Tumor Necrosis Factor Inhibits the Transcriptional Rate of Glucose-6-Phosphatase In Vivo and In Vitro

Shula Metzger, Nachum Begleibter, Varda Barash, Olga Drize, Tamar Peretz, Eitan Shiloni, and Tova Chajek-Shaul

Recombinant human tumor necrosis factor-alpha (TNF) injection in mice was associated with a reduced blood glucose level, already manifest 6 hours following cytokine administration. Insulin levels were not affected. Glycogen content was decreased in a dose-dependent and time-response manner. The activity of glucose-6-phosphatase (G6Pase) was already reduced 6 hours after TNF injection and was sustained 12 hours afterward. Phosphoenolpyruvate carboxykinase (PEPCK) activity was not affected initially (6 hours after injection), but a 50% reduction was observed 12 hours following cytokine administration compared with levels in fasting controls. Both liver G6Pase and PEPCK mRNAs were markedly reduced due to an inhibition of the transcriptional rate. A direct inhibitory effect of TNF on G6Pase promoter activity was demonstrated using HuH-7 cells transiently transfected with G6Pase promoter, fused to a reporter gene.

ADMINISTRATION of lipopolysaccharides or tumor necrosis factor-alpha (TNF) to experimental animals causes hypoglycemia, which is associated with a depletion of hepatic glycogen content and an impairment of the gluconeogenic capacity of the liver.[1,2] A reduction in hepatic activity of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) has been reported in endotoxin- and TNF-treated rats, both intact and adrenalectomized.[2-6] Down-regulation of PEPCK both in vivo and in vitro was due to reduced gene expression caused by inhibition of transcription initiation. However, the reduction in PEPCK activity is not considered the principal cause of the resultant hypoglycemia.[4] G6Pase plays an important regulatory role in glucose homeostasis. The murine G6Pase gene has recently been cloned.[7] Several reports indicate that G6Pase activity is closely correlated with G6Pase mRNA levels. It has been shown that after a partial hepatectomy, rats are able to maintain glucose homeostasis despite the loss of two thirds of the hepatic tissue. During liver regeneration and changes in the hormonal milieu, there is an immediate induction of G6Pase enzyme activity and gene expression, suggesting an important role for G6Pase as a regulator of glucose homeostasis.[8] Streptozotocin-induced diabetes in rats results in an increase in G6Pase hepatic enzyme activity due to an elevation in G6Pase mRNA levels in the liver. There is also a significant correlation between blood glucose level and G6Pase activity or mRNA content.[9] In this research, we study the effect of acute administration of TNF to mice on blood glucose and insulin levels, and describe the inhibitory effect of TNF administration on G6Pase activity and gene expression.

MATERIALS AND METHODS

Animals and Experimental Design

C57BI/6 male mice weighing 18 to 24 g were used. The mice received an injection into the femoral vein (under light diethyl-ether anesthesia) with recombinant human TNF (10^7 U/mg; Cetus, Emeryville, CA) dissolved in 0.2 mL 0.9% NaCl. Control mice received 0.2 mL 0.9% NaCl. Blood was taken from the retro-orbital plexus under light anesthesia for glucose and insulin determination as indicated. The mice were killed, and the livers were excised and processed for enzymatic assays and RNA preparation.

Analytical Procedures

Blood glucose and insulin were determined as previously described.[2]

Hepatic glycogen content. Two hundred milligrams of liver was removed and immediately digested with 2 mL 33% KOH for glycogen determination. Glycogen was isolated by ethanol precipitation and subsequently hydrolyzed to glucose by amyloglucosidase.[10]

Preparation of liver homogenates for enzymatic assays. Liver homogenate 20% wt/vol was prepared in ST buffer (10 mmol/L Tris hydrochloride, pH 7.4, 0.25 mmol/L sucrose, and 0.5 mmol/L EDTA) at 5°C. The homogenate was centrifuged at 12,000 × *g* for 20 minutes, and the resulting supernatant was recentrifuged for 45 minutes at 105,000 × *g*.[2]

