Nutritional and Hormonal Regulation of Fatty Acid Synthase Gene Expression

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Abstract

The maximum 30-fold level of fatty acid synthase(FAS) mRNA was achieved by 6hr after intraperitoneal injection of insulin. The kinetics and maximum effect of insulin were most evident on the 7.2kb mRNA. In six hours after insulin administration there was about 100-fold increase in steady-state mRNA level. We observed a sharp decrease in 7.2kb mRNA by 8hr after insulin administration while there was no change in FAS mRNA content between the 6hr and 8hr-sampling periods. In contrast, a maximum induction of 4-fold was shown in the level of 5.1kb mRNA after insulin injection in streptozotocin-diabetic mice.

Key words: fatty acid synthase(FAS), positive and negative control

INTRODUCTION

Diabetic animals show abnormalities in carbohydrate and lipid metabolism in liver. There is an increase in gluconeogenesis, a decrease in glycolysis and glycogen production and fatty acid synthesis is impaired. Administration of insulin restores the rates of these processes to normal levels. Discovery of insulin and its structure was a major event in the history of biology. However, the mechanism of insulin action remains enigmatic.

A major breakthrough in elucidating insulin action came from the discovery that tyrosine kinase activity is associated with the β-subunit of the receptor and that the activation of the kinase is stimulated by insulin binding to the α-subunit(1,2). The insulin receptor was recently cloned and showed homology with many oncogenes(3). This led to a new hypothesis about the mechanism of insulin action. However, it is not clear how insulin binding to its receptor brings about many biological responses. Some of the effects of insulin on metabolism are due to a phosphorylation-dephosphorylation mechanism involving pre-existing regulatory enzymes. The insulin effects on enzyme biosynthesis have not been studied in depth due to the lack of probes to investigate molecular mechanisms of induction or deinduction of specific enzymes by the hormone.

Recently Andreono et al.(4) and Cimbala et al.(5) independently reported that insulin rapidly decreases the mRNA level for phosphoenolpyruvate carboxykinase(PEPCK), a rate limiting gluconeogenic enzyme, after mRNA for PEPCK is elevated by treating either rat liver or hepatoma cells with cAMP analogs. Furthermore, the negative control of mRNA for PEPCK by insulin is later shown to be transcriptional(6) However, positive control of insulin on gene transcription has not been studied extremely well. It has been reported that insulin plays a role in increasing steady-state mRNA level in several proteins, such as pyruvate kinase(7), casein(8), albumin(9), amylase(10) and the unidentified P33 proteins(11), but it has not yet been shown that insulin alone increases the transcription rate of the genes coding for these proteins.

Fatty acid synthase(FAS) is one of the few enzymes whose synthesis is regulated by insulin(12). Fatty acid synthase plays a central role in *de novo* lipogenesis in animals by catalyzing all the reactions involved in the conversion of acetyl CoA and malonyl CoA to palmitate. Enzyme activity of FAS is not subject to regulation by allosteric effectors or by covalent modification. However, the content of FAS in liver changes drastically when animals are subjected to different nutritional and hormonal

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states. When rats are fasted for 1~2 days the rate of FAS synthesis declines 4-fold while refeeding previously fasted animals a high carbohydrate diet increases the rate of FAS synthesis by 20-25-fold(12). Both lipogenic and lipolytic hormones may participate in regulating FAS content. Administration of glucagon or dibutyryl cAMP during refeeding of fasted animals completely blocks elevation of the rate of FAS synthesis. Fasted, diabetic rats have a low FAS level and cannot increase hepatic FAS activity in response to refeeding. Treatment of these animals with insulin, however, elicits a rapid, 20-fold rise in the relative rate of FAS synthesis and restores FAS activity to a normal level with the same kinetics of recovery as obtained for fasted/refed control animals(12). The above cited changes in FAS activity and the rate of synthesis were later shown to be due to changes in translatable mRNA levels(13). Goodridge and coworkers, working with chick embryo hepatocytes, did not detect an appreciable stimulatory effect of insulin on mRNA level despite a large increase in FAS synthesis and concluded that the regulation was translational(14). However, we have cloned cDNA sequences to murine FAS mRNA. Using the cloned cDNA sequences as probes, we detected a 10-fold increase in mRNA level for FAS within 1hr and a maximum of 30-fold increase in 6hr when diabetic mice without change in nutrient intake were injected with insulin.

