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Polyunsaturated fatty acids inhibit the expression of the glucose-6-phosphate dehydrogenase gene in primary rat hepatocytes by a nuclear posttranscriptional mechanism

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Abstract **Expression of the glucose-6-phosphate dehydrogenase (G6PD) gene is inhibited by the addition of polyunsaturated fatty acids to the medium of primary hepatocytes in culture. To define the regulated step, we measured the abundance of G6PD mRNA both in the nucleus and in total RNA and measured the transcriptional activity of the G6PD gene. Insulin and glucose stimulated a 5- to 7-fold increase in G6PD mRNA in rat hepatocytes. This increase was attenuated by 60% due to the addition of arachidonic acid. These changes in mRNA accumulation occurred in the absence of changes in the rate of transcription. Amounts of precursor mRNA (pre-mRNA) for G6PD in the nucleus changed in parallel with the amount of mature mRNA. The decrease in G6PD pre-mRNA accumulation caused by arachidonic acid was also observed with other long chain polyunsaturated fatty acids but not with monounsaturated fatty acids. In addition, this decrease was not due to a generalized toxicity of the cells due to fatty acid oxidation. These changes in G6PD expression in the primary hepatocytes are qualitatively and quantitatively similar to the changes observed in the intact animal due to dietary carbohydrate and polyunsaturated fat. Regulation of G6PD expression by a nuclear posttranscriptional mechanism represents a novel form of regulation by fatty acids.—**Stabile, L. P., S. A. Klautky, S. M. Minor, and L. M. Salati. **Polyunsaturated fatty acids inhibit the expression of the glucose-6-phosphate dehydrogenase gene in primary rat hepatocytes by a nuclear posttranscriptional mechanism. *J. Lipid Res.* 1998.** 39: **1951–1963.**

Supplementary key words glucose-6-phosphate dehydrogenase • polyunsaturated fatty acids • rat hepatocytes • posttranscriptional regulation • nutritional regulation • precursor mRNA • RNA processing

Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) is the rate-determining enzyme of the pentose phosphate pathway, which provides a source of NADPH for many biosynthetic reductive reactions and ribose-5-phosphate for nucleic acid synthesis. The reaction catalyzed by G6PD provides 50–75% of NADPH needed for fatty acid biosynthesis in rat liver (1). All cell types contain G6PD activity; however, regulation of the enzyme only occurs in liver and adipose tissue (2, 3). G6PD activity in the regulated tissues correlates with the rate of fatty acid biosynthesis and thus it is a member of the lipogenic family of enzymes. Like other lipogenic enzymes, G6PD is regulated by both nutritional and hormonal stimuli. For example, when rodents are fed a high-carbohydrate, low-fat diet after a period of starvation, G6PD activity increases 10- to 16-fold (3, 4). Conversely, G6PD activity decreases 80% in mice and rats consuming a high-fat diet versus those fed a low-fat diet (5, 6). Hormonal aspects of G6PD regulation include a decrease in G6PD activity in diabetic and adrenalectomized rats that is restored by hormone replacement (7). In addition, in primary rat hepatocytes, insulin increases G6PD activity in a dose-dependent manner (8). Curiously, treatment of rat hepatocytes with dexamethasone does not change G6PD activity (8), suggesting that the effect of adrenalectomy is indirect.

Regulation of G6PD by nutritional factors occurs at a nuclear posttranscriptional step. In this regard, the 80% decrease in G6PD mRNA amount is accompanied by an 80% decrease in nuclear precursor mRNA (pre-mRNA) for G6PD in the nucleus. Similarly, the increase in G6PD mRNA due to refeeding starved mice is accompanied by comparable increases in the amount of G6PD pre-mRNA (9). Nevertheless, the rate of transcription of the G6PD gene is not altered despite large changes in the abundance of pre-mRNA (5). Further characterization of the regulation of the G6PD gene indicates that transport of the mRNA from the nucleus to the cytoplasm and splicing of the primary transcript are not regulated steps in its expression. Thus, regulation of the amount of G6PD pre-mRNA probably occurs very soon after transcription of the primary transcript.

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; TBA, thiobarbituric acid; RPA, ribonuclease protection assay; PEPCK, phosphoenolpyruvate carboxykinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; pC3, stearoyl-CoA desaturase I; FAS-17, fatty acid synthase; pre-mRNA, precursor mRNA; UTR, untranslated region.

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The nuclear posttranscriptional regulation of G6PD contrasts with the large changes in transcriptional activity observed with other lipogenic enzymes, such as fatty acid synthase and stearoyl-CoA desaturase I (10, 11). However, changes in pre-mRNA stability may be a common mechanism for all these genes. In this regard, S14, a protein of unknown function that is thought to be involved in lipogenesis, undergoes large transcriptional changes in response to dietary carbohydrate and polyunsaturated fatty acids (10). In addition to these transcriptional changes, dietary carbohydrate enhances the accumulation of processing intermediates of S14 pre-mRNA (12,13) suggesting that enhanced expression of the S14 gene involves increased efficiency of the processing of its pre-mRNA.

The goal of these studies is to further analyze the mechanism by which polyunsaturated fat inhibits G6PD expression. Studies in the intact animal are hampered by an inability to distinguish a direct effect of fatty acids on expression of a gene from an indirect effect due to changes in the hormonal environment of the animal. Cell culture models provide a mechanism to control the hormonal environment while examining the action of fatty acids. Incubation of primary rat hepatocytes with polyunsaturated fatty acids mimics the effect of dietary fat on the transcription of S14 and pyruvate kinase (14, 15). The ability of this cell model to mimic posttranscriptional actions of polyunsaturated fatty acids has not been described. In this paper we demonstrate that the action of polyunsaturated fatty acids on G6PD pre-mRNA accumulation in rat hepatocytes mimics the action of dietary fat in the intact animal both qualitatively and quantitatively.

MATERIALS AND METHODS

Materials

Williams' medium E (Gibco-BRL), newborn calf serum (Gibco-BRL), fatty acids (Nu-Chek Prep), collagenase H (EC 3.4.99.5, Boehringer Mannheim), insulin (Eli Lilly Research Laboratories), Matrigel (Collaborative Biomedical Research), Percoll (Sigma), pBluescriptKS+ (Stratagene), RNase-free DNase I (Gibco-BRL), and RPA II kit (Ambion) were obtained from the indicated sources. Bovine serum albumin fraction V (fatty acid-free), α-[^{32}P]UTP, 3000 Ci/mmol and α-[^{32}P]dCTP, 3000 Ci/mmol were from ICN Biochemicals. Other chemicals were from Fisher Biotech or of the highest purity commercially available. Rats were purchased from Harlan Laboratories (Indianapolis, IN). Standard chow diet was Harlan Teklad and the fat-free, high-glucose diet was from Purina Mills. Rat genomic DNA was obtained from Clontech.

Animal care and cell culture

Male Sprague-Dawley rats (approximately 200 g) fed a standard chow diet were used for all experiments. Rats used for dietary experiments were starved for 24 h or starved for 24 h and then refed a high-glucose, fat-free diet (5) for 15 h prior to killing. Rats were starved for 48 h prior to use as hepatocyte donors.

Hepatocytes were isolated by a modification of the technique of Seglen (16). The livers were perfused with 350 ml of calcium-free buffer (0.14 m NaCl, 6.7 mm KCl, 0.02 m HEPES, pH 7.4, 25 mm glucose, 250 μm EGTA) at 40 ml/min followed by 100 ml of a buffer containing collagenase (67 mm NaCl, 6.7 mm KCl, 0.1 m HEPES, pH 7.4, 6 mm $CaCl_2 \cdot 2H_2O$, 25 mm glucose, 1 mg/ml collagenase H, 0.05 mg/ml trypsin inhibitor). The hepatocytes were then teased from the capsid of the liver and washed into cold, well-oxygenated Williams' medium E supplemented with 23 mm HEPES, pH 7.4, 26 mm sodium bicarbonate, penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml). Glucose was added to a final concentration of 27 mm. The cells were spun through a Percoll gradient for 10 min at 50 *g* to remove non-parenchymal cells (17). Hepatocytes (4×10^6) were placed in 60-mm Primaria dishes preincubated (37°C, 5% CO_2) with medium containing 5% newborn calf serum. Cell viability in all experiments was 90% or greater as estimated by Trypan Blue (0.4%) exclusion. After 4 h, the medium was replaced with serum-free medium. After an additional 16 h of incubation, the medium was replaced with medium containing the treatments indicated in the figure legends and a Matrigel overlay (0.3 mg/ml) (18). Subsequently, the medium was changed every 12 to 24 h to one of the same composition but without Matrigel. The hepatocytes were maintained in a humidified chamber at 37°C in 5% CO_2/95% air. Fatty acids were bound to bovine serum albumin (19). The fatty acid (4 mm)–albumin (1 mm) stocks contained butylated hydroxytoluene (0.01%) and Williams' medium E contains α-tocopherol phosphate, disodium (10 μg/L) to minimize oxidation of fatty acids.