The supernatant was used for determination of PEPCK activity by measuring the exchange between $KH[^{14}C]O_3$ and unlabeled oxaloacetate.[11] The microsomal pellets were used for determination of G6Pase activity according to the method of Burchell et al.[12] The G6Pase activity assay was performed using intact or disrupted microsomes. Disrupted microsomes were obtained by incubation of the microsomal fraction with 0.25% Na-deoxycholate for 15 minutes at 5°C. This procedure results in fully disrupted microsomes. G6Pase activity was corrected to account for any disrupted component contaminating the untreated microsomes (by assaying low-K_m mannose-6-phosphatase activity), and percent latency was calculated according to the equation, % latency = (1 − G6Pase intact/G6Pase disrupted) × 100.

RNA isolation and Northern blotting. RNA was extracted from liver using the acid guanidinium thiocyanate-phenol-chloroform extraction method.[13] Ten micrograms of RNA was electrophoresed on 1% agarose gel containing 2% formaldehyde, in 3-[*N*-morpholino]propanesulfonic acid buffer. RNA was transferred to Amersham (Buckinghamshire, UK) Hybond nylon membranes and subjected to UV cross-linking and hybridization. Probes were radiolabeled using a Random Primed DNA Labelling kit (Boehringer, Mannheim, Germany). DNA probes used for Northern blot analysis were as follows: a 1.6-kb cDNA fragment of rat PEPCK (a gift from Professor L. Reshef, The Hebrew University) and a 283-bp polymerase chain reaction (PCR) product of mouse G6Pase sequence derived from exon 1. Primers for the amplifica-

From the Division of Medicine, Department of Clinical Biochemistry, and Department of Oncology, Division of Surgery, Hadassah University Hospital Ein-Karem; and the Department of Surgery, Hadassah University Hospital Mount Scopus, Jerusalem, Israel.

Submitted September 6, 1996; accepted December 22, 1996.

Supported in part by the Hebrew University and Hadassah University Hospital Joint Research Fund (N.B.).

Address reprint requests to Tova Chajek-Shaul, MD, Division of Medicine, Hadassah University Hospital, PO Box 12000, Jerusalem 91120, Israel.

0026-0495/97/4605-0019$03.00/0

tion were 5′-GCTTGGACTCACTGCAC-3′ (sense) and 5′-GAAGAC-GAGGTTGAACC-3′ (antisense).[7] The PCR product was confirmed by sequencing.

Nuclear run-on transcription. Nuclei were prepared from mouse liver as described by Meisner et al,[14] and resuspended in 50 mmol/L Tris, 5 mmol/L $MgCl_2$, 0.1 mmol/L EDTA, and 40% glycerol. Elongation of nascent RNA was performed at 26°C for 20 minutes. The reaction mixture (200 μL) contained 15×10^6 nuclei, 30% glycerol, 2.5 mmol/L dithiothreitol, 3 mmol/L $MgCl_2$, 70 mmol/L KCl, adenosine, cytidine, and guanosine triphosphates, (0.5 mmol/L each), 7.5 μmol/L uridine triphosphate (UTP), 1 mg/mL heparin, 500 U/mL RNasin, and 100 μCi UTP (800 Ci/mmol). Labeled RNA was extracted using Tri-reagent solution (Molecular Research Center, Cincinnati, OH) and hybridized to Hybond Nylon (Amersham) membrane–bound DNAs: rat PEPCK cDNA and the *SalI* fragment (~3,000 bp) of G6Pase genomic DNA containing sequences from the second intron to the fifth exon. The genomic DNA was isolated from the mouse genomic library in Lambda Fix II vector (Stratagene, La Jolla, CA) using the G6Pase PCR product described earlier as a probe. The isolated clone was analyzed by partial sequencing and restriction mapping.[7]

Plasmid construction. A 266-bp PCR product of the human genomic G6Pase promoter sequence (from −211 to +55) was inserted upstream of the luciferase coding region of plasmid pXP1[15] (ATCC no. 37576) to create plasmid pLH211. Primers for the amplification were 5′-CTGCTGAGTACATGGCCGAT-3′ (sense) and 5′-AGATGTCAG-CAGAGCCCTT-3′ (antisense).[16] The PCR product was confirmed by sequencing.

