNA binding activity of Sp1 are increased by insulin and glucose ingestion and decreased by fasting (20, 26). In addition, the binding of SREBP-1 to its DNA recognition sequence within the insulin response region of fatty-acid synthase may enhance the binding of Sp1 to its response element at −80, which may in turn enhance the interaction of NF-Y with its recognition sequence at −90 (14, 24).

Despite the fact that the proximal promoter region of the fatty-acid synthase gene contains numerous cis-acting elements that interact with transcription factors that appear to be regulated by insulin and glucose, the 2–3-fold enhancement of fatty-acid synthase promoter activity attributable to the insulin and glucose response elements is relatively weak in comparison with the 20–30-fold induction in hepatic fatty-acid
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RESULTS

Identification of a Glucose Response Region within the Distal Promoter of the Fatty-acid Synthase Gene—Nuclei isolated from the livers of rats that had been fasted for 48 h and refed a high glucose diet for 6 h displayed greater sensitivity to DNase I cleavage than did nuclei from fasted rats. Three DNase hypersensitivity sites were identified in the 5'-flanking regions of the fatty-acid synthase gene: (a) −7382 to −6970; (b) −600 to −400; and (c) −100 to +50 (27). Although the proximal sites (b and c) correspond to the regions that are involved with the gene’s response to insulin (18, 19, 22), these regions do not appear to impart glucose responsiveness to the fatty-acid synthase promoter (Fig. 1, note −7150 and −250). However, transfection-reporter assays using primary cultures of rat hepatocytes revealed that constructs that included the distal region of −7382 to −6970 with the proximal insulin elements yielded 7–10-fold more promoter activity than did constructs containing only the insulin response region of the proximal promoter (27). More importantly, the region between −7382 and −6970 imparted glucose responsiveness to the fatty-acid synthase promoter (Fig. 1). Specifically, hepatocytes that had been transfected with a luciferase reporter construct that included the distal enhancer region indicated that the proximal insulin response element of the fatty-acid synthase promoter expressed 2-fold more luciferase when the medium contained 27.5 mM glucose than when it contained only 5.5 mM glucose. More importantly this response was independent of insulin. Deleting the sequences between −7382 and −7242 had no effect on the glucose stimulation of fatty-acid
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**DISCUSSION**

The cis-acting elements responsible for the carbohydrate/insulin induction of fatty-acid synthase gene transcription have been attributed to sequences within the proximal promoter region (i.e., −644 to −50) of the gene (18–22). Of particular interest has been the sequence between −68 and −57. This area contains an E-box binding site for USF-1 and -2 and a binding site for SREBP-1c that involves sequences on either side of the E-box (14, 23). Disruption of either the USF-1 or SREBP-1c genes, and the sequences located between −7214 and −7190 of the fatty-acid synthase gene (Fig. 3), we hypothesized that nuclear protein binding to this site would change with fasting and fasting-refeeding. *In vitro* footprint analysis of the fatty-acid synthase gene between −7350 and −7130 revealed that refeeding fasted rats a high glucose diet resulted in protection of the candidate ChoRE site from DNase I cleavage (Fig. 4, *sites 2 and 3*). Fasting reinstated DNase I cleavage within the ChoRE (Fig. 4). Fasting also resulted in protein binding at two sites: *a*, −7210 to −7190 and *b*, −7170 to −7160 (Fig. 4). Interestingly, *site a* appears to involve sequences that are components of the ChoRE, and *site b* was equivalent to *site 4* of the fed. *Site 1* in the fed animals remains to be identified, but *site 5* corresponds to the C/EBPα site (Fig. 4).