Thiobarbituric acid (TBA) assay

The TBA assay was performed as described by Hostmark and Lystad (20) with minor modifications. Phenol red typically found in cell culture medium interfered with this assay, thus for these experiments Williams' medium E without phenol red was used. To measure TBA-reactive material in the cell culture medium, 4.5 ml of a 0.4% TBA solution in water was mixed with 0.4 ml of medium from hepatocytes incubated with various fatty acids. Glacial acetic acid (100 μl) containing 0.5% butylated hydroxytoluene was added, the mixture was vortexed vigorously, and heated to 100°C for 20 min. Insoluble material was removed by extraction with 3 ml of chloroform–methanol 5:1 (v/v). The absorbance at 532 nm of the aqueous phase was determined. The amount of TBA reactive material was calculated using the molar extinction coefficient (156,000 $m^{-1}cm^{-1}$).

Measurement of G6PD activity

Hepatocytes were prepared as described above and incubated 72 h with the treatments indicated in the figure legends. Cells were washed one time with phosphate-buffered saline, and lysed by three cycles of freezing and thawing in 0.5 ml of buffer (50 mm Tris-acetate, pH 7.4, 300 mm mannitol, 5 mm dithiothreitol) per 60 mm Primaria plate. The lysates were centrifuged for 10 min at 11,000 *g*. Supernatants were used for measurement of G6PD enzyme activity (8) and total protein concentration (21). G6PD activity is reported as mU/mg protein in the cell supernatant; 1 mU equals 1 nmol of NADPH produced per minute. Statistical analysis was with ANOVA and *t*-test.

Isolation of total RNA and Northern analysis

Total RNA from 2–3 plates per treatment was isolated by the method of Chomczynski and Sacchi (22). The denatured cell mix was forced through an 18-gauge syringe two to three times to sheer genomic DNA. Quantitation of RNA using Northern analysis was done as previously described (5).

Isolation of nuclear RNA

Nuclei from hepatocytes were isolated by a modification of the method of Leppard and Shenk (23). Hepatocytes and medium from 15–20 plates per treatment were collected into 50-ml centri-

fuge tubes and the cells were pelleted in a Beckman JS 4.2 rotor at 490 rpm (50 *g*) for 5 min. The cell pellet was washed two times with ice-cold phosphate-buffered saline, then homogenized in 3 ml of isotonic buffer (10 mm Tris, pH 7.5, 150 mm NaCl, 1.5 mm $MgCl_2$, 175 μg phenylmethylsulfonylfluoride/ml, 0.6% nonidet NP-40) by 12 strokes in a Dounce homogenizer using a tight-fitting pestle. The homogenate was incubated on ice for 5 min. Nuclei were pelleted in a Beckman JS 4.2 rotor at 2000 rpm (800 *g*) for 5 min at 4°C and washed one time with 2 ml of isotonic buffer without nonidet NP-40. RNA was purified from the final nuclear pellet by the method of Chomczynski and Sacchi (22); 3 ml of denaturing solution were used per nuclear pellet. The denatured nuclear RNA mix was forced through an 18-gauge syringe five times to sheer genomic DNA. In experiments using intact rat liver (Fig. 2), nuclei were purified through a 2 m sucrose cushion as previously described (5) prior to isolation of nuclear RNA.

Nuclear run-on assays and DNA probes

Nuclei were isolated from hepatocytes using the method of Leppard and Shenk (23) as described above. In one experiment, nuclei were isolated by the method of Milsted, Cox, and Nilson (24). The same results were obtained in the run-on assays regardless of the method of nuclei preparation. The final nuclear pellet was resuspended and washed into nuclei storage buffer (50 mm HEPES, pH 7.4, 75 mm NaCl, 0.1 mm EDTA, 5 mm dithiothreitol, 0.12 mm phenylmethylsulfonylfluoride, and 50% glycerol). Nuclei were purified from intact rat liver by centrifugation through a 2 m sucrose cushion as previously described (5). The nuclei were stored at −70°C in 100 μl of storage buffer prior to use in a nuclear run-on assay.

The nuclear run-on assay was performed on both hepatocyte and liver nuclei as previously described (5, 25). DNA used as probes were obtained as follows: mouse G6PD cDNA (pMZ3) from M. Zollo (26), rat phosphoenolpyruvate carboxykinase (pPCK10) cDNA from R. Hanson (27), mouse albumin cDNA (pmalb-2) from S. Tilghman (28), rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (pRGAPDH1) from F. Rottman (29), mouse stearoyl-CoA desaturase I cDNA (pC3) from J. Ntambi (30), rat fatty acid synthase cDNA (pFAS-17) from S. Clarke (31). The G6PD cDNA probe is the BamHI (exon 2) to XbaI (exon 13) fragment of pMZ3 which was subcloned into pBluescriptKS+ thereby eliminating exon 1 which is GC-rich, and most of exon 13 which is dissimilar between rats and mice.

Probe design for ribonuclease protection assay (RPA)

Two probes were designed for use in the ribonuclease protection assay (**Fig. 1**). The first probe, intron 3–exon 4, was derived from PCR amplification of rat genomic DNA using primer 1, 5′-GGGGTACCGGTAATATCTCTACACTACCCCCCAATC-3′ and primer 2, 5′-GGAATTCGCTCACTCTGTTTGCGGATGTC-3′. Primer 1 contained a KpnI restriction site at its 5′ end (underline) for subcloning of the PCR amplified product into pBluescriptKS+ and the remaining sequence is from intron 3. Primer 2 contained an EcoRI site at its 5′ end (underline) and the G6PD sequence begins 11 nt from the end of exon 4. The second probe, exon 9–intron 9, was derived from PCR amplification of rat genomic DNA using primer 3, 5′-GGGGTACCCAG AGGTGGAAACTGACAACGTG-3′ and primer 4, 5′-GGAATTC TAGCCCTCCTTCTCCAGCATTC-3′. The same restriction sites are present as for the previous probe. Primer 3 contained exon 9-specific sequences beginning 22 nt 3′ from the start of exon 9. Primer 4 contained intron 9-specific sequences.

The DNA fragments, after PCR amplification, were subcloned into pBluescriptKS+ and the authenticity of these sequences was verified by sequencing. The subclones were linearized with HindIII and used in an in vitro transcription reaction. The transcripts produced with T7 polymerase were designed to be larger than the protected fragments so that incompletely digested probe could be differentiated from the target signal in the ribonuclease protection assay. As a control for strand-specific hybridization, RNA probes were also generated from the T3 promoter in pBluescriptKS+ which resulted in a probe whose sequence was identical to G6PD mRNA (sense probe) and thus should not hybridize to cellular RNAs. No bands were detected in a ribonuclease protection assay using these probes (data not shown) therefore only T7-generated probes could hybridize to G6PD mRNA.

Ribonuclease protection assay

Antisense RNA probes were synthesized in an in vitro transcription reaction. Template DNA (0.5 g) was added to a reaction mixture containing 40 mm Tris-HCl, pH 7.5, 6 mm $MgCl_2$, 2 mm spermidine, 10 mm NaCl, 10 mm dithiothreitol, 14 U RNasin, 400 μm each ATP, CTP, and GTP, 20 μm cold UTP, 20 U T7 RNA polymerase and 50 μCi α-[^{32}P]UTP (sp. act. 3000 Ci/mmol) and incubated at 37°C for 1 h. RNase-free DNase I (100 U) was added and the reaction was incubated for 15 min at 37°C. The probes were purified in a 5% denaturing polyacrylamide gel and eluted overnight at 37°C in 0.5 μm ammonium acetate, 1 mm EDTA, and 0.2% SDS.