Cell culture and transfection experiments. HuH-7 cells[17] were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. For DNA transfection experiments, cells were plated in 35-mm culture dishes at approximately 40% confluency and cultured for 24 hours before transfection. DNA was transfected into cells by the calcium phosphate coprecipitation method.[18] Sixteen hours posttransfection, cells were rinsed and the medium was replaced. Cells were incubated for an additional 32 hours. During the last 16 hours of incubation, cells were treated with 10 ng/mL human TNF. Cells were lysed with 200 μL lysis buffer (Luciferase assay kit; Promega, Madison, WI). A 50-μL aliquot was taken for β-galactosidase assay[19] to normalize for an equal efficiency of transfection. A 20-μL aliquot was taken for luciferase assay, which was performed according to the manufacturer's instructions (Promega).

Statistical Analysis

Experimental values represent the mean ± SE. Data were analyzed using ANOVA, followed by Dunnett's test for comparing a control mean with each group mean.[20] Statistical significance was set at P less than .05.

RESULTS

Effect of TNF on Serum Glucose and Insulin Levels

Mice were injected with TNF and deprived of food for 6 hours (8 AM to 2 PM) or 12 hours (10 PM to 10 AM). Serum glucose levels determined 6 hours following a single injection of 2.5, 5, or 10 μg TNF were reduced to 72%, 41%, and 41%, respectively, compared with glucose levels in control mice injected with saline. Twelve hours following injection, serum glucose levels were 77%, 64%, and 41% of the control glucose level, respectively. Insulin levels in TNF-treated mice and fasted mice were similar (Table 1).

Effect of TNF on Liver Glycogen Content

Liver glycogen levels were decreased in a time- and dose-dependent manner. Six hours after a single injection of 2.5, 5, or 10 μg TNF, glycogen levels were 69%, 41%, and 35% of control levels, respectively (Table 1). Twelve hours after injection, glycogen levels were reduced 50%, 27%, and 11%, respectively.

Table 1. Effect of TNF on Serum Glucose and Insulin Levels in the Circulation and on Liver Glycogen Content

TNF (μg/mouse)	Glucose (mg/dL)		Insulin (μU/mL)		Liver Glycogen (mg/g)	
	6 h	12 h	6 h	12 h	6 h	12 h
0	197 ± 11	151 ± 14	7 ± 2	4 ± 2	46 ± 5	44 ± 3
2.5	142 ± 26*	116 ± 4*	4 ± 2	ND	32 ± 2*	22 ± 7*
5.0	81 ± 10*	97 ± 8*	4 ± 2	4 ± 2	19 ± 2*	12 ± 3*
10.0	81 ± 1*	62 ± 8*	5 ± 1	ND	16 ± 1*	5 ± 1*

NOTE. Mice were injected with TNF at the indicated doses and deprived of food for 6 or 12 hours, after which blood was withdrawn for glucose and insulin determination. Mice were killed and liver glycogen content was determined. Results are the mean ± SE of 5 to 11 animals.

Abbreviation: ND, not determined.

*$P < .05$ *v* control of the same group (0 TNF).

Effect of TNF on G6Pase and PEPCK Activities

A significant decrease in PEPCK and G6Pase activity was observed in mice injected with a single dose of 2.5, 5, or 10 μg TNF. Six hours after TNF injection (8 AM to 2 PM), during which time mice were deprived of food, there was a substantial reduction (~30%) in G6Pase activity (Table 2). At 6 hours, the effect of 2.5 μg TNF was not significantly different from the effect of 5 or 10 μg TNF. However, 12 hours after injection (7 PM to 7 AM), a significant dose-response reduction in G6Pase activity was observed ($P < .05$). TNF administration inhibited G6Pase activity in both intact and disrupted microsomes, but no significant change in percent latency was observed except for a slight but significant increase due to 12 hours of starvation (Table 2). Six hours after injection, PEPCK activity remained unchanged (Fig 1). The effect of TNF on PEPCK activity was observed only 12 hours after injection, with the maximal effect achieved after a single injection of 2.5 μg TNF (~60% reduction; Fig 1). However, the level of PEPCK activity in TNF-treated mice 12 hours after injection was not significantly