The ChoRE of the Fatty-acid Synthase Gene Binds a Carbohydrate Response Factor and Imparts Carbohydrate Responsiveness—The ChoRE of the rat 1-type pyruvate kinase and S14 genes binds a novel hepatic carbohydrate responsive transcription factor (ChoRF) (25, 29). Electrophoretic mobility shift assays using a partially enriched ChoRF fraction prepared from the hepatic nuclei of fed rats revealed that the ChoRE of the rat fatty-acid synthase gene binds the ChoRF (Fig. 5, *lane 1*). Protein binding activity for the fatty-acid synthase ChoRE was equal to or even greater than for the ChoRE of 1-type pyruvate kinase or two S14 ChoRE variants that support a glucose response (Fig. 5, *lanes 1–3*). In addition to binding the novel ChoRF, the ChoRE sequence of the fatty-acid synthase gene also interacts with USF, a transcription factor possibly involved with the carbohydrate/insulin induction of fatty-acid synthase and 1-type pyruvate kinase (19, 34). The similarity of the ChoRE of fatty-acid synthase to that of the 1-type pyruvate kinase gene was reinforced by the observation that the fatty-acid synthase ChoRE effectively competed for the binding of the ChoRF to the ChoRE of the 1-type pyruvate kinase gene (Fig. 6, *lanes 1, 4, and 5*). The competitive action of the fatty-acid synthase ChoRE was comparable with that observed for the ChoRE of the S14 gene (Fig. 6, *lanes 4–7*). On the other hand, the sterol response element and the hepatic nuclear factor-4 response element were ineffective as competitors for ChoRF binding (Fig. 6, *lanes 8–11*).

Finally, in addition to binding a unique ChoRF, the ChoRE of the fatty-acid synthase gene was found to impart carbohydrate responsiveness to a basal 1-type pyruvate kinase promoter that is otherwise unresponsive to glucose (Table 1). Specifically, when a tandem repeat of the fatty-acid synthase ChoRE was inserted into the 1-type pyruvate kinase promoter-reporter construct (−96/+12) PK.LUC (lacking the PK ChoRE sequence), luciferase expression was increased nearly 5-fold; and when ligated to (−40/+12) PK.LUC, luciferase activity was increased 30-fold (Table 1). The greater glucose induction with the (−40/+12) PK.LUC construct was due to the fact that the (−40) lower promoter activity than does the −96 1-type pyruvate kinase promoter when hepatocytes are maintained in low glucose media. Finally, the enhancer activity of the fatty-acid synthase ChoRE was very comparable with that of the S14 ChoRE (Table 1).

The cis-acting elements responsible for the carbohydrate/insulin induction of fatty-acid synthase gene transcription have been attributed to sequences within the proximal promoter region (i.e., −644 to −50) of the gene (18–22). Of particular interest has been the sequence between −68 and −57. This area contains an E-box binding site for USF-1 and -2 and a binding site for SREBP-1c that involves sequences on either side of the E-box (14, 23). Disruption of either the USF-1 or
USF-2 genes reduced the hepatic abundance of fatty-acid synthase mRNA to a level that was only 10–15% of that found in wild type mice (35). Moreover, an early report by Sul and co-workers (19) indicated that the DNA binding activity and protein abundance of USF-1 was reduced by fasting and increased by carbohydrate refeeding. However, subsequent studies have clearly demonstrated that the abundance of hepatic USFs is not altered by nutritional manipulations (7). In contrast to USF, the hepatic abundance of SREBP-1c was rapidly induced by refeeding fasted rats a high glucose diet, and by administering insulin to diabetic rats, or by treating rat hepatocytes in primary culture with insulin (36–38). This induction of hepatic SREBP-1c expression was paralleled by an increase in hepatic fatty-acid synthase gene transcription (20). The importance of SREBP-1c to fatty-acid synthase gene transcription was further suggested by the observation that disrupting the binding site for SREBP-1c without affecting the E-box site for USF eliminated the insulin/glucose activation of the fatty-acid synthase promoter (24). Thus, the nuclear abundance of SREBP-1c and the binding of SREBP-1c to its recognition sequences within the area of -68 to -57 appear to be important determinants for hepatic fatty-acid synthase promoter activity. However, disruption of the SREBP-1 gene only partially prevented the increase in hepatic fatty-acid synthase mRNA that results from feeding fasted rats a high glucose diet (27). This suggested that nutritional regulation of fatty-acid synthase gene transcription required factors other than SREBP-1c. Using transgenic mice to characterize 5’-flanking sequences between -2100 and +67, Sul and co-workers (19, 21) recently discovered that a USF binding site at -332 and an SREBP-1 binding site at -150 of the fatty-acid synthase gene were essential for insulin and carbohydrate regulation of the gene. Moreover, the investigators proposed that the region between

