Insulin and Dietary Fructose Induce Stearoyl-CoA Desaturase 1 Gene Expression in Liver of Diabetic Mice*

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Recently, we showed that the increase in SCD1 mRNA due to feeding a fat-free, high carbohydrate diet to mice is due to enhancement of the transcriptional activity of the SCD1 gene (13).

Since the carbohydrate-mediated induction of SCD1 transcription in the fasted mouse can be partially explained by stimulation of endogenous insulin released from the pancreas into the bloodstream, we used the streptozotocin-induced diabetic mouse model to study the role of carbohydrate and insulin in the regulation of expression of the SCD1 gene in liver. It is shown here that both fructose and insulin administration cause induction of the SCD1 mRNA in the livers of diabetic mice. The induction of the SCD1 mRNA in response to fructose or insulin is only partly a consequence of the transcriptional activation of the gene, indicating that effects of fructose or insulin also occur at the posttranscriptional level. In addition, the insulin-mediated induction of SCD1 mRNA is blocked by cAMP and theophylline, implicating a negative role for cAMP in the regulation of the SCD1 gene in vitro. Furthermore, we report that the effects of insulin on SCD1 mRNA are also inhibited by cycloheximide administration, indicating that ongoing protein synthesis is necessary for induction of SCD1 mRNA by insulin. Overall, our results suggest positive control by both insulin and fructose on SCD1 gene expression in mouse liver.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and other nucleic acid-modifying enzymes were obtained from Promega. The fat-free, high-carbohydrate diet was from U. S. Biochemical Corp. Fructose, dextrose, and starch diets were made in our laboratory using the appropriate sugars obtained from Fisher. Bovine insulin was from Sigma, and porcine insulin was from Eli Lilly Co. The origin of the cDNA probes for phosphoenolpyruvate carboxykinase (pPck-10) and albumin (pmalb2) were previously described (13). All plasmid DNAs were isolated by a modification of the SDS-NaOH method (14) or using Wizard Maxi prep columns from Promega. Nucleic acid-modifying enzymes were purchased from Promega, Boehringer Mannheim, and Ambion Inc. Streptozotocin, dibutyryl-cAMP, theophylline, and cycloheximide were all obtained from Sigma.

Animals and Treatments—Male CD-1 mice, used throughout this study, were obtained from the Department of Biochemistry of the University of Wisconsin and from Harlan Sprague-Dawley. Mice (4–6 weeks old) were made diabetic by four daily intraperitoneal injections of streptozotocin (5 mg/100 g) in sodium citrate (pH 4.5), as described by Ziegler et al. (15). Mice were used within 7 days after the last injection. Diabetes was confirmed by high fasting glucose level determined using the hexokinase-glucose-6-phosphate dehydrogenase assay (Sigma) and Clinetex (Fisher). Animals were considered diabetic when blood glucose levels exceeded 250 mg/dl, usually on the 7th day from the first injection. The animals were maintained on a complete diet of Purina Chow throughout the entire course of streptozotocin injections. 24 h before insulin was administered, the diabetic mice were switched to an 80% starch diet and were maintained on this diet for the remainder of the study. Insulin was administered to diabetic animals at a combined dose of regular bovine insulin (5 units/100 g) intraperitoneally and porcine insulin (50 units/100 g) subcutaneously. For other diabetic dietary stud-

The abbreviation used is: SCD, stearoyl-CoA desaturase.

The transcription and mRNA levels of murine liver stearoyl-CoA desaturase 1 (SCD1) are induced 11- and 45-fold, respectively, by feeding fasted normal mice with a fat-free, high carbohydrate diet (Ntambi, J. M. (1992) J. Biol. Chem. 267, 10925–10930). In this study, we used streptozotocin-induced diabetic mice to study the regulatory role of carbohydrate and insulin on expression of the SCD1 gene in liver. Fructose administration to fasted diabetic mice induced a 2-fold increase in SCD1 mRNA within 6 h and a 25-fold increase within 24 h. Similarly, insulin administration to diabetic mice induced SCD1 mRNA from 4-fold within 4 h to 22-fold within 24 h. Insulin plus fructose, however, achieved full induction, with a 45-fold increase of SCD1 mRNA and a 10-fold increase in SCD1 transcription within 24 h. Additionally, the effect of insulin on SCD1 mRNA was inhibited 75% with dibutyryl-CAMP and theophylline administration and 70% by cycloheximide administration.