Table 2. Effect of TNF on Hepatic G6Pase Activity

Time (h)	TNF (μg/mouse)	G6Pase (nmol/min/mg protein)		Latency (%)
		Intact	Disrupted	
6	0	178 ± 10	411 ± 20	56 ± 2
	2.5	131 ± 21*	279 ± 55*	52 ± 2
	5.0	127 ± 4*	323 ± 30*	60 ± 4
	10.0	109 ± 3*	239 ± 7*	54 ± 1
12	0	196 ± 11	532 ± 24	66 ± 1†
	2.5	147 ± 7*	370 ± 25*	60 ± 1
	5.0	133 ± 12*	285 ± 24*	53 ± 2
	10.0	106 ± 9*	237 ± 10*	55 ± 2

NOTE. Mice received a single injection of TNF at the indicated dose, were deprived of food, and were then killed 6 or 12 hours later for determination of hepatic G6Pase activity. Results are the mean ± SE of 5 to 11 mice.

*$P < .05$ *v* control of the same group.

†$P < .05$ *v* control at 6 hours.

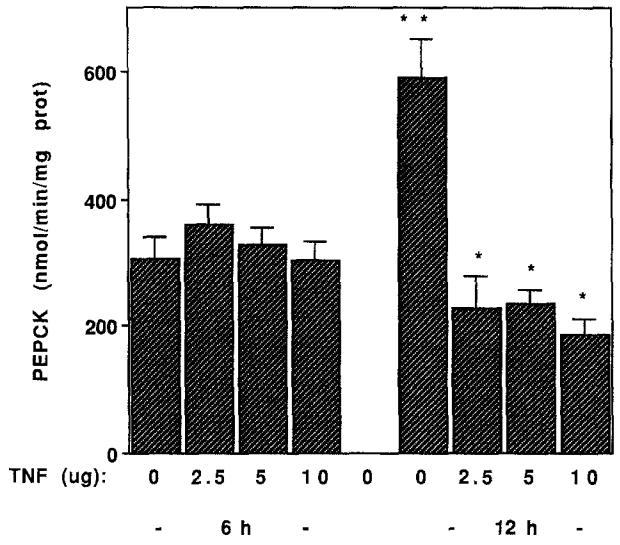

Fig 1. Effect of TNF on PEPCK activity. Mice were injected with different doses of TNF as indicated. After injection, they were starved for 6 hours (8 AM to 2 PM) or 12 hours (7 PM to 7 AM) and then killed. Livers were excised and processed for determination of PEPCK activity. Results are the mean ± SE of 5 to 11 mice. *$P < .05$ *v* control at 12 hours; **$P < .05$ *v* control at 6 hours.

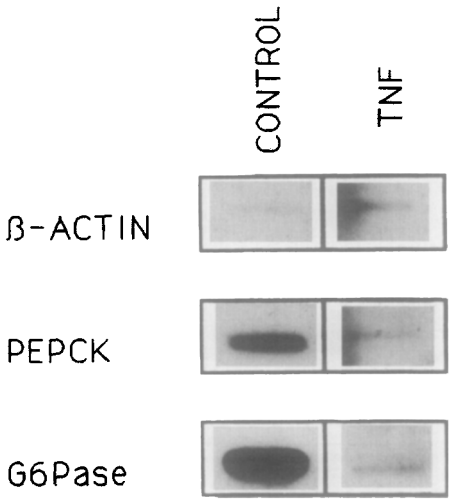

Fig 3. Nuclear run-on analysis of PEPCK and G6Pase transcriptional activity. Mice were killed 5 hours after a single injection of 5 μg TNF. Filters contained 5 μg linearized and denatured rat β-actin cDNA, rat PEPCK cDNA, or mouse G6Pase genomic DNA.

different from the activity measured 6 hours after TNF injection. Thus, it seems that TNF prevents the 12-hour fasting–induced increase in PEPCK activity.