MATERIALS AND METHODS

Construction of cDNA library and screening

Poly(A⁺) RNA was prepared from livers of previously fasted mice which were refed a high carbohydrate, fat-free diet and from 3T3-L1 adipocytes. The mRNA(5μg each) was employed as a template for the first strand synthesis catalyzed by avian myeloblastosis virus reverse transcriptase after oligo(dT) priming. Second strand was synthesized by the Klenow fragment of DNA polymerase 1 and RNase H(15). The double strand cDNAs were inserted into the EcoR1 site of λgt10. The resultant libraries were screened by plaque hybridization with the two EcoR1-Pst1 fragments of 5.3-kb cDNA sequence, first labeled with ³²P by random priming.

Northern blot analysis

Poly(A⁻) RNA was prepared by subjecting total RNA prepared from mouse tissues or 3T3-L1 cells by a phenol extraction method(16) to oligo(dT) chromatography. Northern blot hybridization was performed(17), using pFAS-1 and p7.2 as probes, after labeling by Nick-translation or by random priming. An actin cDNA sequence (pAM-91) was used to confirm equivalent mRNA contents of each sample.

Nuclear run-on transcription assays

Livers from three mice were homogenized in 5 volumes of buffer containing 0.32M sucrose, 3mM MgCl₂, 5mM Hepes(pH 6.9), and 0.5mM β-mercaptoethanol. Nuclei collected by centrifugation were washed once by centrifugation through a 2.1M sucrose cushion at 20,000 rpm(52,900×g) for 60min in a Beckman SW 28 rotor. The nuclei were stored in liquid nitrogen in 50mM Tris(pH 7.9), 5mM MgCl₂, 0.5mM β-mercaptoethanol, and 40% glycerol. Run-on transcription was carried out at 25°C for 45min in a reaction mixture containing 10⁷ nuclei and 100μ Ci of [α-³²P]UTP(3000Ci/mmol) in a final volume of 0.5 ml. Labeled RNA was isolated and hybridized to 5μg of plasmids fixed on nitrocellulose(18).

RESULTS AND DISCUSSION

Northern blot analysis of mRNAs

The purified mRNA was used as template to construct partial cDNA libraries. The first strand was synthesized by reverse transcription. Then the second strand was syn-
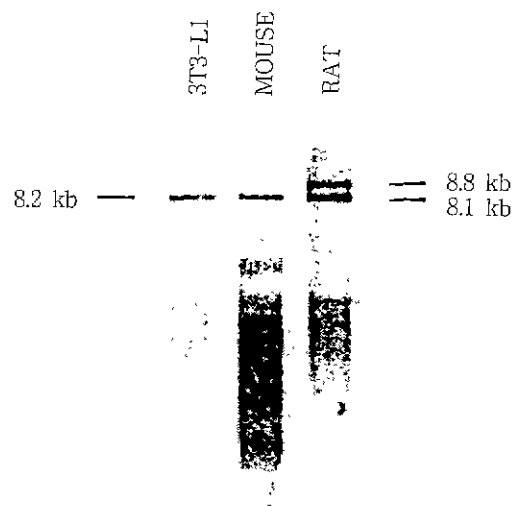

Fig. 1. Northern blot analysis of mouse liver, 3T3-L1 adipocytes and rat liver mRNAs. A 3.1kb insert of FAS cDNA clone was used as a probe. For size estimation HindIII digests of λ DNA were used as standards.

thesized with DNA polymerase 1 and RNase H according to Gubler and Hoffman(15). The first library was constructed using dG/dC tailing and the *Pst*1 site of pBR322. The second library was constructed using the *Eco*R1 site of λgt10. We employed a differential hybridization technique because we observed that the levels of translatable mRNAs coding for FAS in liver were increased 20-fold when starved mice were fed a high carbohydrate, fat-free diet. We have selected approximately 50 clones which are expressed differentially. We have identified a total of 22 clones which code for FAS mRNA by hybrid-selected translation. There was a single FAS mRNA of 8.2kb in size that codes for mouse FAS by Northern blot analysis under stringent conditions. This is in contrast to rat tissue which shows two mRNAs with sizes of 8.8 and 8.1kb(Fig. 1).

