Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 17, Issue of April 26, pp. 9871–9874, 1996

Glucose Regulates *in Vivo* Glucose-6-phosphatase Gene Expression in the Liver of Diabetic Rats*

(Received for publication, January 31, 1996, and in revised form, March 6, 1996)

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Overproduction of glucose by the liver is the major cause of fasting hyperglycemia in both insulin-dependent and non-insulin-dependent diabetes mellitus. The distal enzymatic step in the process of glucose output is catalyzed by the glucose-6-phosphatase complex. We show here that 90% partially pancreatectomized diabetic rats have a >5-fold increase in the messenger RNA and a 3–4-fold increase in the protein level of the catalytic subunit of glucose-6-phosphatase in the liver. Normalization of the plasma glucose concentration in diabetic rats with either insulin or the glycosuric agent phlorizin normalized the hepatic glucose-6-phosphatase messenger RNA and protein within ~8 h. Conversely, phlorizin failed to decrease hepatic glucose-6-phosphatase gene expression in diabetic rats when the fall in the plasma glucose concentration was prevented by glucose infusion. These data indicate that *in vivo* gene expression of glucose-6-phosphatase in the diabetic liver is regulated by glucose independently from insulin, and thus prolonged hyperglycemia may result in overproduction of glucose via increased expression of this protein.

Excessive production of glucose is the major cause of fasting hyperglycemia in human (1, 2) and experimental diabetes mellitus (3, 4). The hydrolysis of hepatic glucose-6-phosphate is the "final common pathway" for the release of glucose into the circulation. Recent experimental evidence supports the notion that this final step is rate-determining for the increased rate of hepatic glucose output in diabetic states. In fact, marked changes in the rate of formation of hepatic glucose 6-phosphate through gluconeogenesis fail to alter hepatic glucose production (5–9), and in experimental diabetes hepatic glucose production is markedly elevated in the presence of a significant *decrease* in the hepatic glucose 6-phosphate pool (4, 10).

The catalytic portion of the glucose-6-phosphatase (Glc-6-Pase)[1] complex has recently been cloned (11–13). Studies in FAO rat hepatoma cells (13) indicate that insulin down-regulates Glc-6-Pase mRNA levels, while glucocorticoid induces it. Haber *et al.* (14) have recently reported a marked increase in hepatic Glc-6-Pase mRNA and protein in diabetic BB rats. In the same study, *in vivo* treatment with 0.5 unit of insulin normalized the plasma glucose concentration and the hepatic Glc-6-Pase mRNA levels in diabetic rats within 4 h. Similarly, Liu *et al.* (15) have shown increased hepatic Glc-6-Pase mRNA and activity in streptozotocin-induced diabetic rats. Since experimental diabetes and insulin treatment are associated with simultaneous and opposite changes in the plasma glucose and plasma insulin concentrations, we have attempted to discern the relative role of glucose and insulin in the short-term regulation of the hepatic Glc-6-Pase gene expression in (90% partially pancreatectomized) diabetic rats.

Our results confirm that prolonged insulin deficiency and hyperglycemia (experimental diabetes) cause a marked increase in the hepatic Glc-6-Pase mRNA and protein and indicate that short-term (~8 h) correction of hyperglycemia in diabetic rats leads to normalization of the hepatic gene expression of this enzyme, regardless of the circulating insulin concentrations.

MATERIALS AND METHODS

Animals—Four groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were studied: group I, controls (n = 9); group II, 90% partially *pancreatectomized* rats (n = 10); group III, 90% partially pancreatectomized rats acutely treated with low dose insulin (n = 10); group IV, 90% partially pancreatectomized rats acutely treated with phlorizin (n = 16). At 3–4 weeks of age, all rats (80–100 g) were anesthetized with pentobarbital (50 mg/kg of body weight, intraperitoneally), and in groups II–IV, 90% of their pancreas was removed according to the technique of Foglia (16), as modified by Bonner-Weir *et al.* (17). Immediately after surgery rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)–dark (6 p.m. to 6 a.m.) cycle. All rats received *ad libitum* water and standard rat laboratory chow (Ralston-Purina, St. Louis, MO). After surgery rats were weighed twice weekly, and tail vein blood was collected for the determination of nonfasting plasma glucose and insulin concentrations at the same time (8 a.m.). Five weeks following pancreatectomy rats were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg of body weight), and indwelling catheters were inserted into the right internal jugular vein and in the left carotid artery, as described previously (18, 19). Food intake was carefully monitored following catheter placement, and only rats gaining weight and eating >12 g of chow during the night prior to the study were included in the study.