Effect of TNF on G6Pase and PEPCK mRNA Levels

Mice received a single injection of 0, 5, or 10 μg TNF at 7 PM and were deprived of food for 12 hours. They were killed for RNA extraction and Northern blot analysis (Fig 2). Blots were analyzed by a phosphoimager, and hybridization signals were corrected to β-actin mRNA levels. Twelve hours after injection of 5 or 10 μg TNF, G6Pase mRNA levels were approximately 5% of control levels. PEPCK mRNA levels were 10% and 6% after injection of 5 or 10 μg TNF, respectively.

To understand the regulation of G6Pase and PEPCK gene expression, nuclear run-on experiments were performed. Labeled PEPCK and G6Pase mRNA levels were markedly reduced using nuclei preparations from mice 5 hours after a single injection of 5 μg TNF (Fig 3). These experiments demonstrate that the effects of TNF on G6Pase and PEPCK mRNA levels are due to inhibition of the transcriptional rates.

Direct Effect of TNF on G6Pase Proximal Promoter Activity

To determine if G6Pase transcription is directly affected by TNF, promoter activity was studied in the hepatoma cell line HuH-7. Plasmid pLH211 containing the human G6Pase promoter (from −211 to +55) upstream of the luciferase reporter gene was transiently transfected into HuH-7 cells together with a reference plasmid containing the bacterial β-galactosidase gene under the cytomegalovirus promoter. The results show that the proximal region of the G6Pase promoter contains liver-specific elements. Cells transfected with the test plasmid contained 50-fold greater luciferase activity than cells transfected with the basic plasmid pXP1 (Fig 4). In epithelial cells (HR9), G6Pase promoter caused only a threefold increase in luciferase activity (data not shown). Treatment with 10 ng/mL TNF significantly decreased G6Pase promoter activity in hepatoma cells, to 60% of the activity determined in untreated cells (Fig 4).

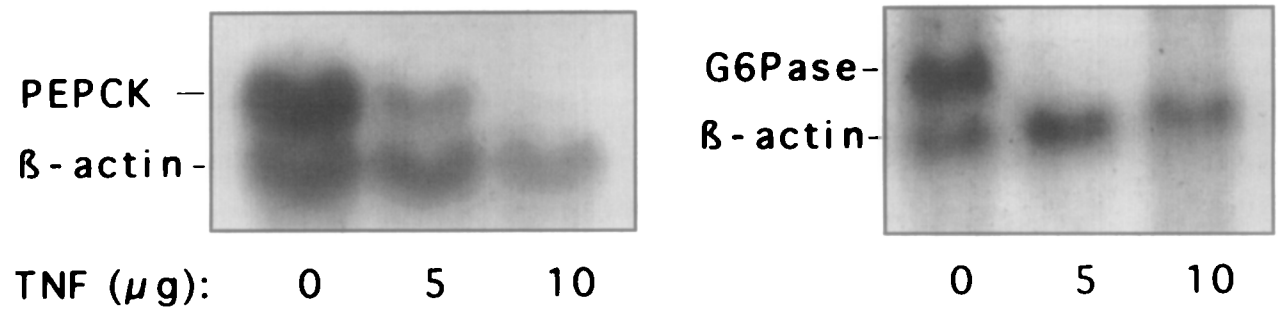

Fig 2. Effect of TNF on hepatic mRNA levels of PEPCK and G6Pase. Animals received a single injection of 0 (control), 5, or 10 μg TNF at 7 PM, were deprived of food for 12 hours, and were then killed. Livers were excised for RNA extraction, and Northern blot analysis was performed. Membranes were hybridized with ^{32}P-labeled rat β-actin, rat PEPCK, and mouse G6Pase as indicated. Figure represents 1 of 3 similar experiments.