Ribonuclease protection assays were performed using the RPA II kit. Nuclear or total RNA (25 or 30 μg) was hybridized to 2×10^4 cpm of ^{32}P-labeled RNA in 20 μl of hybridization solution (80% deionized formamide, 100 mm sodium citrate, pH 6.4, 300 mm sodium acetate, pH 6.4, 1 mm EDTA) at 45°C for 16 h. After hybridization, the solution was treated with a mixture of 0.5 U RNase A and 20 U RNase T_1 for 30 min at 37°C. The resulting

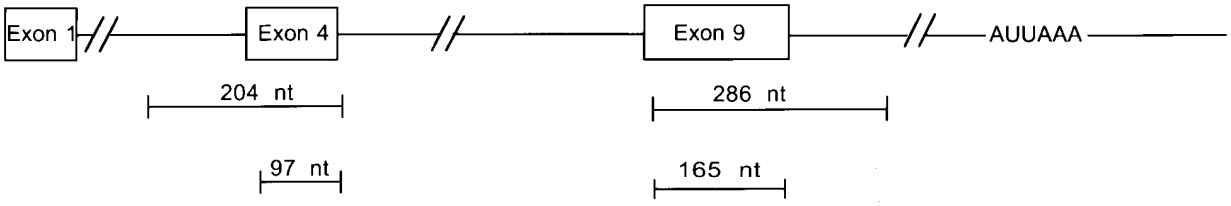


Fig. 1. Probes and predicted fragments for the ribonuclease protection assay. The top line represents a simplified diagram of the G6PD primary transcript showing three of the genes 13 exons. The lower lines represent G6PD specific mRNA fragments that are detected in the ribonuclease protection assays by specific G6PD probes. Two probes were designed to hybridize across intron–exon boundaries of target mRNA such that pre-mRNA with and without introns could be detected in the assay. The first probe (intron 3–exon 4) was designed to cross the intron 3–exon 4 boundary and protects a 204 nt fragment of pre-mRNA containing intron 3. In addition, this probe recognizes processed transcripts (97 nt fragment) that have undergone splicing of intron 3. The second probe (exon 9–intron 9) spans the exon 9–intron 9 boundary. It protects a 286 nt fragment of the transcript containing intron 9, and nuclear mRNA without intron 9 (165 nt fragment).

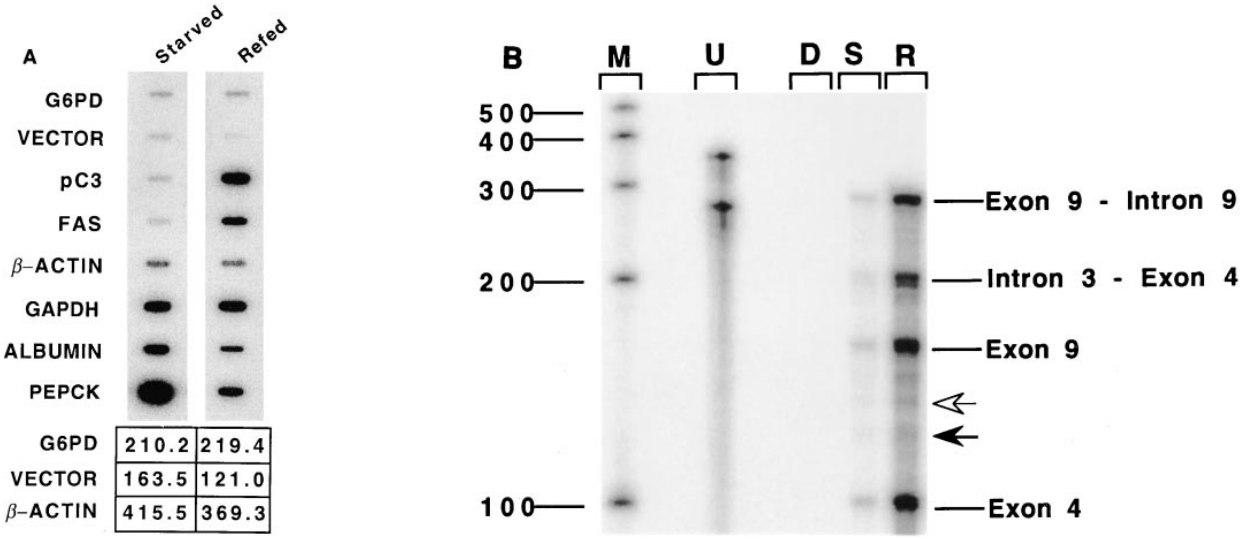
hybridization products protected from RNase digestion were separated in a 5% denaturing polyacrylamide gel. The gel was dried and placed in a storage phosphor cassette for 1 to 3 days. Images were quantified using ImageQuaNT software by Molecular Dynamics.

RESULTS

Effect of starvation and refeeding on transcriptional activity of G6PD in rat

Refeeding starved mice results in a 15-fold or more increase in G6PD mRNA, while the addition of polyunsaturated fat to a high-carbohydrate diet results in an 80% decrease in G6PD mRNA (5). In mice, dietary-induced changes in G6PD mRNA accumulation are caused by changes in the amount of pre-mRNA for G6PD via a posttranscriptional regulatory mechanism in the nucleus (9). Mouse hepatocytes in primary culture do not provide a hormonally responsive system to study the molecular basis for this regulation (32; S. A. Klautky and L. M. Salati, unpublished results). In contrast, G6PD activity is regulated by both insulin and fatty acids in rat hepatocytes in primary culture (8). Prior to choosing rat hepatocytes as a model to further define the molecular basis of G6PD regulation, we verified that regulation of G6PD expression by diet occurred by a posttranscriptional regulatory mechanism as observed in mice. No difference in G6PD transcription was observed in rats that were starved versus starved and then refed a high-carbohydrate diet (**Fig. 2A**). In contrast, refeeding increased the transcription of the stearoyl-CoA desaturase I (pC3) and fatty acid synthase (FAS) genes, by 85- and 77-fold, respectively, and transcription of the phosphoenolpyruvate carboxykinase gene was inhibited by 93%. Thus, the expected transcriptional changes were observed for genes regulated at the level of transcription while regulation of G6PD expression occurred primarily at a posttranscriptional step in rats as in mice.

We next measured nuclear pre-mRNA for the G6PD gene using a ribonuclease protection assay and probes that hybridized across two different intron/exon boundaries in the G6PD primary transcript (Fig. 1). Because the G6PD gene contains 13 exons and the primary transcript



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S	R	Fold Increase	
63	1192	18.9	Exon 9 - Intron 9
48	938	19.5	Intron 3 - Exon 4
86	1490	17.3	Exon 9
86	1627	18.9	Exon 4

Fig. 2. Effect of starvation and refeeding on expression of G6PD in the rat. (A) Nuclei were isolated from the pooled livers of two rats that had been starved for 15 h or from the livers of two rats that had been starved for 24 h followed by refeeding a high-glucose, low-fat diet for 15 h. Nuclear run-on assays were performed as described in Materials and Methods. Hybridization to the probes was quantified using ImageQuaNT. Quantitation of the hybridization signals for G6PD, vector (pBluescriptKS+), and β-actin are listed below each strip. The values are the integration units ($\times 10^{-2}$) from ImageQuaNT. cDNA clones for fatty acid synthase (FAS) and stearoyl-CoA desaturase I (pC3) were used as positive controls to show transcriptional activation due to refeeding a high-carbohydrate diet. A PEPCK cDNA probe was used as a negative control to show transcriptional inhibition by refeeding. β-Actin, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), albumin, and phosphoenolpyruvate carboxykinase (PEPCK) cDNA probes were used as controls for selectivity of the response. The assay was done in duplicate with identical results. (B) Nuclear RNA was isolated from the same batch of nuclei as described above. G6PD nuclear RNA (25 μg) was analyzed using a ribonuclease protection assay and two G6PD specific probes. M, RNA Century marker (Ambion); U, undigested probe (hybridization of probes to 25 μg yeast RNA without subsequent RNase digestion); D, digested probe (hybridization to 25 μg yeast RNA followed by RNase digestion); S, nuclear RNA isolated from livers of starved rats; R, nuclear RNA isolated from livers of refed rats. (C) Quantitation of the signals was done using ImageQuaNT. The values shown in the table are the integration units ($\times 10^{-2}$) and the calculated fold-increase due to refeeding.

is 18 kb in length it cannot be examined in total. The probes represent discrete locations within the primary transcript and permit quantitative analysis of the amount of pre-mRNA. The intron 3–exon 4 probe hybridized across a 3′-splice site and the exon 9–intron 9 probe hybridized across a 5′-splice site. RNase digestion of each hybrid of probe and target RNA resulted in two protected fragments. These included RNA containing the intron and RNA from which the intron had been spliced. The 204 and 286 nt bands represented pre-mRNAs that contained introns 3 and 9, respectively (Fig. 2B). The 97 and 165 nt bands represented pre-mRNAs from which introns 3 and 9, respectively, had been spliced. Protected fragments representing only intron 9 (121 nt, closed arrow) and the exon 4 and intron 3 lariat (33; 133 nt, open arrow) also appeared to be detected. The relative intensity of these fragments was much less than the bands containing exon sequences. This may reflect that introns, once spliced, were rapidly degraded in the nucleus. In some experiments (Fig. 2B and 6A) a doublet in the intron 3–exon 4 band was observed. The sequence at one end of the intron 3–exon 4 probe is AU-rich which may allow local denaturation of the hybrid making it susceptible to ribonuclease digestion. This doublet disappeared when the RNase digestion conditions were altered to use either 50% less RNase A and RNase T1 or only RNase T1 (data not shown) confirming that the doublet was due to "breathing" at the ends of the hybrid.