Regulation of mRNAs by insulin

To find out if insulin regulates the expression of genes coding for FAS and other specific mRNAs *in vivo*, we
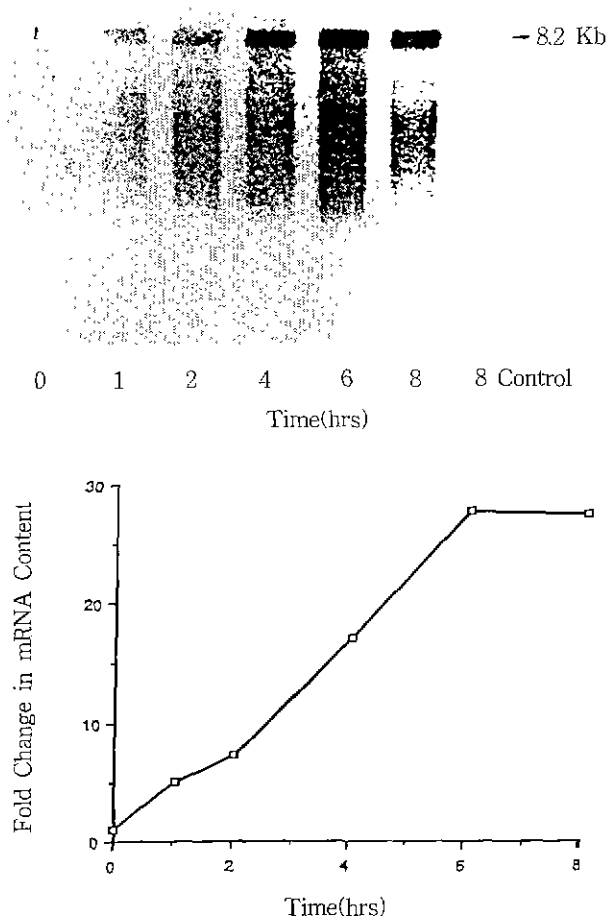

Fig. 2. Effect of insulin on mRNA level for FAS in streptozotocin-diabetic mice.
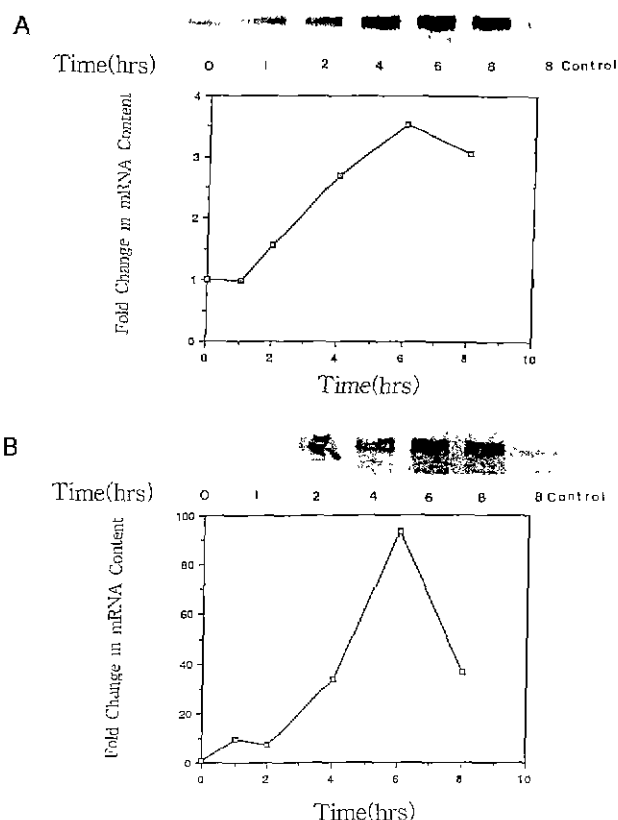

Fig. 3. Effect of insulin on levels of 5.1kb(A) and 7.2kb (B) mRNAs in streptozotocin-diabetic mice.

utilized the streptozotocin-diabetic mice model. Time courses of specific mRNA induction were followed after insulin administration. The level of fatty acid synthase mRNA increased 5-fold 1hr after intraperitoneal injection of insulin. The maximum level was achieved by 6 hr reaching a 30-fold induction(Fig. 2). The kinetics and maximum effect of insulin on other specific mRNAs were different, the 7.2kb mRNA being the most responsive to insulin. In six hours after insulin administration there were about two orders of magnitude(100-fold) increase in steady-state mRNA level. We observed a sharp decrease in 7.2kb mRNA in 8hr after insulin administration while there was no change in FAS mRNA content between the 6hr and 8hr sampling periods(Fig. 2 and Figs. 3A, 3B). In contrast, a maximum induction of 4-fold was shown in the level of 5.1kb mRNA after insulin injection in streptozotocin-diabetic mice(Figs. 3A and 3B). This indicates that regulation of gene expression during refeeding is the result of complex metabolic modifications. It is possible that *in vivo* insulin plays a dominant role in the regulation of FAS, 7.2 and 5.1kb mRNAs, while glucagon may play such a role in other cases. These results agree with that of Paulauskis and Sul(19). The individual con-

tributions of hormonal and nutritional factors will be dissected by using cultured cells. Moreover, further experiments are necessary to determine whether the control is at transcriptional, RNA processing, RNA stability or in combination.

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