Synthesis of liver albumin mRNA showed little change upon dietary manipulation or insulin treatment. Our data demonstrate that insulin and dietary fructose or a metabolite of fructose positively regulate the expression of the SCD1 gene in mouse liver.

Stearoyl-CoA desaturase is a key enzyme involved in the transcription of unsaturated fatty acids and is a point of regulation of this process. The enzyme catalyzes the Δ9-cis desaturation of C18 and C16 fatty acyl-CoAs, leading to the synthesis of oleyl and palmitoleyl-CoA as major products (1). Hepatic stearoyl-CoA desaturase enzyme levels, like a number of lipogenic enzymes, are increased by a high carbohydrate diet (2) and decreased by starvation and diabetes (3). Insulin administration to diabetic rats restores the enzyme level to normal (4, 5). Fructose, but not glucose, increases the enzyme level in diabetic rats (6). While insulin and fructose activate the transcription of several rat genes, such as fatty acid synthase and S14 (7, 8), leading to the stimulation of lipogenesis in rat liver, the mechanism of insulin and fructose action on stearoyl-CoA desaturase activity in liver tissue is not known. Studies on hepatic pyruvate kinase and malic enzyme suggest that dietary carbohydrates act independently of insulin to induce the corresponding mRNA through posttranscriptional regulatory mechanisms (9–11). In insulin rats, the increase in stearoyl-CoA desaturase enzyme activity in response to a fat-free, high carbohydrate diet correlates positively with an increase in abundance of stearoyl-CoA desaturase mRNA in total liver (12).

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ies, diabetic mice were fasted for 12 h and then received either 80% fructose or 80% glucose pellets with or without the combined insulin administration. Dibutyryl-cAMP (6 mg/100 g) and theophylline (3 mg/100 g), given as a combined dose, and cycloheximide (1 mg/100 g) were intraperitoneally administered 6 h after insulin administration. Control animals received sodium citrate instead of insulin, and all animals were given free access to tap water.

Measurement of mRNA and Nuclear Run-on Activity—Total hepatic RNA was extracted as described by Chirgwin et al. (16). SCD1 mRNA and albumin mRNA expression were measured by RNase protection as previously described (17) and quantified by laser densitometric scanning of autoradiograms. The integrity of the RNA used in the RNase protection assays was verified by fractionating 30 μg of total liver RNA on a 1% agarose gel containing 6.7% formaldehyde and visualizing the RNA by ethidium bromide staining. Nuclei were isolated from fresh liver tissue and used for gene transcription studies as previously described (13).

RESULTS

Induction of SCD1 mRNA by Fat-free, High Carbohydrate Diet in Diabetic Mouse Liver—We have previously shown that refeeding of a fat-free, high carbohydrate diet to fasted normal mice increases the transcription of the SCD1 gene (13). These nutritional manipulations of fasting and refeeding cause fluctuations in the circulating levels of insulin and glucagon, which themselves act as regulating agents. To determine whether the carbohydrate regulation of the SCD1 gene was insulin-dependent, we examined the effects of the fat-free, high carbohydrate diet on hepatic SCD1 mRNA expression in fasted diabetic mice. SCD1 mRNA levels were analyzed by RNase protection using a radiolabeled SCD1-specific complementary riboprobe (17). The protected probe segments were then subjected to denaturing gel electrophoresis, and the autoradiogram is shown in Fig. 1A. Hybridization signals for SCD1 mRNA were quantitated by laser densitometric scanning of autoradiograms with different exposure times to verify the -fold induction (Fig. 1B). The fat-free, high carbohydrate diet caused a 10-fold increase in SCD1 mRNA in the diabetic mouse. Diabetic mice receiving only fructose showed a 23-fold increase after 24 h, while these mice showed no increase in SCD1 mRNA after receiving glucose alone. Fructose plus insulin and glucose plus insulin induced a 45- and 46-fold increase, respectively, in SCD1 mRNA within the same time period. This -fold induction is similar to that previously reported (13) when a fasted normal mouse is fed a fat-free, high carbohydrate diet for the same period of time. These results indicate that normal circulating insulin levels are not required for fructose induction of SCD1 mRNA but that insulin is, indeed, required for complete induction of the gene.