To determine whether the abundance of G6PD pre-mRNA was regulated in the rat, RNA was isolated from the nuclei of the same rats as were used for transcriptional measurements (Fig. 2B). In the livers of rats that had been starved for 24 h, G6PD pre-mRNA was barely detectable. G6PD pre-mRNA was 18.9- and 19.5-fold higher as detected by the exon 9–intron 9 and intron 3–exon 4 protected fragments, respectively, in the livers of rats that had been refed a high-carbohydrate, low-fat diet relative to the rats that had been starved. Pre-mRNAs in which introns 9 and 3 had been spliced were increased 17.3- and 18.9-fold, respectively. The changes in amount of exon-only protected fragments were similar to the full-length protected fragments, suggesting that splicing was not a regulated step. Thus, refeeding starved rats increased the abundance of pre-mRNAs for G6PD in the nucleus in the absence of an observable change in transcriptional activity of the gene. Thus, the regulated step mediating the effects of starvation and refeeding on G6PD expression in the rat appears to be the same as that in the mouse. Rat hepatocytes should provide a useful model system for further characterizing the molecular mechanisms involved in regulation of G6PD expression.

Regulation of enzyme activity and mRNA accumulation by glucose, insulin, and fatty acids in rat hepatocytes in primary culture

G6PD activity has been shown to be maximally increased by insulin, and this increase is attenuated by arachidonic acid in rat hepatocytes (8). To determine whether fatty acids regulated G6PD at a pretranslational step, mRNA accumulation and enzyme activity were measured in rat hepatocytes incubated with insulin or insulin and arachidonic acid. Incubation with insulin (0.1 μm or 1 μm) for 72 h resulted in a 5.7- to 5.9-fold increase in G6PD activity (**Fig. 3A**). Coincubation with arachidonic acid significantly inhibited ($P < 0.05$) the insulin-dependent increase in G6PD activity by 56% and 51%, for 0.1 μm and 1 μm insulin, respectively. These results are consistent with a previous report (8). G6PD mRNA accumulation was examined using Northern analysis. The increase in G6PD activity in hepatocytes incubated with insulin was accompanied by a 4.2-fold increase in mRNA for G6PD (Figs. 3B and 3C). This increase in G6PD expression was not exclusively due to the addition of insulin. In this regard, incubation with 27 mm glucose in the medium for 48 h resulted in a 2.9 ± 0.5-fold increase in G6PD mRNA ($n = 6$); the addition of insulin (0.1 μm) resulted in a further increase in G6PD mRNA of 1.6 ± 0.06-fold ($n = 6$; Fig. 3B). Incubation with 250 μm arachidonic acid or eicosapentaenoic acid decreased the amount of G6PD mRNA by approximately 50% relative to hepatocytes incubated with glucose and insulin (Fig. 3C). The concentration of arachidonic acid used in these experiments has previously been shown to maximally inhibit G6PD expression (8). Arachidonic acid or eicosapentaenoic acid did not inhibit the increase in G6PD mRNA caused by 27 mm glucose alone (data not shown) suggesting that inhibition of G6PD expression by fatty acids may be caused by an inhibition of the action of insulin. Thus, changes in G6PD enzyme activity due to long chain polyunsaturated fatty acids were accompanied by comparable changes in the amount of mRNA for G6PD indicating that regulation was pretranslational.

To determine whether the effect of glucose, insulin, and arachidonic acid on G6PD expression was specific, we also measured the amount of GAPDH and β-actin mRNA with these treatments. Expression of GAPDH mRNA was consistently increased 2-fold by insulin and glucose, but the amount of GAPDH mRNA was not changed by incubation with polyunsaturated fatty acids indicating that the effect of fatty acids was specific (Fig. 3B). Further, the amount of β-actin mRNA did not change with any of the treatments (data not shown) indicating that insulin, glucose, and long chain polyunsaturated fatty acids do not have generalized positive or negative effects within the hepatocytes.

Time course of the effect of arachidonic acid on G6PD mRNA accumulation

In the intact mouse, inhibition of G6PD mRNA amount by dietary fat is very rapid; a 20% decrease is observed within 4.5 h after providing a high-fat diet compared to mice consuming a low-fat diet (5). To test whether inhibition by fatty acids in culture was as rapid as the inhibition by dietary fat, hepatocytes were incubated in the presence of glucose and insulin for 48 h to induce the amount of G6PD expression prior to the addition of arachidonic acid (**Fig. 4A**). Incubation of hepatocytes for 48 h with 27 mm glucose in the medium increased the expression of G6PD mRNA 4.1-fold, compared to time 0. Insulin further enhanced this accumulation 1.5-fold relative to glucose-

