J. Biochem. 119, 162-166 (1996)

Relationship between the Concentrations of Glycolytic Intermediates and Expression of the L-Type Pyruvate Kinase Gene in Cultured Hepatocytes[1]

Rujun Kang,* Kazuya Yamada,* Takashi Tanaka,* Ting Lu,* and Tamio Noguchi*,†,[2]

*Department of Nutrition and Physiological Chemistry, Osaka University Medical School, Suita, Osaka 565; and †Department of Biochemistry, Fukui Medical School, Matsuoka, Fukui 910-11

Received for publication, September 20, 1995

Previous studies have suggested that some glycolytic intermediates are involved in the regulation of L-type pyruvate kinase gene expression by carbohydrates such as glucose and fructose. To find such intermediates, we examined the relationship between the levels of L-type pyruvate kinase mRNA and glycolytic metabolites in hepatocytes cultured under various conditions. Of the metabolites, the levels of 3-phosphoglycerate and phosphoenolpyruvate only increased significantly under conditions under which the expression of the L-type pyruvate kinase gene was stimulated. The level of glucose 6-phosphate, which was reported to be involved in dietary stimulation of this gene expression, was not correlated with the mRNA level since marked accumulation of deoxyglucose 6-phosphate occurring on the addition of deoxyglucose, a nonmetabolizable glucose analog, was not accompanied by an increase in the L-type pyruvate kinase mRNA level. In addition, we found that fructose at a low concentration in the presence of glucose failed to increase the mRNA and metabolite levels in contrast to other reports that the promoter activity of the L-type pyruvate kinase gene is stimulated by this treatment. Thus we propose that 3-phosphoglycerate and/or phosphoenolpyruvate are involved in the carbohydrate regulation of L-type pyruvate kinase gene expression.

Key words: carbohydrate metabolism, glycolytic metabolites, L-type pyruvate kinase gene expression.

Hormones and carbohydrates regulate the gene expression of glycolytic and lipogenic enzymes at both the transcriptional and post-transcriptional levels (*1-3*). The L-type isozyme of pyruvate kinase (PK), an important glycolytic enzyme, is one such enzyme. This isozyme is expressed mainly in parenchymal hepatocytes in the liver, and also in kidney, small intestine, and pancreatic B-cells (*4, 5*). The hepatic expression of the L-type PK is stimulated by dietary fructose as well as glucose *in vivo* (*6, 7*). This occurs at both the transcriptional and post-transcriptional levels (*8-10*). The transcriptional stimulation of the L-type PK gene by dietary glucose requires increased secretion of insulin and occurs much more slowly than that by dietary fructose (*8, 10*). Since dietary glycerol has the same effect as fructose, and since neither compound stimulates insulin secretion, it has been postulated that some metabolite common to the metabolism of these compounds is involved in this stimulation (*7, 10, 11*). Further analysis using transgenic mice indicated that an enhancer unit of the L-type PK gene, comprising −170 to −76, is responsive to dietary glucose as well as fructose (*12*). One of the three elements constituting the enhancer unit, PKL-III (*13*), has been found to be responsive to glucose/insulin in cultured hepatocytes using a transient expression assay (*14, 15*). Thus, it is very likely that PKL-III is also an element responsive to fructose, suggesting that both glucose and fructose share a common mechanism of transcriptional stimulation of the L-type PK gene. This also raises the possibility that an intermediate common to the metabolism of both glucose and fructose is involved in this effect.

In cultured hepatocytes, in fact, an effect of insulin on the L-type PK gene was observed in the presence of glucose, but not in the presence of 2-deoxyglucose, a nonmetabolizable glucose analog, or pyruvate (*16*). Thus, the role of insulin in stimulation of the L-type PK gene appears to increase the concentration of some glycolytic metabolite by stimulating glucose metabolism. This metabolite may also be formed from fructose in the presence of insulin, since fructose could partially replace glucose as to the effect on the L-type PK gene (*16*).

Therefore, we propose that a glycolytic intermediate between fructose 6-phosphate and phosphoenolpyruvate is the candidate molecule involved in transcriptional stimulation of the L-type PK gene. However, it has been reported that glucose-dependent gene activation of glycolytic and

[1] This study was supported by Grants-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture of Japan.