CHO-RE sequence

|    | FAS | CCACA GGCCTG |
|----|-----|-------------|
|    | L-PK| GCACCC CTGCA |
|    | S 14| GTGCG CCTGTG |

![In vitro footprint of the fatty-acid synthase gene between -7240 and -7120.](image1)

**FIG. 2.** *In vitro* footprint of the fatty-acid synthase gene between -7240 and -7120. The fragments of the fatty-acid synthase gene between -7190 and -7120 (A) and -7240 and -7180 (B) were subjected to *in vitro* DNase I footprinting as described under “Experimental Procedures.” The radiolabeled probes were incubated with DNase I plus 0 (lanes N), 10, and 50 µg of liver nuclear proteins extract (lanes 1 and 2). The DNase I protected regions are marked by the brackets at the right and are described in C.

![The fatty-acid synthase ChoRE sequence similarity with the ChoRE sequence of L-type pyruvate kinase and S 14 genes.](image2)

**FIG. 3.** The fatty-acid synthase ChoRE sequence similarity with the ChoRE sequence of L-type pyruvate kinase and S 14 genes.
The fatty-acid synthase ChoRE (−7214/−7198) binds ChoRF. Electrophoretic mobility shift assays employed the following radiolabeled oligonucleotides: fatty-acid synthase (−7214/−7198) (lane 1), rat t-type pyruvate kinase ChoRE (−171/−142) (lane 2), rat S₁₄ ChoRE (−1448/−1422) (lane 3), “mut 3/5” ChoRE (lane 4), and “mut 3-6” ChoRE (lane 5). The “mut 3/5” ChoRE is derived from the rat S₁₄ ChoRE (34) and the “mut 3-6” ChoRE is derived from the mouse S₁₄ ChoRE (29). Each oligonucleotide was labeled to approximately the same specific activity and used with partially enriched ChoRF fraction from rat liver (29). Arrows indicate the ChoRF and the USF complexes.

(Fig. 1). On the other hand, when the distal region of −7382 to −6970 was linked to the proximal sequences of the fatty-acid synthase gene, fatty-acid synthase promoter activity was increased 3–4-fold (27), and the transcriptional activity of the promoter was comparable with the rate of gene transcription observed in rats fed a high glucose diet (5–9). Interestingly, the 3–4-fold enhancement of fatty-acid synthase promoter activity contributed by the sequences between −7382 and −6970 was approximately that amount required for the proximal promoter region of −444 to +67 to achieve in vivo rates of fatty-acid synthase gene transcription (19, 21, 27).

The above observations suggested to us that perhaps the proximal promoter region imparted insulin control to the fatty-acid synthase promoter, while the distal sequences between −7382 and −6970 conferred carbohydrate stimulation to the promoter. Consequently the sequences between −7382 and −6970 were examined more closely for their role in the glucose induction of fatty-acid synthase gene transcription. Functional mapping studies indicated that the −7382 to −6970 region contained glucose-responsive sequences. More importantly, the glucose enhancement of fatty-acid synthase promoter activity appeared to be independent of insulin (Fig. 1). The ability of glucose to enhance fatty-acid synthase promoter activity by an insulin-independent mechanism is consistent with early observations showing that the feeding of fructose to diabetic rats induced hepatic fatty-acid synthase gene expression (39, 40).