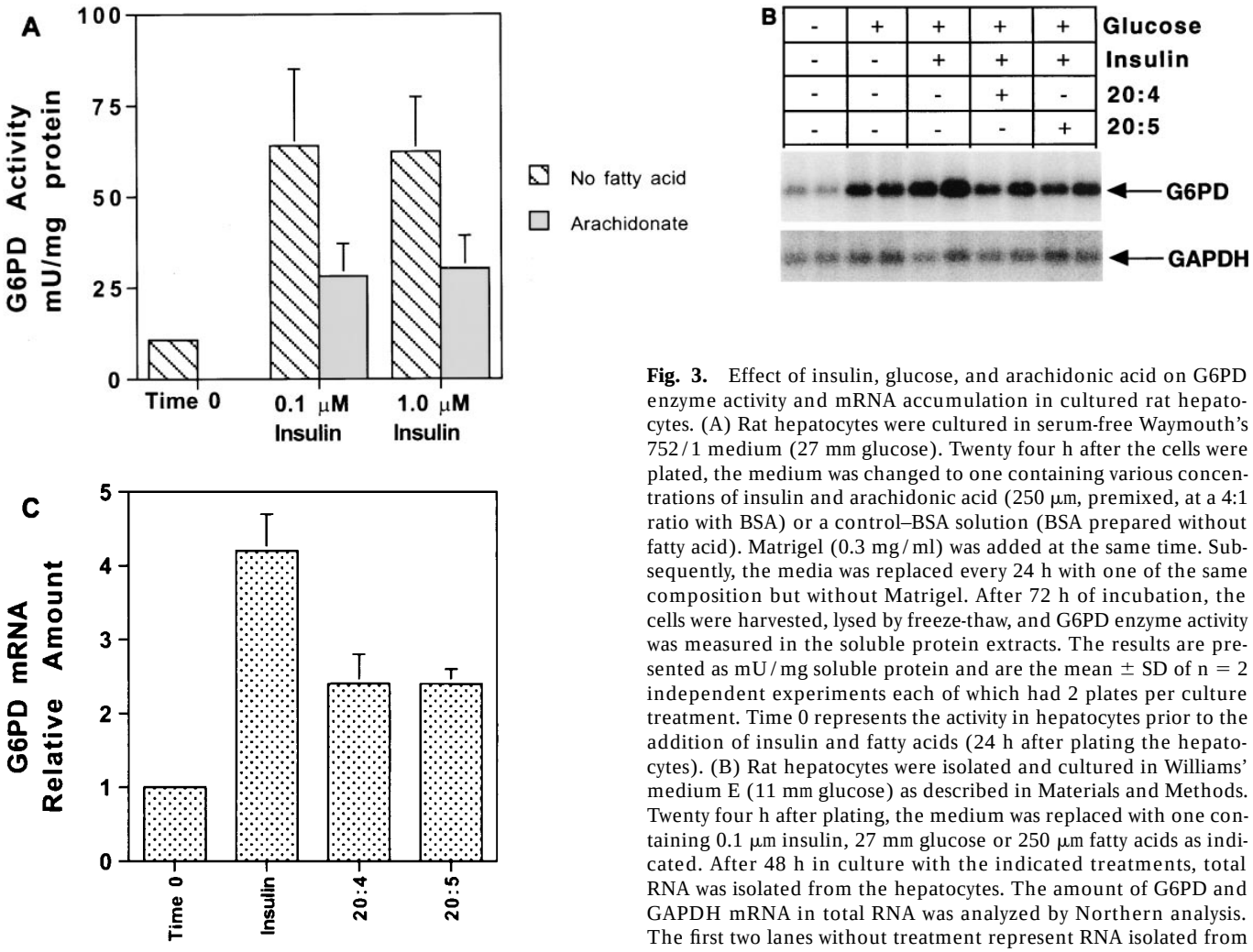


Fig. 3. Effect of insulin, glucose, and arachidonic acid on G6PD enzyme activity and mRNA accumulation in cultured rat hepatocytes. (A) Rat hepatocytes were cultured in serum-free Waymouth's 752/1 medium (27 mm glucose). Twenty four h after the cells were plated, the medium was changed to one containing various concentrations of insulin and arachidonic acid (250 μm, premixed, at a 4:1 ratio with BSA) or a control–BSA solution (BSA prepared without fatty acid). Matrigel (0.3 mg/ml) was added at the same time. Subsequently, the media was replaced every 24 h with one of the same composition but without Matrigel. After 72 h of incubation, the cells were harvested, lysed by freeze-thaw, and G6PD enzyme activity was measured in the soluble protein extracts. The results are presented as mU/mg soluble protein and are the mean ± SD of n = 2 independent experiments each of which had 2 plates per culture treatment. Time 0 represents the activity in hepatocytes prior to the addition of insulin and fatty acids (24 h after plating the hepatocytes). (B) Rat hepatocytes were isolated and cultured in Williams' medium E (11 mm glucose) as described in Materials and Methods. Twenty four h after plating, the medium was replaced with one containing 0.1 μm insulin, 27 mm glucose or 250 μm fatty acids as indicated. After 48 h in culture with the indicated treatments, total RNA was isolated from the hepatocytes. The amount of G6PD and GAPDH mRNA in total RNA was analyzed by Northern analysis. The first two lanes without treatment represent RNA isolated from hepatocytes after 24 h of incubation, prior to the addition of the treatments (time 0). The signal for GAPDH was enhanced 1.5-fold over the signal for G6PD using ImageQuaNT. The size of the mRNAs for G6PD and GAPDH are 2.3 and 1.8 kb, respectively. Part B is a representative Northern analysis and part C is the mean ± the SE of n = 8 experiments for the time 0, insulin (0.1 μm insulin and 27 mm glucose), and arachidonic acid (20:4; 250 μm) treatments and n = 3 experiments for the eicosapentaenoic acid (20:5; 250 μm)) treatment. Quantitation was done using ImageQuaNT analysis and the results are expressed as relative amounts where the amount of RNA at time 0 was set to one.

treated cells. Within 2 h after the addition of arachidonic acid, the amount of G6PD mRNA was decreased 13.8% (average of 2 experiments). The maximum decrease of 50% in G6PD mRNA abundance was observed by 8 h. This decrease in mRNA was similar to the 60% decrease observed in hepatocytes incubated with arachidonic acid coincidentally with glucose and insulin for the entire period (Fig. 4A, 48 h lane). The expression of GAPDH and β-actin were not affected by arachidonic acid in these cells (Fig. 4A). The inhibition of G6PD mRNA accumulation was also rapid when arachidonic acid was added coincidentally with glucose and insulin (Fig. 4B). The amount of G6PD mRNA was decreased 52% within 12 h of treatment in the absence of changes in the expression of GAPDH and β-actin (Fig. 4B). Thus, polyunsaturated fatty acids inhibit the insulin stimulation of G6PD mRNA accumulation when added coincidentally with insulin and glucose, or when added to cells that were previously incubated with insulin and glucose for 48 h. Further, the kinetics of inhibition by polyunsaturated fatty acids in hepatocytes in culture was similar to the kinetics of inhibition by dietary polyunsaturated fat in the intact mouse.

G6PD is regulated at a posttranscriptional step in the nucleus of rat hepatocytes

We next tested whether fatty acids regulated G6PD expression by a posttranscriptional mechanism. Nuclei were isolated from hepatocytes and transcriptional activity of the G6PD gene was measured using nuclear run-on assays. Transcriptional activity of the G6PD gene was low in rat hepatocytes and little or no change was observed due to either glucose and insulin or arachidonic acid (**Fig. 5A**). The apparent increase in G6PD transcription due to glucose and insulin shown in Fig. 5A was only observed in this experiment. In multiple experiments, transcriptional activity of the G6PD gene was not significantly increased by

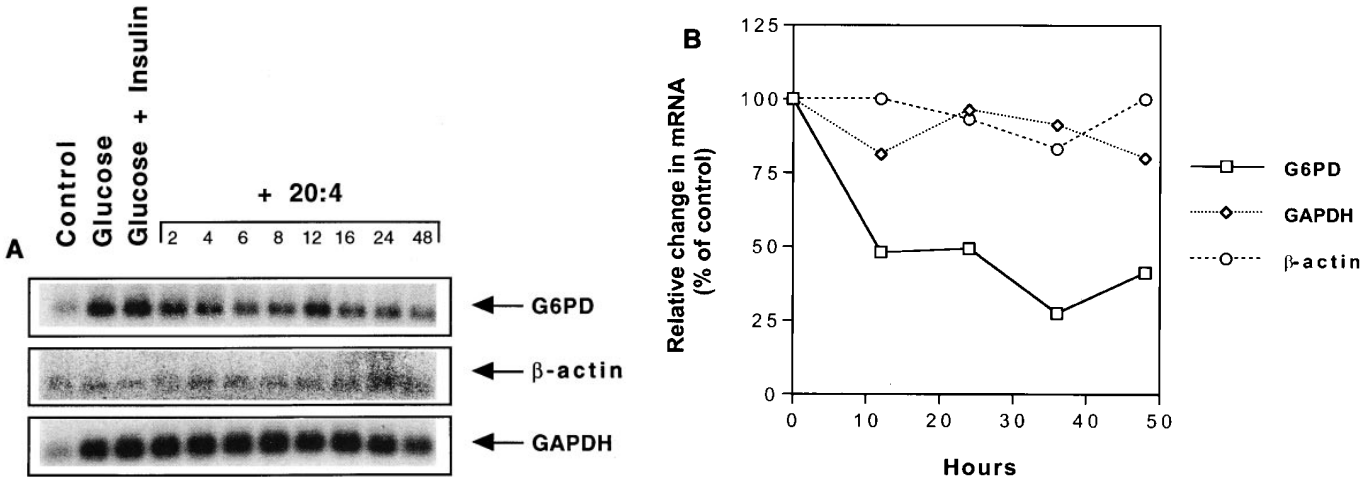


Fig. 4. Time course of the inhibition of G6PD mRNA accumulation by arachidonic acid. Rat hepatocytes were isolated and cultured as described. (A) Twenty four h later, the medium was replaced with one containing 0.1 μm insulin, 27 mm glucose, and Matrigel (0.3 mg/ml). The medium was replaced every 24 h with one of the same composition but without Matrigel. Arachidonic acid (250 μm) was added after 48 h of incubation with hormones. RNA was isolated at the indicated times (2–24 h) after the addition of fatty acid. The 48-h lane represents RNA isolated from hepatocytes in which arachidonic acid was added with glucose and insulin after 24 h in culture. In addition, cells prior to treatment (control), cells incubated with glucose alone for 48 h, and cells incubated with glucose plus insulin for 48 h were harvested and total RNA was isolated. Northern analysis was performed as described and results were quantified using ImageQuaNT. The signal for β-actin was enhanced 4 times over the signals for G6PD and GAPDH. Similar results were obtained in two independent experiments. (B) Twenty four h after hepatocyte isolation, the medium was replaced with one containing 0.1 μm insulin, 27 mm glucose, 250 μm arachidonic acid, and Matrigel (0.3 mg/ml). The medium was replaced every 12 with one of the same composition but without Matrigel. At the indicated times, RNA was isolated and G6PD mRNA was measured by RNase protection assay as described. GAPDH and β-actin were measured by Northern analysis. Quantitation was done using ImageQuaNT and the results are expressed as relative amounts where the amount of the specific mRNA in cells incubated without fatty acid was set to 100 (0 h). The data are representative of two separate experiments with similar results.