[2] To whom correspondence should be addressed: Department of Biochemistry, Fukui Medical School, 23 Shimoaizuki, Matsuoka, Fukui 910-11. Phone: +81-776-61-3111 (Ext. 2225), Fax: +81-776-61-8102, E-mail: tnoguchi@fmsrsa.fukui-med.ac.jp

Abbreviations: DAP, dihydroxyacetone phosphate; dG6P, 2-deoxyglucose 6-phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; 3PG, 3-phosphoglycerate; PK, pyruvate kinase.

lipogenic enzymes including L-type PK requires the accumulation of glucose 6-phosphate (*17-19*). In the present study, we determined glycolytic intermediates in cultured hepatocytes under various conditions to find a candidate intermediate involved in transcriptional stimulation of the L-type PK gene. In addition, we examined the effect of a low fructose concentration on the L-type PK mRNA level in cultured hepatocytes, since it has been found, using a transient expression system, to stimulate the promoter activity of the L-type PK gene in hepatocytes (*19*).

EXPERIMENTAL PROCEDURES

Materials—Insulin was obtained from Sigma. Dexamethasone phosphate, collagenase, and Williams' medium E were purchased from Koch-Light, Wako Pure Chemical Ind., and Flow Laboratories, respectively. Fetal bovine serum was obtained from Hyclone Laboratories. Glucose, fructose, and 2-deoxy-D-glucose were products of Katayama Chemical Ind. Glucose 6-phosphate dehydrogenase, phosphoglucose isomerase, glycerol 3-phosphate dehydrogenase, triosephosphate isomerase, aldolase, glyceraldehyde 3-phosphate dehydrogenase, PK, enolase, and phosphoglucomutase were obtained from Boehringer Mannheim. Nylon membranes (Hybond N) were purchased from Amersham International. [α-^{32}P]dATP was a product of Du Pont-New England Nuclear.

Preparation and Primary Culture of Rat Hepatocytes—Parenchymal hepatocytes were isolated from male Sprague-Dawley rats (190-240 g body weight) given laboratory chow *ad libitum*. The liver was perfused with collagenase and the isolated hepatocytes were cultured as described previously (*16*). Briefly, the hepatocytes were dispensed into 15 cm tissue culture dishes at a density of 1×10^5 cells/cm^2 with 20 ml of Williams' medium E containing 10% fetal bovine serum and 1 μM dexamethasone. This medium also contained 11 mM glucose. The cultures were incubated at 37°C under 5% CO_2 in air. After 4 h, the medium was replaced with serum-free medium and the incubation was continued for 16 h. Then the medium was replaced with the same medium containing the indicated concentrations of carbohydrates in the presence or absence of 0.1 μM insulin. The medium was changed every 24 h. The cells were harvested at the indicated times for determination of the L-type PK mRNA and glycolytic metabolites. Control samples were taken at zero time.

Isolation of RNA and Northern Hybridization—Total cellular RNA was extracted from hepatocytes using the acid guanidine-phenol-chloroform method (*20*). The RNA for L-type PK was analyzed by Northern blotting using 20 μg of total RNA, followed by autoradiography as described (*8, 10, 16*). The integrity and amount of RNA applied were determined by ethidium bromide staining. The probe for L-type PK was as described (*10*), and labeled with [α-^{32}P]-dATP (3,000 Ci/mmol) using a random oligonucleotide primer (*21*).

Determination of Metabolite Concentrations—The concentrations of metabolites including glucose 6-phosphate (G6P), 2-deoxyglucose 6-phosphate (dG6P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (FBP), glyceraldehyde 3-phosphate, dihydroxyacetone phosphate (DAP), 1,3-bisphosphoglycerate, 3-phosphoglycerate (3PG), 2-phosphoglycerate (2PG), and phosphoenolpyruvate (PEP), in cultured hepatocytes were assayed enzymatically. Briefly, after removing a culture medium, 0.5 ml of ice-cold 0.6 M $HClO_4$ was immediately added to a culture dish. The extract was centrifuged and then the acid supernatant was neutralized with 5 M K_2CO_3. The supernatant after centrifugation was used for spectrophotometric determination of the metabolite concentrations as described (*22*), except for dG6P, which was measured as described by Manchester *et al.* (*23*).