Time Course of SCD1 mRNA Induction by Dietary Fructose—Given the results in Fig. 1, it appears that fructose rather than glucose is the component of the fat-free, high carbohydrate diet that causes induction of the SCD1 mRNA in the diabetic mouse. To examine the induction time course of SCD1 mRNA by fructose in the diabetic state in vivo, mice were fasted and refed an 80% fructose diet for various periods of time. SCD1 mRNA accumulation in liver tissue was analyzed using the RNase protection assay, and the results are shown in Fig. 2. Based on the quantitation of hybridization signals by laser densitometry, the SCD1 mRNA showed a 2-fold increase in 6 h, which rose to a maximum 23-fold increase within 24 h (Fig. 2, A and B). No further induction was obtained at longer time points (data not shown). These results show that fructose can induce SCD1 mRNA independently of insulin. The level of liver albumin mRNA, which serves as a control for liver-specific gene expression, was also analyzed by RNase protection (Fig. 2C) and did not significantly change during the time course of fructose feeding.

Time Course of SCD1 mRNA Induction by Insulin—To examine the induction of SCD1 mRNA by insulin alone in vivo, SCD1 mRNA accumulation was determined in livers of diabetic mice that had been injected with insulin for various periods of time. SCD1 mRNA levels were analyzed by RNase protection and quantified as described above. Based on the time course of induction, insulin administration increased the SCD1 mRNA from 4-fold within 4 h and accumulated more rapidly to about 22-fold within the next 20 h (Fig. 3A). Similar patterns of SCD1 mRNA accumulation were observed in two separate experiments. Again, the level of liver albumin mRNA was also analyzed by RNase protection (Fig. 3C), and it did not significantly change during the time course of insulin administration. Control animals receiving sodium citrate instead of insulin showed no change in SCD1 or albumin mRNA (data not shown). This control study shows that the effect of insulin on SCD1 mRNA levels in mouse liver is specific.

Effects of Insulin and Dietary Fructose on the Transcriptional Rate of the SCD1 Gene—Nuclear run-on experiments were performed to determine whether the increase in steady state levels
of SCD1 mRNA in response to dietary fructose and insulin administration was due to an increase in gene transcription.

This was accomplished using nuclei isolated from livers of mice that had been injected with insulin or fed fructose for the amounts of time that resulted in a maximum increase in mRNA. SCD1 transcriptional activity was measured as hybridization of the labeled RNA to pC3, a 2-kilobase cDNA of the SCD1 gene including the entire coding region, as was previously described (13). Fig. 4 demonstrates that a 3- and 2-fold increase in the transcriptional activity of the SCD1 gene occurred in response to fructose and insulin, respectively, while fructose plus insulin resulted in a 10-fold increase in the transcription of the SCD1 gene. The increase caused by fructose plus insulin correlates well with the 11-fold increase in the transcriptional activity of the SCD1 gene obtained with a fat-free, high carbohydrate diet fed to a fasted normal mouse (13). This result suggests that both carbohydrate and insulin are required to obtain full transcriptional induction of the SCD1 gene in liver. Attempts to establish a time course of transcriptional induction with either fructose or insulin alone were unsuccessful because the -fold induction in each case was very low. Since the change in transcription cannot explain the increase in mRNA for fructose and insulin alone, these effects must be primarily posttranscriptional.

To ensure that the change in transcriptional run-on activity measured for the SCD1 gene does not reflect a general increase in transcription, in response to feeding or insulin administration, similar measurements were performed with pmalb2 and pPck-10. The pmalb2 is an albumin cDNA clone for which the corresponding mRNA does not change in abundance (Figs. 2C and 3C), and pPck-10 is a cDNA clone for phosphoenolpyruvate carboxykinase that decreases with insulin administration in vivo and in vitro (18). No significant change in transcriptional run-on activity was detected with the albumin cDNA plasmid, while the transcription of the phosphoenolpyruvate carboxykinase gene decreased in response to insulin administration and did not change with fructose feeding. The phosphoenolpyruvate carboxykinase gene was also induced by diabetes, possibly due to high levels of cAMP. This is, again, in agreement with published data (19). Labeled RNA from the transcriptional assays showed virtually no hybridization to pGEM. These control studies, therefore, show both sequence specificity of the transcriptional assay and reflect the in vivo status of the SCD1 gene. These studies also provide further proof that fructose and insulin act together at a transcriptional level to regulate the expression of the SCD1 gene in vivo.