glucose and insulin (Fig. 5B). In the same preparation of cells, the abundance of G6PD mRNA was increased 5-fold by glucose and insulin, whereas arachidonic acid attenuated this increase by 47% (Fig. 5B). Expression of the β-actin gene was not regulated by any of the treatments (Figs. 5A and 5B), thus, the transcriptional activity of the other genes was expressed relative to transcription of the β-actin gene. Transcriptional activity of the GAPDH gene was increased 3-fold by glucose and insulin, consistent with the change in accumulation of GAPDH mRNA. Although PEPCK transcriptional activity was low in the absence of transcriptional activators such as cAMP, PEPCK transcription was still decreased 31–94% in hepatocytes incubated with glucose and insulin. Arachidonic acid did not change the transcriptional activity of the PEPCK gene and had only a minor effect on transcription of the GAPDH gene which was not reflected by a change in its mRNA. In one experiment (shown), glucose and insulin caused a 2-fold increase in G6PD transcription; this was not consistently observed. Thus, we conclude that transcription of the G6PD gene is not regulated by polyunsaturated fatty acids.

Posttranscriptional regulation of G6PD by diet in the intact mouse and rat involves changes in pre-mRNA accumulation in the nucleus. We tested whether arachidonic acid inhibited G6PD expression in rat hepatocytes by changing the amount of pre-mRNA for G6PD in the nucleus or by changing the amount of mRNA for G6PD in the cytoplasm. Nuclear RNA was purified from hepatocytes treated with glucose and insulin with or without arachidonic acid. G6PD pre-mRNA was measured using the ribonuclease protection assay as previously described. Hybridization of each probe in the assay with nuclear RNA resulted in two protected fragments corresponding to the full-length protected fragment and the exon-only protected fragment (**Fig. 6A**). Incubation of the hepatocytes with glucose and insulin for 48 h resulted in a 3- to 4-fold increase in pre-mRNA for G6PD. This increase was observed with both the exon 9–intron 9 and intron 3–exon 4 probes. Inhibition of G6PD pre-mRNA accumulation by arachidonic acid was approximately 60% as quantified with each of the protected fragments (Fig. 6B). The increase in signal intensity observed in protected fragments representing pre-mRNA without the intron versus pre-mRNA with the intron may reflect either the detection of cytoplasmic RNA co-purifying with the nuclei. Using more highly purified nuclei, the difference in these signals decreased (data not shown). Both pre-mRNA and mature mRNA was decreased by arachidonic acid to a similar extent suggesting that regulation occurs early in the nuclear processing pathway, perhaps prior to splicing.

To determine whether changes in the pre-mRNA pool could account for the changes in mRNA for G6PD, total RNA was prepared from the same hepatocytes and the amount of G6PD mRNA was measured using the ribonuclease protection assay (Fig. 6A). Total RNA is primarily cytoplasmic RNA (9). The amount of mature G6PD mRNA was increased 3- to 4-fold by incubation with glucose and insulin, and the inhibition of G6PD mRNA amount was 50% or more (Fig. 6B). Thus changes in the amount of pre-mRNA for

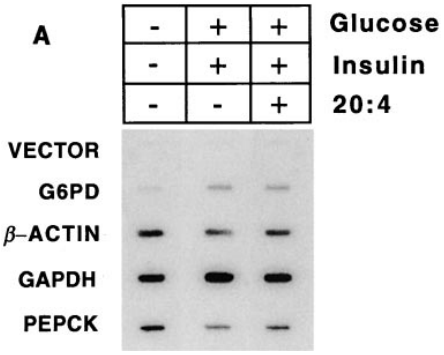


Fig. 5. G6PD is regulated at a posttranscriptional step in rat hepatocytes. (A) Hepatocytes were isolated and maintained in culture as described in the legend to Fig. 3B. Nuclei were isolated from 16 plates per treatment and nuclear run-on assays were performed as described in Materials and Methods. Lane 1 represents nuclei isolated from hepatocytes at 24 h after isolation of the cells. Lanes 2 and 3 represent the results from nuclei isolated after 48 h of incubation with 27 mm glucose and 0.1 μm insulin (lane 2), or 27 mm glucose, 0.1 μm insulin, and 250 μm arachidonic acid (lane 3). The amount of hybridization of the transcripts to the DNA probes was quantified using ImageQuaNT. Part A is a representative blot. (B) Quantitative results of 3 independent experiments comparing transcriptional activity with mRNA abundance (n = 2 plates per treatment per experiment) within the same hepatocyte population. Transcriptional activity of the G6PD, GAPDH and PEPCK genes are expressed relative to the transcriptional activity of the β-actin gene, which was not regulated by these treatments. Fold increase is the amount of mRNA in hepatocytes treated with glucose and insulin divided by the amount of mRNA in hepatocytes prior to these treatments. The % decrease is the amount of mRNA in hepatocytes treated with arachidonic acid divided by the amount of mRNA in hepatocytes that were not incubated with arachidonic acid ×100.

B

	Transcription		
	No Addition	Glucose+Insulin	Glucose+Insulin+20:4
G6PD	0.35 ± 0.2	0.55 ± 0.1	0.51 ± 0.2
GAPDH	1.74 ± 0.5	5.65 ± 0.6	3.23 ± 0.1
PEPCK	0.91 ± 0.2	0.33 ± 0.1	0.45 ± 0.1

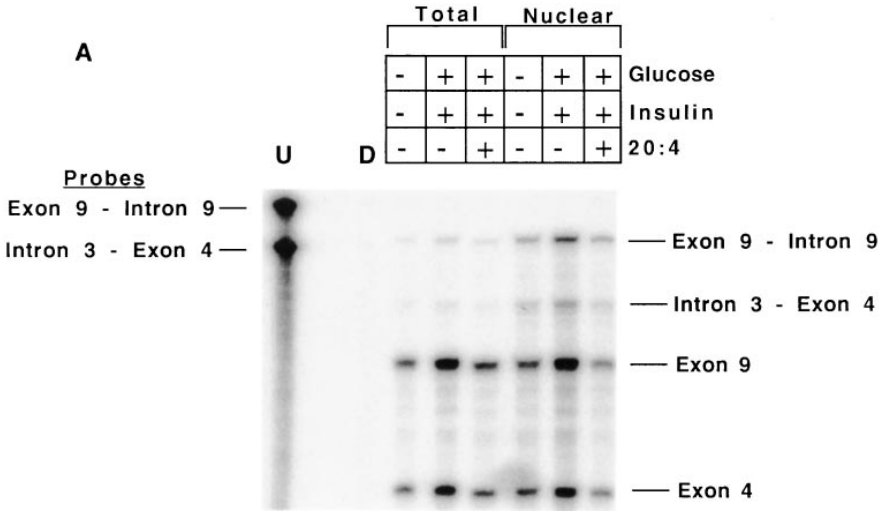
	mRNA	
	Fold increase	% Decrease
G6PD	5.0 ± 1.0	47.0 ± 1.0
GAPDH	2.5 ± 0.07	9.0 ± 3.2
β-Actin	1.1 ± 0.08	4.0 ± 0.05

G6PD due to insulin, glucose, or arachidonic acid can account for all of the change in G6PD mature mRNA.

Effect of monounsaturated versus polyunsaturated fatty acids on G6PD expression

Two characteristics of the inhibition of gene expression by fatty acids are that *1*) only polyunsaturated fatty acids of the n–6 and n–3 families of fatty acids are inhibitors, and *2*) the inhibition is not a consequence of toxic actions such as an increase in peroxide formation. We tested whether the inhibition of G6PD expression by fatty acids fulfilled these two criteria. Hepatocytes were incubated with glucose and insulin, or glucose, insulin and various fatty acids for 48 h. RNA was isolated from nuclei and cells and the amount of pre-mRNA for G6PD was measured using the ribonuclease protection assay (**Fig. 7A**). Arachidonate (n–6) and eicosapentaenoate (n–3) inhibited G6PD pre-mRNA amount by 88% and 57%, respectively. In contrast, oleate (n–9 and one double bond) had little or no effect. A 45% inhibition was observed with α-linolenate and γ-linolenate, the metabolic precursors of arachidonate and eicosapentaenoate, respectively. The lower potency of these 18 carbon fatty acids as inhibitors was not unexpected. Previous work has shown that conversion of α-linoleate to arachidonate is attenuated in primary hepatocyte cultures (34). Thus, inhibition of G6PD expression at a nuclear posttranscriptional step is specific for long chain polyunsaturated fatty acids of the n–3 and n–6 classes.