RESULTS AND DISCUSSION

We determined the L-type PK mRNA level, and the concentrations of glycolytic intermediates between G6P and PEP in hepatocytes cultured under various conditions to determine the relationship between the levels of mRNA and glycolytic intermediates. L-type PK mRNA was measured at 30 h after the start of treatment since the mRNA level reaches a maximum after 24 to 36 h, as reported (*16*). Control samples were taken at zero time. As shown in Fig. 1A, 30 mM glucose only induced an increase in the L-type PK mRNA level in the presence of insulin. Fructose at 5 mM also increased the mRNA level in the presence of insulin, but the enhancement was less than 15% of that with glucose. Substitution of 30 mM 2-deoxyglucose for glucose completely abolished the effect of insulin on L-type PK mRNA (Fig. 1B). These results coincide with those reported previously (*16*).

We determined the concentrations of glycolytic metabolites in hepatocytes treated with 30 mM glucose in the presence or absence of 0.1 μM insulin for 0.5, 2, or 6 h. These time points were chosen because L-type PK mRNA

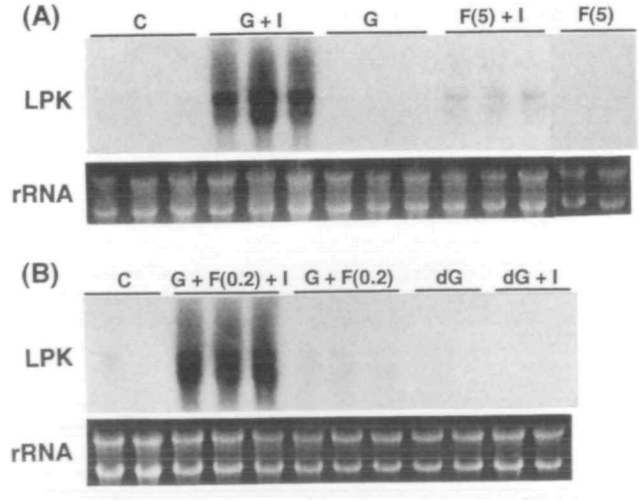

Fig. 1. Effects of various carbohydrates and insulin on the level of L-type pyruvate kinase mRNA in cultured hepatocytes. Hepatocytes were incubated for 30 h with the indicated carbohydrates in the presence or absence of insulin. Control cells were harvested at zero time. Total RNA was isolated from the cells and separated by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde (20 μg per lane) after staining with ethidium bromide. The RNA was then transferred to a nylon membrane and hybridized with ^{32}P-labeled L-type pyruvate kinase cDNA. (A) C, control; G, glucose (30 mM); F(5), fructose (5 mM), I, insulin (0.1 μM) (B) C, control; G, glucose (30 mM); F(0.2), fructose (0.2 mM); dG, 2-deoxyglucose (30 mM); I, insulin (0.1 μM). LPK, L-type pyruvate kinase

starts to increase after 6 h under these conditions (*16*). The values were expressed relative to those for hepatocytes at zero time that had been incubated for 16 h in the presence of 11 mM glucose. The concentrations of G6P, F6P, FBP, DAP, glyceraldehyde 3-phosphate, 1,3-bisphosphoglycerate, 3PG, 2PG, and PEP at zero time were 757±163, 578±129, 662±370, 948±279, 430±138, 480±245, 3227±251, 885±196, and 1692±170 nmol/10^7 cells. Of these metabolites, the levels of G6P, FBP, and DAP increased significantly after incubation for 0.5 h with 30 mM glucose (Table I). The increased level of G6P continued for at least 6 h. The addition of insulin to the medium resulted in a further 1.5-fold increase in the G6P concentration after 0.5 h. But this hormone did not increase the levels of FBP and DAP as compared with those in hepatocytes treated with a high glucose concentration. The concentrations of 3PG, 2PG, and PEP were also significantly increased by insulin treatment after 0.5 h, although the levels of these intermediates did not change after 0.5 h and decreased significantly after 2 h on incubation with high glucose only. The F6P level tended to increase in both groups, but the differences were not statistically significant.

Based on these results, we further determined the concentrations of G6P, 3PG, 2PG, and PEP in hepatocytes cultured for 0.5 h under various conditions. As shown in Table II, these intermediates did not significantly increase in hepatocytes incubated with 5 mM fructose. Treatment with 5 mM fructose and 0.1 μM insulin caused significant increases in the levels of these metabolites, but only the levels of 3PG and PEP were significantly higher than those in hepatocytes incubated with fructose alone. When 2-deoxyglucose was added to the medium at 30 mM, the dG6P concentration was measured after 0.5 h and its level was expressed relative to that of G6P at zero time. Although substitution of deoxyglucose for glucose abolished the effect of insulin on L-type PK mRNA, the level of dG6P increased 3.5-fold in the absence of insulin and 5.8-fold in its presence. Insulin caused significant accumulation of this metabolite. No other metabolites increased under these conditions.