Effect of cAMP and Cycloheximide on SCD1 mRNA Induction by Insulin—Many genes that are stimulated by insulin are inhibited by cAMP (7, 8). We therefore examined the effect of cAMP on the insulin-mediated induction of SCD1 gene expression using dibutyryl-cAMP and theophylline. Dibutyryl-cAMP and theophylline were administered 6 h after insulin adminis-
Stearoyl-CoA Desaturase 1 Gene Regulation

Fig. 4. Effect of an insulin and dietary fructose on the transcriptional rate of the SCD1 gene in diabetic mouse liver. A, nuclei were isolated from livers of fasted diabetic mice that had been refeed fructose or injected with insulin for various periods of time for in vitro run-on transcription. 32P-Labeled mRNA was isolated, and hybridizations to 5 μg of immobilized DNA probes were carried out as described under "Experimental Procedures." pGEM, pGEM plasmid; pC3, cDNA for mouse SCD1; pmalb2, cDNA for mouse liver albumin; pPck-10, cDNA for rat liver phosphoenolpyruvate carboxykinase. B, densitometric scan of the autoradiogram shown in A. The results were normalized to the control value (fasted diabetic mouse) obtained for each plasmid DNA and are presented as -fold change in transcription. The data are representative of two different experiments yielding essentially identical results.

DISCUSSION

Stearoyl-CoA desaturase genes (SCD1 and SCD2) encode a key enzyme involved in the biosynthesis of unsaturated fatty acids as well as the regulation of this process. The transcription of the SCD1 gene was previously shown to increase dramatically in the livers of normal starved mice upon feeding a fat-free, high-carbohydrate diet (13). Because the carbohydrate-mediated induction of SCD1 gene transcription in the fasted normal mouse can, at least in part, be explained by an increase in circulating levels of insulin, we used diabetic mice to study the role that carbohydrate and insulin play in the regulation of expression of the SCD1 gene in liver. Our studies show that both fructose and insulin induced the expression of the SCD1 gene in vivo (Figs. 2 and 3). Dietary fructose increased the level of SCD1 mRNA in diabetic liver from 2-fold in the first 6 h to 23-fold in 24 h. Insulin administered to diabetic mice induced a 4-fold increase of SCD1 mRNA levels within the first 4 h and a 22-fold increase within 12 h, exhibiting a delay in response characteristic of insulin for some genes. In this respect, the regulation of the SCD1 gene is very similar to that of the pyruvate kinase gene as has been reported (20, 21) but is different from that of the mouse fatty acid synthase gene and the rat S14 protein gene, each of which respond rapidly to insulin (7, 8). The full induction of SCD1 mRNA previously observed in the normal mouse fed a fat-free, high carbohydrate diet could not be restored by either insulin or fructose alone but instead required a combination of the two or a combination of glucose and insulin (Fig. 1).

We then examined the mechanism of induction of the SCD1 mRNA by insulin and dietary fructose in diabetic mouse liver using a nuclear run-on transcription assay. As shown in Fig. 4, both fructose and insulin enhanced the rate of transcription of this gene but to a small extent when independently administered. When combined, fructose plus insulin caused a 10-fold increase in the transcription of the SCD1 gene in the same time period. However, full induction of SCD1 mRNA cannot be solely accounted for by transcription of the SCD1 gene, implicating regulatory roles for both carbohydrate and insulin at the post-transcriptional level. Again, the regulation of the SCD1 gene by fructose and insulin is similar to that reported for the pyruvate kinase and malic enzyme gene expression (9, 11, 22), which require insulin to observe significant transcriptional activation upon carbohydrate feeding. Whether the SCD1 and pyruvate kinase genes are regulated by similar molecular signals remains to be determined. The difference in kinetics of transcription and mRNA expression of the SCD1 gene, from that of the S14 and fatty acid synthase genes, may suggest that the molecular basis for insulin induction of several groups of hepatic genes involved in lipid metabolism is different. However, these effects were specific because transcription of the phosphoenolpyruvate carboxykinase gene was not affected by fructose and, as expected, was repressed by insulin treatment. No significant hybridization to pGEM was observed, again showing the specificity of the assay.