Long chain polyunsaturated fatty acids can be subject to oxidation within cells releasing toxic peroxides into the medium (35). Thus we determined whether the inhibition of G6PD expression was due to a specific action of long chain polyunsaturated fatty acids or a more generalized effect due to toxicity of these fatty acids. Toxicity was assessed by measurement of TBA-reactive substances in the medium of the hepatocytes. TBA-reactive materials provide an estimate of malondialdehyde levels in the culture medium, a primary product of peroxidation of polyunsaturated fatty acids. The amount of TBA-reactive material was 1.44 nmol and 2.1 nmol/plate after 24 and 48 h of incubation, respectively, in the absence of fatty acids. Regardless of the presence or the degree of unsaturation of the fatty acid, little or no change in the amount of TBA-reactive substances was observed in the medium of the hepatocytes (range of 1.24–1.75 nmol/plate at 24 h and 1.09–1.87 nmol/plate at 48 h). These results coupled with the lack of change in the expression of GAPDH and β-actin (Fig. 7B) indicate that the effects of long chain polyun-



B

Nuclear RNA

Protected Fragment	Fold-increase due to glucose and insulin	% inhibition due to 20:4
Intron 3- Exon 4	3.5 ± 0.3	58 ± 5.7
Exon 4	3.2 ± 0.6	57 ± 6.8
Exon 9- Intron 9	5.6 ± 1.5	62 ± 6.4
Exon 9	3.7 ± 0.8	60 ± 7.3

Total RNA

Protected Fragment	Fold-increase due to glucose and insulin	% inhibition due to 20:4
Exon 4	3.1 ± 0.2	51 ± 2.5
Exon 9	3.6 ± 0.3	52 ± 2.8

Fig. 6. Posttranscriptional regulation of G6PD by arachidonic acid occurs in the nucleus. Hepatocytes were isolated and incubated with 27 mm glucose, 0.1 μm insulin with or without 250 μm arachidonic acid (20:4) as described in Fig. 5. The lane indicating the absence of these additions represents RNA from cells prior to the addition of these treatments (time 0; 24 h after hepatocyte isolation). After 48 h of treatment, nuclear RNA was isolated from 17 plates per treatment as described in Materials and Methods. Total RNA was isolated from 2 plates per treatment within the same experiment. G6PD mRNA in nuclear and total RNA was analyzed using a ribonuclease protection assay. (A) Representative experiment; U, undigested probe; D, RNase digested probe. (B) Quantitative results of 4 independent experiments (mean ± SE). Hybridization to the G6PD probes was quantified using ImageQuaNT analysis. Fold increase is the amount of mRNA in hepatocytes treated with glucose and insulin divided by the amount of mRNA in hepatocytes prior to these treatments. The % decrease is the amount of mRNA in hepatocytes treated with arachidonic acid divided by the amount of mRNA in hepatocytes that were not incubated with arachidonic acid ×100.

saturated fatty acids on G6PD expression are not due to a generalized toxicity within the cells.

DISCUSSION

G6PD activity and mRNA accumulation undergo large changes with starvation/refeeding and during consumption of a high-fat diet versus a low-fat diet in rats and mice (5, 36). Transcription rate of the gene does not change under these conditions (5; Fig. 2A) and thus, regulation is posttranscriptional. Further, this regulation occurs in the nucleus at an early step, perhaps prior to processing events (9; Fig. 2B). In the present report we present several lines of evidence that fatty acids directly mediate the effect of dietary fat on the expression of G6PD. First, long chain polyunsaturated fatty acids inhibit the expression of G6PD in primary hepatocyte cultures at the same posttranscriptional step observed with dietary fat in the intact animal. In this regard, G6PD expression was inhibited by arachidonic acid by decreases in the amount of pre-mRNA for G6PD in the nucleus in the absence of transcriptional

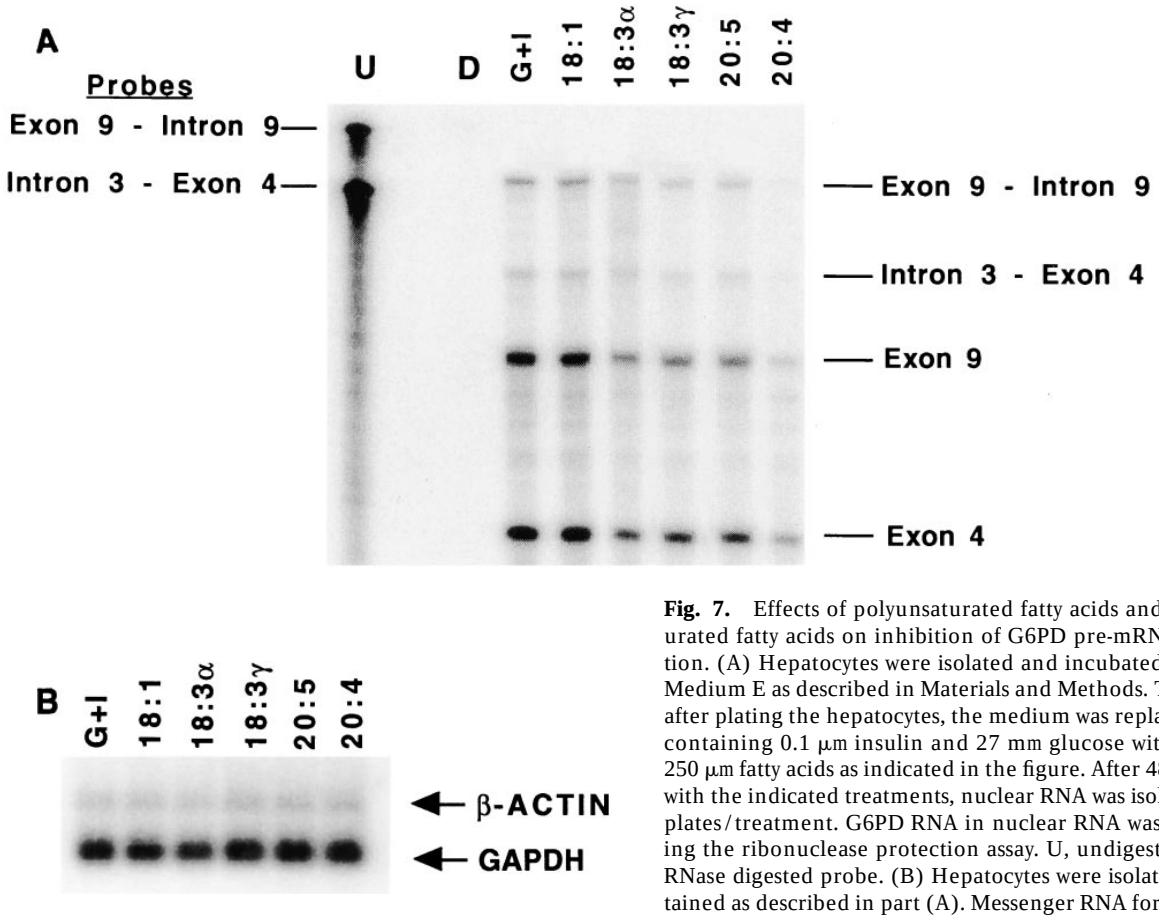


Fig. 7. Effects of polyunsaturated fatty acids and monounsaturated fatty acids on inhibition of G6PD pre-mRNA accumulation. (A) Hepatocytes were isolated and incubated in Williams' Medium E as described in Materials and Methods. Twenty-four h after plating the hepatocytes, the medium was replaced with one containing 0.1 μm insulin and 27 mm glucose with or without 250 μm fatty acids as indicated in the figure. After 48 h in culture with the indicated treatments, nuclear RNA was isolated from 12 plates/treatment. G6PD RNA in nuclear RNA was analyzed using the ribonuclease protection assay. U, undigested probe; D, RNase digested probe. (B) Hepatocytes were isolated and maintained as described in part (A). Messenger RNA for GAPDH and β-actin was measured by Northern analysis.

regulation of the G6PD gene. Second, the inhibition of G6PD expression by arachidonic acid in hepatocyte cultures occurs rapidly. A decrease in G6PD mRNA was detected as early as 2 h after the addition of fatty acid and the decrease was maximal by 8 h, identical to the time course of the decrease of G6PD mRNA observed with the intact animal (5). Last, the specificity of the effect of fatty acids on G6PD expression is the same as that observed in the intact animal. For example, polyunsaturated fatty acids but not monounsaturated fatty acids inhibit G6PD in intact animals and in primary rat hepatocyte cultures (6, 8; Fig. 7).