Recently, Doiron *et al.* found, using a transient expression assay, that 0.2 mM fructose in the presence of 20 or 40 mM glucose stimulated the promoter activity of the L-type PK gene in cultured hepatocytes regardless of the presence of insulin (*19*). This observation was explained by the ability of a low concentration of fructose to activate glucokinase mediated through the formation of fructose 1-phosphate, which interacts with a regulatory protein of glucokinase (*24*). This leads to the accumulation of G6P, which is believed to be involved in transcriptional activation of the L-type PK gene. This regulatory protein with a molecular mass of 62 kDa inhibits glucokinase by binding to the enzyme in the presence of F6P (*25*). Fructose 1-phosphate, which is produced from fructose by fructokinase, prevents this inhibition by causing dissociation of the enzyme-regulatory protein complex (*25*). However, the extent of glucokinase activation in cultured hepatocytes on the addition of 0.2 mM fructose was reported to be only 2- to 4-fold in the presence of 5 mM glucose, and this fructose effect essentially disappears in the presence of over 30 mM

TABLE I. **Effects of glucose and insulin on the concentrations of glycolytic metabolites in cultured hepatocytes.** Hepatocytes were cultured with 30 mM glucose in the presence or absence of 0.1 μM insulin for 0.5, 2, or 6 h. Control samples were obtained at zero time. The metabolite concentrations were determined as described under "EXPERIMENTAL PROCEDURES." Data are normalized as to the control value at zero time, and expressed as means±SD for 3 to 5 independent experiments. The values significantly different from controls are indicated by superscript letters a ($p<0.05$) and b ($p<0.01$), and from glucose by superscript letters c ($p<0.05$), d ($p<0.01$), and e ($p<0.001$).

Metabolite	Glucose (30 mM)				Glucose (30 mM)+insulin		
	0 h	0.5 h	2 h	6 h	0.5 h	2 h	6 h
Glucose 6-phosphate	100	226±32[b]	195±59[a]	189±53[a]	343±56[b,e]	227±22[b]	216±33[b]
Fructose 6-phosphate	100	226±75	210±105	227±107	264±112	232±87	195±30
Fructose 1,6-phosphate	100	260±37[b]	163±60	119±33	176±41	160±43	170±54
Glyceraldehyde 3-phosphate	100	77±29	75±25	69±5	82±21	73±32	78±5
Dihydroxyacetone phosphate	100	140±33[a]	104±8	100±12	135±13	123±23	142±16[a]
1,3-Bisphosphoglycerate	100	133±33	133±88	169±119	133±62	150±50	72±26
3-Phosphoglycerate	100	110±20	45±11[b]	75±35	183±34[b,e]	116±25[d]	80±17
2-Phosphoglycerate	100	98±11	69±20[a]	46±10[b]	127±23[a,c]	82±15	57±9[b]
Phosphoenolpyruvate	100	91±10	62±18[b]	67±20[b]	136±9[b,e]	92±7[c]	80±20

TABLE II. **Effects of various carbohydrates and insulin on the concentrations of glycolytic metabolites in cultured hepatocytes.** Hepatocytes were incubated with various carbohydrates at the concentrations indicated in parentheses and 0.1 μM insulin for 0.5 h. Control samples were obtained at zero time. The metabolite concentrations were determined as described under "EXPERIMENTAL PROCEDURES." Data are normalized as to the control value at zero time, and expressed as means±SD for 3 to 5 independent experiments. The concentration of deoxyglucose 6-phosphate was normalized as to that of glucose 6-phosphate at zero time. The values significantly different from controls are indicated by superscript letters a ($p<0.05$) and b ($p<0.01$), and from fructose (5 mM) or deoxyglucose (30 mM) by superscript letters c ($p<0.05$), d ($p<0.01$), and e ($p<0.001$). Glu, glucose; Fru, fructose; Deoxyglu, deoxyglucose.