The glucose-responsive element within the distal region of the fatty-acid synthase gene appears to reside within the area

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* C. Rufo, M. Teran-Garcia, M. T. Nakamura, and S. D. Clarke, unpublished data.
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The ChoRF of fatty-acid synthase confers glucose responsiveness

The type of transcription factor that interacts with the ChoRE of the fatty-acid synthase gene and functions as the target for the glucose signal is unknown, but the 5'-CAGGTG sequence is a consensus binding sequence for basic/helix-loop-helix/leucine zipper transcription factors (28). Hasegawa et al. (41) recently reported that a “glucose response element-binding protein” interacted with the CACGTG motifs located in the ChoRE of rat l-type pyruvate kinase, and in the insulin response element (~71 to ~50) of the fatty-acid synthase gene. However, this unknown protein interacted weakly with the ChoRE of the rat S14 gene and did not interact at all with comparable motifs in the acetyl-CoA carboxylase and citrate lyase genes. Moreover the insulin response element between ~71 and ~50 is not a glucose response sequence for the fatty-acid synthase gene (Fig. 1). In contrast we found that the unique ChoRF of rat liver nuclei (25, 29) readily interacted with the ChoRE l-type pyruvate kinase, S14, and fatty-acid synthase genes (Fig. 6). While the identity of ChoRF is unclear, it has now been shown to recognize specific glucose-responsive sequences common to three genes: l-type pyruvate kinase, S14, and fatty-acid synthase. It also recognizes variant versions of these elements, such as the mut3/5 oligonucleotide (Fig. 6), that retain glucose responsiveness but have greatly reduced binding affinity for USF. On the other hand, mutations of these naturally occurring ChoREs that fail to support a response to glucose do not bind ChoRF. Likewise, ChoRF does not bind to a consensus sterol response element or the adenovirus major late promoter USF binding site. Based on these correlations, ChoRF appears to be a strong candidate for mediating the effects of glucose on lipogenic gene expression. At present the identity of ChoRF is unknown, but it does not appear that ChoRF is identical to SREBP-1c or USF. The migration of the ChoRF complex is significantly slower than the migration of either of these factors. In addition, antibodies to USF or SREBP-1 fail to inhibit binding of ChoRF to the ChoRE (25). We thus propose that ChoRF is a novel hepatic factor, most likely of the basic/helix-loop-helix family, that is regulated by changes in glucose metabolism.

In summary, we have shown that glucose regulation of fatty-acid synthase transcription is mediated at least in part by a distal enhancer located over 7,000 bp upstream from the transcriptional start site. Within this enhancer a specific ChoRE binds the ChoRF and confers glucose regulation. The ChoRF appears to work in conjunction with SREBP-1c, and possibly USF-1 and -2, to impart glucose and insulin responsiveness to the fatty-acid synthase promoter. Hence, regulation of fatty-acid synthase expression in response to carbohydrate requires both insulin and glucose signaling pathways working synergistically to provide the overall dramatic effects observed by carbohydrate diet. Recently, a similar situation has been found for the S14 gene (25). It is not unreasonable to speculate that many other lipogenic enzyme genes regulated by these two effectors may utilize a similar dual regulatory pathway for glucose and insulin stimulation.

Table I

| Construct | Luciferase -Fold glucose induction |
|-----------|-----------------------------------|
| Fatty-acid synthase | | |
| FAS (~250) | 1870 2017 1.1 |
| ~7382/~4606 FAS (~250) | 4308 8072 1.9 |
| ~7382/~4606m FAS (~250) | 3161 4309 1.4 |
| L-pyruvate kinase | | |
| PK (~96) | 704 733 1.0 |
| FAS (~7382/~6970) PK (~96) | 2436 5364 2.2 |
| 2x~FAS (~7218/~7194) | 2761 12699 4.6 |
| PK (~96) | | |
| 2x~FAS (~7218/~7194) PK (~40) | 132 3529 26.7 |
| 2x~S14 (~1448/~1422) PK (~40) | 260 9874 38.0 |