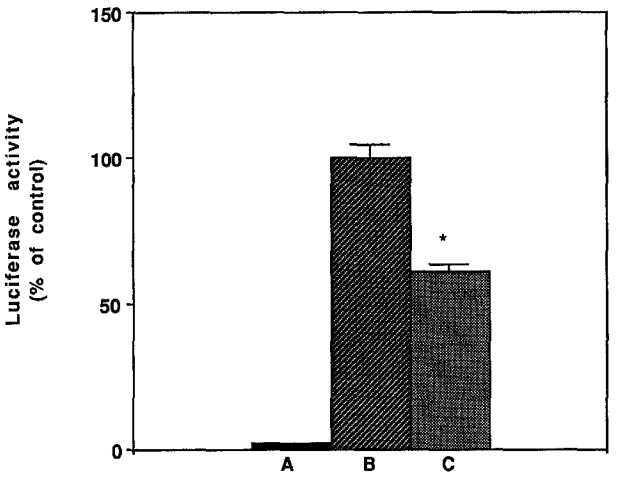

Fig 4. Effect of TNF on G6Pase promoter activity in HuH-7 cells. Plasmid pLH211, containing the human G6Pase promoter (−211 to +55) upstream of the luciferase reporter gene was transfected into HuH-7 cells. Transfected cells were incubated with or without 10 ng/mL recombinant human TNF for 16 hours. The cells were harvested, and luciferase activity in cell extracts was determined and normalized to β-galactosidase activity. Results are the mean ± SE of 5 transfected dishes and represent 1 of 4 similar experiments. *$P <$ .05 *v* control. (A) Transfection with the basic plasmid without a promoter; (B) transfection with pLH211; (C) transfection with pLH211 and incubation with 10 ng/mL recombinant human TNF for 16 hours.

DISCUSSION

The current study demonstrates that intravenous administration of TNF reduces blood glucose levels in mice. Since the decrease in serum glucose levels is not associated with increased serum insulin levels, the reduction of blood glucose was mediated by factors other than insulin, as suggested previously by our group[2] and others.[21,22]

Glycogen reservoirs are reduced in a dose-dependent manner, and gluconeogenic capacity is impaired. Six hours after TNF administration, G6Pase activity was already reduced by 30% but PEPCK activity remained unaffected. However, 12 hours after injection, PEPCK activity was inhibited by about 60% compared with the level in starved mice. Regulation of G6Pase and PEPCK activity is associated with a marked reduction in their mRNA levels, due to reduced transcriptional rates.

A total absence of G6Pase activity, as in glycogen-storage disease type 1, or inhibition of G6Pase activity by metabolites or pharmacological agents is associated with glycogen accumulation in the liver.[23] In our model, the reduction in G6Pase activity was associated with a depletion of hepatic glycogen. This may be due to changes in hormone levels or responsiveness induced by TNF,[2-4] or may be a potential direct effect on enzymes involved in glycogen metabolism. The reduction in PEPCK activity might affect gluconeogenesis. However, PEPCK inhibition is not a major contributor to glycogen depletion, since the latter is already evident 6 hours after TNF administration even though PEPCK activity is not yet affected. A depletion of liver glycogen content and an impairment of hepatic gluconeogenic capacity and hormonal responsiveness following lipopolysaccharide treatment in rats have been previously described.[1-4] Glucose-6-phosphate (G6P) may be channeled via an increased activity of G6P dehydrogenase to the pentose cycle for NADPH synthesis or to the formation of acetyl coenzyme A, as previously suggested.[24] NADPH and acetyl coenzyme A are both needed for fatty acid and cholesterol synthesis. Indeed, fatty acid and cholesterol synthesis is elevated following TNF administration, as is TG secretion from the liver.[25,26]