Metabolite	Control	Glu (30 mM)	Glu (30 mM) +Fru (0.2 mM)	Fru (5 mM)	Fru (5 mM) +insulin	Deoxyglu (30 mM)	Dexoyglu (30 mM) +insulin
Glucose 6-phosphate	100	230±59[a]	259±79[b]	206±142	273±105[a]		
Deoxyglucose 6-phosphate						349±20[b]	576±89[b,c]
3-Phosphoglycerate	100	149±32	172±27[a]	80±39	143±23[a,c]	49±7[b]	75±4[b,d]
2-Phosphoglycerate	100	133±21	156±32[a]	112±11	166±51[b]	109±25	132±46
Phosphoenolpyruvate	100	106±12	121±12	90±5	133±9[b,d]	65±14[a]	96±21

glucose (*24*). Therefore, we examined whether 0.2 mM fructose actually stimulates expression of the endogenous L-type PK gene in cultured hepatocytes in the presence of 30 mM glucose. As shown in Fig. 1B, the L-type PK mRNA level remained unchanged under these conditions. Further addition of insulin induced a remarkable increase in the mRNA level. Glycolytic metabolites, including G6P, 3PG, and 2PG, accumulated significantly in hepatocytes cultured with 30 mM glucose and 0.2 mM fructose (Table II). However, the effects of fructose at this concentration on the levels of these metabolites were not significant, since the increased levels of these metabolites were not significantly different from the values in hepatocytes treated with 30 mM glucose alone. This is consistent with the observation mentioned above that a low concentration of fructose failed to stimulate glucokinase activity in the presence of over 30 mM glucose. Thus, we consider that the activation of glucokinase on the addition of fructose without alteration in the enzyme protein level is trivial, if any, as to activation of the L-type PK gene, and that therefore the major role of fructose in stimulation of the L-type PK gene is different from that proposed by Vaulont and Kahn (*26*). They insisted that fructose stimulates transcription of the L-type PK gene by activating glucokinase through fructose 1-phosphate *in vivo*, as mentioned above, while we propose that fructose acts through a metabolite common to glucose. Moreover, although glycerol does not stimulate glucokinase activity in cultured hepatocytes (*24*), it shows a similar effect to fructose in stimulating expression of the L-type PK gene *in vitro* as well as *in vivo* (*7, 10, 11, 16*).

Taken together, these results suggest that 3PG and PEP are candidate compounds involved in transcriptional stimulation of the L-type PK gene. It appears that the difference in the extent of the L-type PK gene expression stimulated by glucose and insulin from that by fructose and insulin in cultured hepatocytes is correlated with changes in the 3PG level rather than ones in the PEP level. There have been reports that an increase in the G6P level is important for stimulation of the gene expression of glycolytic and lipogenic enzymes including L-type PK by carbohydrates (*17-19*). However, the present evidence does not support this and suggests that if it is the case, the accumulation of this metabolite alone is not sufficient for activation of the L-type PK gene, since the addition of deoxyglucose in the presence of insulin failed to increase the L-type PK mRNA level in spite of the concomitant accumulation of dG6P. Obviously further experiments are required to verify our proposal.