of ~7242 to ~7150 (Fig. 1). Sequence analysis revealed that this 100-nucleotide region contains candidate recognition sites for several transcription factors, including C/EBPα and NF-1, but the sequence located between ~7240 and ~7190 was of particular interest, because it possesses characteristics that are similar to the ChoRE of the l-type pyruvate kinase and S14 genes. Like the ChoRE of the l-type pyruvate kinase and S14 genes, the E-box motif of the ChoRE for fatty-acid synthase exists as palindromic sequences that are separated by a 5-bp spacer (Fig. 3). In the case of the fatty-acid synthase ChoRE, one motif contains a five out of six nucleotide match and the other displays a four out of six nucleotide match with the ChoRE of the other two genes. Mutation analysis of the rat S14 and l-type pyruvate kinase genes indicates that the first 4 bp (CAGGTG) of the E-box are critical to confering glucose responsiveness, while mutations in the fifth and sixth positions did not disrupt the glucose response (41). Spacing between the E-boxes is critical to the function of the ChoRE, because shortening the spacer to 4 bp resulted in a loss of the glucose response, and lengthening the spacing sequence to 6 bp blunted the glucose response (28).
REFERENCES

1. Zammit, V. A. (1999) Biochem. J. 343, 505–515
2. McGarry, J. D., and Brown, N. F. (1997) Eur. J. Biochem. 244, 1–14
3. Clarke, S. D., Armstrong, M. K., and Jump, D. B. (1990) J. Nutr. 120, 218–224
4. Clarke, B. A., and Clarke, S. D. (1982) Arch. Biochem. Biophys. 218, 92–100
5. Paulauskas, J. D., and Sul, H. S. (1989) J. Biol. Chem. 264, 574–577
6. Katsurada, A., Iritani, H., Fukuda, Y., Matsumura, Y., Nishimoto, T., Noguchi, T., and Tanaka, T. (1990) Eur. J. Biochem. 190, 427–433
7. Towle, H. C., Kaytor, E. N., and Shihi, H. M. (1997) Annu. Rev. Nutr. 17, 405–433
8. Girad, J., Ferré, P., and Foufelle, F. (1997) Annu. Rev. Nutr. 17, 325–352
9. Jump, D. B., and Clarke, S. D. (1999) Arch. Biochem. Biophys. 365P, 1–14
10. Jump, D. B., Bell, A., Lepar, H., and Hu, D. (1990) Biochem. J. 269, 521–527
11. Williams, B. H., and Berdanier, C. D. (1983) J. Nutr. 112, 534–541
12. Armstrong, M. K., Blake, W. L., and Clarke, S. D. (1991) Biochem. Biophys. Res. Commun. 177, 1056–1061
13. Jump, D. B., Clarke, S. D., Peters, J., and Etherton, T. D. (1998) J. Biol. Chem. 273, 2307–2312
14. Yin, D., Clarke, S. D., and Berdanier, C. (1983) J. Nutr. 112, 534–541
15. Moustaid, N., Beyer, R. S., and Sul, H. S. (1994) J. Biol. Chem. 269, 521–527
16. Mouatt, N., Beyer, R. S., and Sul, H. S. (1994) J. Biol. Chem. 269, 5829–5834
17. Latasa, M. J., Moon, Y. S., Kim, K. H., and Sul, H. S. (2000) J. Biol. Chem. 275, 10211–10217
18. Roder, K., Wolf, S., Beck, K. F., and Schweizer, M. (1997) J. Biol. Chem. 272, 21616–21624
Involvement of a Unique Carbohydrate-responsive Factor in the Glucose Regulation of Rat Liver Fatty-acid Synthase Gene Transcription
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J. Biol. Chem. 2001, 276:21969-21975.
doi: 10.1074/jbc.M100461200 originally published online March 28, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100461200

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