G6Pase is a multicomponent enzyme system containing a catalytic subunit, T1, T2, T3, and T4 transporters, and a Ca^{2+} binding protein.[23] The reduction in G6Pase activity in disrupted microsomes indicates a quantitative reduction in the catalytic unit protein. This is supported by the reduction of the G6Pase mRNA level and transcriptional activity. The changes in gene expression of G6Pase exceed the effect on enzymatic activity. Recently, it has been shown that liver regeneration or the diabetic state are associated with an increased G6Pase activity and gene expression.[8,9]

Several hormonal stimuli are known to affect liver G6Pase and PEPCK activities.[8,9,27] Therefore, a possible mechanism for the reduction in the hepatic activities and mRNA levels could result from changes in the hormonal milieu or hormonal responsiveness induced by TNF.[28,29] However, it has been previously reported that TNF inhibits the PEPCK transcriptional rate in vitro.[4] In the current study, we demonstrate a direct effect of TNF on G6Pase promoter activity. Transfection experiments in HuH-7 cells indicate that the human G6Pase promoter (from −211 to +55) is sufficient to direct hepatic transcription. Addition of 10 ng/mL recombinant human TNF to the culture medium results in a 40% inhibition of proximal promoter activity.

TNF is one of the mediators of the hepatic acute-phase response and is known to activate NF_kB, a pleiotropic transcription factor.[30,31] In hepatic cells, it activates transcription of several genes through the NF_kB binding site.[32-34] It has also been reported that TNF affects distribution of the different isoforms of CCAAT/enhancer binding protein (C/EBP) and their DNA binding activity, thus changing the effect of C/EBP on hepatic gene expression.[35] Further studies are required to identify the mediators involved in the effect of TNF on G6Pase gene expression. We are currently analyzing the G6Pase promoter region to identify binding sites for transcription factors involved in the regulation of G6Pase transcription.

REFERENCES

1. Lang CH, Bagby GJ, Buday AZ, et al: The contribution of glyconeogenesis to glycogen depletion during glucose infusion in endotoxemia. Metabolism 36:180-187, 1987

2. Chajek-Shaul T, Barash V, Weidenfeld J, et al: Lethal hypoglycemia and hypothermia induced by administration of low doses of tumor necrosis factor to adrenalectomized rats. Metabolism 39:242-250, 1990

3. Yasmineh WG, Theologides A: Effect of tumor necrosis factor on enzymes of gluconeogenesis in the rat. Proc Soc Exp Biol Med 199:97-103, 1992

4. Hill MR, McCallum R: Identification of tumor necrosis factor as a transcriptional regulator of the phosphoenolpyruvate carboxykinase gene following endotoxin treatment of mice. Infect Immunol 60:4040-4050, 1992

5. Hill MR, McCallum R: Altered transcriptional regulation of

phosphoenolpyruvate-carboxykinase in rats following endotoxin treatment. J Clin Invest 88:811-816, 1991

6. Chang CK, Moskal SF, Srivenugopal KS, et al: Altered levels of mRNA encoding enzymes of hepatic glucose metabolism in septic rats. Circ Shock 41:35-39, 1993

7. Shelly LL, Lei K-J, Pan C-J, et al: Isolation of the gene for murine glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1A. J Biol Chem 268:21482-21485, 1993

8. Haber BA, Chin S, Chuang E, et al: High levels of glucose-6-phosphate gene and protein expression reflect an adaptive response in proliferating liver and diabetes. J Clin Invest 95:832-841, 199

9. Liu Z, Barrett EJ, Dalkin AC, et al: Effect of acute diabetes on rat hepatic glucose-6-phosphatase activity and its mRNA level. Biochem Biophys Res Comm 205:680-686, 1994

10. Hujing F: A rapid enzymatic method for glycogen determination in very small tissue samples. Clin Chim Acta 30:567-572, 1970

11. Chang ME, Lane MD: The enzymatic carboxylation of phosphoenolpyruvate. II. Purification and properties of liver mitochondrial phosphoenolpyruvate carboxykinase. J Biol Chem 241:2413-2429, 1966

12. Burchell A, Hume R, Burchell B: A new microtechnique for the analysis of the human hepatic microsomal glucose-6-phosphatase system. Clin Chim Acta 173:183-192, 1988

13. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159, 1987

14. Meisner HM, Loose DS, Hanson RW: Effect of hormone on the transcription of the gene for cytosolic phosphoenolpyruvate carboxykinase (GTP) in rat kidney. Biochemistry 24:421-425, 1985

15. Nordeen SK: Luciferase reporter gene vectors for analysis of promoters and enhancers. Biotechniques 6:454-458, 1988

16. Schmoll D, Allan BB, Burchell A: Cloning and sequencing of the 5′ region of the human glucose-6-phosphatase gene: Transcriptional regulation by cAMP, insulin and glucocorticoids in H4IIE hepatoma cells. FEBS Lett 383:63-66, 1996

17. Nakabayashi H, Taketa K, Miyano K, et al: Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. Cancer Res 42:3858-3863, 1982

18. Graham FL, Van der Ebb AJ: A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52:456-467, 1973

19. Miller H: Experiments in Molecular Genetics. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1972

20. Zar JH: Biostatistical analysis. Englewood Cliffs, NJ, Prentice-Hall, 1974, pp 157-158

21. Lang CH, Bagby GJ, Spitzer JJ: Glucose kinetics and body temperature after lethal and nonlethal doses of endotoxin. Am J Physiol 248:R471-R478, 1985

22. Lang CH, Dobrescu C: Sepsis-induced increases in glucose uptake by macrophage-rich tissues persist during hypoglycemia. Metabolism 40:585-593, 1991

23. Nordlie CR, Bode AM, Foster JD: Recent advances in hepatic glucose-6-phosphatase regulation and function. Proc Soc Exp Biol Med 203:274-285, 1993

24. Kuttner RE, Schumer W, Apantaku FO: Effect of endotoxin and glucocorticoid pretreatment on hexose monophosphate shunt activity in rat liver. Circ Shock 9:37-45, 1982

25. Feingold KR, Grunfeld C: Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. J Clin Invest 80:184-190, 1987

26. Chajek-Shaul T, Friedman G, Stein O, et al: Mechanism of the hypertriglyceridemia induced by tumor necrosis factor administration to rats. Biochim Biophys Acta 1001:316-324, 1989

27. Spagnoli D, Dobrosielski-Vergona K, Widnell CC: Effect of hormones on the activity of glucose-6-phosphatase in primary cultures of rat hepatocytes. Arch Biochem Biophys 226:182-189, 1983

28. Warren RS, Starnes HF Jr, Gabrilove JL, et al: The acute metabolic effect of tumor necrosis factor administration in humans. Arch Surg 122:1396-1400, 1987

29. Tracey KJ, Lowry SF, Fahey TJ III, et al: Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. Surg Gynecol Obstet 164:415-422, 1987

30. Kishimoto T, Taga T, Akira S: Cytokine signal transduction. Cell 76:253-262, 1994

31. Lenardo MJ, Baltimore D: NF-kB: A pleiotropic mediator of inducible and tissue-specific gene control. Cell 58:227-229, 1989

32. Brasier AR, Ron D, Tate JE, et al: A family of constitutive C/EBP like DNA binding proteins attenuate the IL-1 alpha induced NF-kB mediated transactivation of the angiotensinogen gene acute-phase response element. EMBO J 9:3933-3944, 1990

33. Nonaka M, Huang Z-M: Interleukin-1–mediated enhancement of mouse factor B gene expression via NF-kB like hepatoma nuclear factor. Mol Cell Biol 10:6283-6289, 1990

34. Gruber PJ, Torres-Rosado A, Wolak ML, et al: Apo CIII gene transcription is regulated by a cytokine inducible NF-kB element. Nucleic Acids Res 22:2417-2422, 1994

35. Diehl AM, Yang SQ, Yin M, et al: Tumor necrosis factor alpha modulates CCAAT/enhancer binding proteins–DNA binding activities and promotes hepatocyte-specific gene expression during liver regeneration. Hepatology 22:252-261, 1995