REFERENCES

1. Goodridge, A.G. (1987) Dietary regulation of gene expression: Enzymes involved in carbohydrate and lipid metabolism. *Annu. Rev. Nutr.* **7**, 157-185
2. Pilkis, S.J. and Granner, D.K. (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu. Rev. Physiol.* **54**, 885-909
3. Iritani, N. (1992) Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. *Eur. J. Biochem.* **205**, 433-442
4. Imamura, K. and Tanaka, T. (1982) Pyruvate kinase isozymes from rat. *Methods Enzymol.* **90**, 150-165
5. Noguchi, T., Yamada, K., Yamagata, K., Takenaka, M., Nakajima, H., Imai, E., Wang, Z., and Tanaka, T. (1991) Expression of liver type pyruvate kinase in insulinoma cells: Involvement of LF-B1 (HNF1). *Biochem. Biophys. Res. Commun.* **181**, 259-264
6. Tanaka, T., Harano, Y., Sue, F., and Morimura, H. (1967) Crystallization, characterization and metabolic regulation of two types of pyruvate kinase isolated from rat tissues. *J. Biochem.* **62**, 71-91
7. Sillero, A., Sillero, M.A.G., and Sols, A. (1969) Regulation of the level of key enzymes of glycolysis and gluconeogenesis in liver. *Eur. J. Biochem.* **10**, 351-354
8. Noguchi, T., Inoue, H., and Tanaka, T. (1985) Transcriptional regulation of L-type pyruvate kinase in diabetic rat liver by insulin and dietary fructose. *J. Biol. Chem.* **260**, 14393-14397
9. Munnich, A., Lyonnet, S., Chauvet, D., Van Schaftingen, E., and Kahn, A. (1987) Differential effects of glucose and fructose on liver L-type pyruvate kinase gene expression in vivo. *J. Biol. Chem.* **262**, 17065-17071
10. Matsuda, T., Noguchi, T., Takenaka, M., Yamada, K., and Tanaka, T. (1990) Regulation of L-type pyruvate kinase gene expression by dietary fructose in normal and diabetic rats. *J. Biochem.* **107**, 655-660
11. Takeda, Y., Inoue, H., Honjo, K., Tanioka, H., and Daikuhara, Y. (1967) Dietary response of various key enzymes related to glucose metabolism in normal and diabetic rat liver. *Biochim. Biophys. Acta* **136**, 214-222
12. Noguchi, T., Okabe, M., Wang, Z., Yamada, K., Imai, E., and Tanaka, T. (1993) An enhancer unit of L-type pyruvate kinase gene is responsible for transcriptional stimulation by dietary fructose as well as glucose in transgenic mice. *FEBS Lett.* **318**, 269-272
13. Yamada, K., Noguchi, T., Matsuda, T., Takenaka, M., Monaci, P., Nicosia, A., and Tanaka, T. (1990) Identification and characterization of hepatocyte-specific regulatory regions of the rat pyruvate kinase L gene. The synergistic effects of multiple elements. *J. Biol. Chem.* **265**, 19885-19891
14. Shih, H. and Towle, H.C. (1992) Definition of the carbohydrate response element of the rat S14 gene. Evidence for a common factor required for carbohydrate regulation of hepatic genes. *J. Biol. Chem.* **267**, 13222-13228
15. Bergot, M.-O., Diaz-Guerra, M.-J.M., Puzenat, N., Raymondjean, M., and Kahn, A. (1992) *Cis*-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. *Nucleic Acids Res.* **20**, 1871-1878
16. Matsuda, T., Noguchi, T., Yamada, K., Takenaka, M., and Tanaka, T. (1990) Regulation of the gene expression of glucokinase and L-type pyruvate kinase in primary cultures of rat hepatocytes by hormones and carbohydrates. *J. Biochem.* **108**, 778-784
17. Foufelle, F., Gouhot, B., Pegorier, J.-P., Perdereau, D., Girard, J., and Ferre, P. (1992) Glucose stimulation of lipogenic enzyme gene expression in cultured white adipose tissue. A role for glucose 6-phosphate. *J. Biol. Chem.* **267**, 20543-20546
18. Marie, S., Diaz-Guerra, M.-J., Miquerol, L., Kahn, A., and Iynedjian, P.B. (1993) The L-type pyruvate kinase gene as a model for studies of glucose-dependent regulation of gene expression in the endocrine pancreatic β-cell type. *J. Biol. Chem.* **268**, 23881-23890
19. Doiron, B., Cuif, M.-H., Kahn, A., and Diaz-Guerra, M.-J.M. (1994) Respective roles of glucose, fructose, and insulin in the regulation of the liver-specific pyruvate kinase gene promoter. *J. Biol. Chem.* **269**, 10213-10216
20. Chomozynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159
21. Feinberg, A.P. and Vogelstein, B. (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6-13
22. Bergmeyer, H.U. (1984) *Methods of Enzymatic Analysis* (3rd ed.) Vol. IV, *Metabolites 1: Carbohydrate*, Verlag Chemie, Weinheim
23. Manchester, J.K., Chi, M.M.-Y., Carter, J.G., Pusateri, M.E., McDougal, D.B., and Lowry, O.H. (1990) Measurement of 2-deoxyglucose and 2-deoxyglucose 6-phosphate in tissues. *Anal.*

Biochem. **185**, 118-124
24. Van Schaftingen, E. and Vandercrammen, A. (1989) Stimulation of glucose phosphorylation by fructose in isolated hepatocytes. *Eur. J. Biochem.* **179**, 173-177
25. Van Schaftingen, E., Detheux, M., and Da Cunha, M.V. (1994) Short-term control of glucokinase activity: Role of a regulatory protein. *FASEB J.* **8**, 414-419
26. Vaulont, S. and Kahn, A. (1994) Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB J.* **8**, 28-35