The exact mechanism by which dietary fructose regulates the expression of the SCD1 gene remains unclear. One possibility is that the low insulin levels associated with the diabetic state result in greatly depressed glucokinase activity, while fructokinase and triosekinase are unaffected (23). Therefore, unlike glucose, fructose can stimulate lipogenesis in the liver of diabetic mice (24). It is possible then that intermediates unique to the metabolism of fructose, such as glyceral or beyond, may play a significant role in the induction of expression of the SCD1 gene within the 24-h interval of this study. As recently described, it is also possible that this dietary fructose is activating glucokinase activity by binding to its regulatory protein in liver (25). By this mechanism, then, glucose would be metabolized in the diabetic liver in absence of insulin, and the molecule that is regulating SCD1 could be a common metabolite of glucose and fructose.

In addition, our results show that fructose, or a metabolite of fructose, alone can induce SCD1 gene expression independent
of insulin. This result is consistent with the suggestion that insulin and fructose act at two different steps to regulate the expression of the SCD1 gene. However, these considerations do not rule out the direct role of insulin in synergistic regulation of SCD1 gene expression. We showed that fructose plus insulin induced a 45-fold increase in SCD1 mRNA in the same time period. Insulin may function either to accelerate hepatic carbohydrate metabolism or induce functions independent of dietary carbohydrate.

Since many insulin-stimulated genes are inhibited by cAMP (7, 8), we examined the effect of cAMP on SCD1 gene expression. Our results show that the positive action of insulin on SCD1 gene expression was countered by the negative effect of dibutyryl-cAMP and theophylline (Fig. 5). The inhibition of SCD1 gene expression by cAMP suggests that in the diabetic state the inhibition is due, at least in part, to glucagon activation of cAMP-dependent protein kinases. Our results show that the induction of the SCD1 mRNA by insulin also requires protein synthesis (Fig. 5). This is similar to effects of other insulin-sensitive genes (7, 9, 22) but in contrast to the action of insulin on phosphoenolpyruvate carboxykinase, in which expression of the gene is inhibited within 15 min and protein synthesis is not required (26). Therefore, the action of insulin on SCD1 gene expression may not be direct; insulin (or its messenger) may stimulate synthesis of some unknown protein with a very rapid turnover, and this protein may in turn somehow stimulate transcription of the SCD1 gene. Stearoyl-CoA desaturase enzyme synthesis is known to be regulated by insulin in liver (4, 5) and by glycerol in the diabetic liver (6), but the mechanism by which carbohydrate and insulin mediate these effects has not yet been established in liver at the time of this report. The increase in SCD enzyme synthesis in 3T3-L1 preadipocytes during differentiation has been reported (27), and the regulation of the transcription of the SCD1 gene by insulin in adipocytes has also been described (28). Recently, several insulin-responsive elements have been mapped in lipogenic genes, including fatty acid synthase gene, and protein factors that bind to these sequences have been described (29, 30). Whether similar factors mediate the regulation of the SCD1 gene expression by insulin in liver has not been established. It is hoped that more precise identification of the carbohydrate- and insulin-dependent factors that regulate SCD1 gene expression will be best approached with liver cells in culture.

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Fig. 5. Effect of cAMP and cycloheximide (CHX) on insulin-mediated SCD1 mRNA expression. Diabetic mice were injected with insulin as described under "Experimental Procedures." 6 h after insulin administration, either a combined dose of dibutyryl-cAMP and theophylline or a single dose of cycloheximide was intraperitoneally administered. Total liver RNA was isolated and subjected to RNase protection analysis using a SCD1-specific complementary riboprobe 18 h after insulin administration. The autoradiogram is representative of two separate experiments yielding essentially identical results.