Our observations with respect to posttranscriptional regulation by fatty acids are very similar to the observations with respect to transcriptional regulation by fatty acids. Transcriptional activities of the genes for fatty acid synthase, S14, and pyruvate kinase are inhibited by polyunsaturated fatty acids in intact animals or in hepatocytes in primary culture (10, 14, 15). The differential effect of polyunsaturated fatty acids versus monounsaturated fatty acids is observed for these genes. These similarities between the transcriptional and posttranscriptional regulation by polyunsaturated fatty acids suggest that a common fatty acid metabolite is involved in both modes of regulation.

Glucose and insulin increase G6PD expression 5- to 7-fold in the hepatocytes. This change is similar to the increase in G6PD mRNA and pre-mRNA abundance that is observed in mice during their typical feeding cycle (9). In mice consuming a high-carbohydrate, low-fat diet ad libitum, the amounts of G6PD mRNA and pre-mRNA increase 7-fold from a low level prior to the onset of the dark or feeding cycle to the highest level observed 8 h into the dark cycle (9). Thus, factors needed for the positive regulation of G6PD expression appear to be present in the hepatocyte system. Arachidonic acid inhibited G6PD enzyme activity and mRNA accumulation by 50–60%. These changes are somewhat smaller than the 80% decreases observed in the intact animal. The attenuated effect of fatty acids may indicate that the effect of dietary fat is not exclusively due to the fatty acids in the diet but may also involve hormonal regulation. Alternatively, this may indicate an absence of some of the regulatory factors in primary hepatocyte cultures needed to observe the maximal effect.

The inhibition of G6PD expression by arachidonic acid was only observed in cells treated with both glucose and insulin. Arachidonic acid does not decrease the amount of G6PD mRNA in hepatocytes treated only with glucose. The simplest interpretation of these observations is that polyunsaturated fatty acids inhibit signal transduction by insulin. As insulin is also thought to stimulate glucose metabolism in hepatocytes and generation of the glucose-signal (37), the step in signal transduction inhibited by arachidonic acid must be downstream of the step involved in glucose utilization. Alternatively, separate signalling

pathways may be involved in insulin stimulation of glucose metabolism and insulin stimulation of G6PD expression. Formal proof of this hypothesis will require the identification of the specific signals involved in regulation by insulin and fatty acids. It is not clear whether all genes inhibited by arachidonic acid will share a similar signal transduction pathway. In this regard, the activity of fatty acid synthase is inhibited by polyunsaturated fat in diabetic rats (38) suggesting a mechanism independent of insulin. Inhibition of fatty acid synthase by fatty acids with and without insulin has not been tested in primary hepatocyte cultures.

Examples of nutrient regulation at posttranscriptional steps indicate that cytoplasmic mRNA stability is the primary process involved. For example, excess cellular iron leads to a decrease in stability of the transferrin receptor mRNA which is mediated by the binding of an iron regulatory protein to the iron response element in the 3′-UTR of the transferrin receptor mRNA (39, 40). Glucose increases the stability of the fatty acid synthase mRNA. Incubating HepG2 cells with increasing concentrations of glucose stimulates a 5-fold increase in fatty acid synthase mRNA in the absence of transcriptional regulation (41). The mechanism for this stability appears to involve a differential partitioning of the cytoplasmic mRNA between a translated pool protected from degradation and a decay compartment (42). Fatty acids also act posttranscriptionally by decreasing the stability of glut-4 (43) and stearoyl-CoA desaturase I (44) mRNAs in the cytoplasm of 3T3-L1 adipocytes. In contrast, the posttranscriptional mechanism involved in the regulation of G6PD by nutrients is quite different in that it occurs in the nucleus and involves the pre-mRNA (Fig. 6B).

Regulation of pre-mRNA in the nucleus could result from a change in the rate of processing of the pre-mRNA, stability of pre-mRNA in the nucleus, or a block in transport of mRNA from the nucleus to the cytoplasm. Posttranscriptional regulation in the nucleus has been reported for several genes. The putative lipogenic protein, S14, is regulated partly by a nuclear posttranscriptional mechanism which involves enhanced splicing of S14 pre-mRNA during consumption of a high-carbohydrate diet or after insulin administration (12, 13). Fibronectin and liver/kidney/bone alkaline phosphatase genes are both regulated by accumulation of the pre-mRNA for these genes in the nucleus. The fibronectin gene is regulated by dexamethasone (45) or by transformation of TE-85 cells with Ha-*ras* (46), whereas alkaline phosphatase is regulated in a tissue-specific manner (47). Changes in expression of fibronectin and the alkaline phosphatase genes are not mediated by changes in transcription, splicing or polyadenylation. In both of these cases, nuclear stability of the pre-mRNA is the postulated mechanism, although this has not been tested experimentally.

Regulation of G6PD pre-mRNA accumulation most likely occurs early after transcription of the gene, perhaps prior to processing. Accumulation of G6PD mRNA in the cytoplasm due to consumption of a high-carbohydrate diet occurs shortly after an increase in pre-mRNA is observed in the nucleus thereby ruling out nuclear–cytoplasmic transport as a potential regulatory mechanism (9). Whether regulated by diet or nutrients in cultured hepatocytes, the similarity in the rate of change of pre-mRNAs both with and without introns suggests that the efficiency of splicing is also not a regulated step. Regulation could, however, involve any of the other processing reactions occurring within the nucleus such as capping or polyadenylation. Deadenylation and decapping of pre-mRNA can function as a regulatory mechanism in the nucleus (48) but are most often associated with the presence of nonsense codons (49). In previous work (9) we detected a selective disappearance of the 3′-most portion of G6PD pre-mRNA as a result of dietary manipulation.

We hypothesize that nuclear posttranscriptional regulation of G6PD occurs early after transcription by differential partitioning of the pre-mRNA into various compartments in the nucleus. This idea is not without precedent. For example, processing of pre-RNAs is thought to occur in close association with the nuclear matrix (50–52). Further, this type of compartmentalization has been suggested for the regulation of the alkaline phosphatase gene by retinoic acid (53). Stabilization of the nascent alkaline phosphatase mRNA by retinoic acid is hypothesized to occur by enhanced processing of the pre-mRNA in the nuclear matrix compartment thereby facilitating movement through the nucleus to the cytoplasm. Preliminary evidence from our laboratory suggests that this is also the case for G6PD (B. Amir-Ahmady and L. M. Salati, unpublished results). The amount of G6PD pre-mRNA on the nuclear matrix is increased in livers of fed mice relative to livers of mice that have been starved. Perhaps a protein is induced during high-carbohydrate feeding that facilitates the movement of the pre-mRNA through the nuclear processing reactions, thus protecting it from degradation. In the starved animal or animal fed a high-fat diet, the activity or amount of this protein may be decreased and thus association with the nuclear matrix would not occur. Pre-mRNA not associated with the nuclear matrix would be more susceptible to degradation by RNases in the nucleus.

In summary, regulation of G6PD by polyunsaturated fatty acids in primary rat hepatocytes is mediated by a nuclear posttranscriptional mechanism. This mechanism of regulation by fatty acids has not previously been described for other genes. However, nuclear posttranscriptional regulation may be common mechanism for all lipogenic genes. The data with carbohydrate regulation of S14 (12) suggests that such a regulatory mechanism occurs in a gene that also undergoes transcriptional changes to the same stimulus. G6PD provides an ideal model to examine this form of regulation because of the absence of transcriptional changes that would confound the interpretation of nuclear events. Experiments are currently in progress aimed at determining the *cis*-acting elements within the G6PD pre-mRNA sequence involved in regulation by polyunsaturated fat using this cell culture system.

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