Insulin/Phlorizin Administration—Studies were performed in awake, unstressed, chronically catheterized rats. At ~7 a.m. food was removed, and rats received either a vehicle subcutaneous injection (groups I, II, and III) and a variable intra-arterial insulin infusion (group III) or a subcutaneous injection of phlorizin (group IV, 0.3 g/kg of body weight) and an intra-arterial infusion of saline (groups I, II, and IV). The insulin infusion (group III) was designed to gradually lower the plasma glucose concentration in a similar fashion to that observed with a single injection of phlorizin (group IV). The average rate of insulin infusion was 1.8 ± 0.3 milliunits/kg·min during the first 3 h and 0.9 ± 0.3 milliunits/kg·min thereafter. To discern the effect of the normalization of the plasma glucose concentration from the potential direct effect of phlorizin on Glc-6-Pase gene expression, a subgroup (n = 6) of phlorizin-treated diabetic rats also received glucose infusions designed to maintain hyperglycemia throughout the *in vivo* study. Plasma sam-

* This work was supported by National Institutes of Health Grants DK 45024 and DK 48321, by a grant from the Juvenile Diabetes Foundation, and by Albert Einstein Diabetes Research and Training Center Grant DK 20541. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: Glc-6-Pase, glucose-6-phosphatase; FFA, free fatty acid(s); MOPS, 4-morpholinepropanesulfonic acid.

TABLE I

General basal characteristics of control and diabetic rats

In groups 3 and 4, data for plasma glucose and hormone concentrations were collected prior to the insulin and phlorizin administration, respectively.

Group	*n*	Body weight	Glucose	Insulin	Glucagon
		g	*mM*	*microunits/ml*	*pg/ml*
1. Control	9	326 ± 13	8.2 ± 0.2	43 ± 5	148 ± 10
2. Pancreatectomized	9	301 ± 12	22.3 ± 1.7[a]	27 ± 5[a]	175 ± 13
3. Pancreatectomized/insulin	10	352 ± 9	23.1 ± 1.4[a]	25 ± 6[a]	163 ± 12
4. Pancreatectomized/phlorizin	10	328 ± 10	22.8 ± 1.1[a]	21 ± 5[a]	171 ± 13

[a] $p < 0.01$ *versus* control.

ples for determination of plasma insulin, glucagon, and FFA concentrations were obtained at time −30, 0, 30, 60, 90, and 120 min during the final portion of the *in vivo* study. The total volume of blood withdrawn was ~3.0 ml/study; to prevent volume depletion and anemia, a solution (1:1, v/v) of ~4.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (4 units/ml) was infused. At the end of the *in vivo* studies, rats were anesthetized (pentobarbital, 60 mg/kg of body weight, intravenously), the abdomen was quickly opened, portal vein blood was obtained, and the liver was freeze-clamped *in situ* with aluminum tongs precooled in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the liver was less than 45 s. All tissue samples were stored at −80 °C for subsequent analysis.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committees of the Albert Einstein College of Medicine.

Immunoblotting Analysis—Microsomes were prepared according to Lange *et al.* (20). Briefly, liver tissue (100 mg) was homogenized in 10 volumes of a Tris/sucrose/phenylmethylsulfonyl fluoride buffer (50 mM Tris buffer, pH 7.3, 250 mM sucrose, 1 mM phenylmethylsulfonyl fluoride). This homogenate was centrifuged for 10 min at 10,000 × *g*; the cytosol was then centrifuged for 1 h at 100,000 × *g*, and the pellet was resuspended in 1 ml of Tris/sucrose/phenylmethylsulfonyl fluoride buffer. The resuspended pellet was incubated at 4 °C for 30 min in the presence of Triton X-100 at a final concentration of 0.1%. Protein content was measured by the Bio-Rad assay kit using bovine serum albumin as standard. Equal amounts of proteins (20 μg) were subjected to a 10% SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell). After blocking, the membranes were incubated with a 1:1500 dilution of affinity-purified anti-Glc-6-Pase antibody (a gift of Dr. Rebecca Taub, University of Pennsylvania) followed by a 1:1500 dilution of goat anti-rabbit horseradish peroxidase-conjugated secondary antibodies kit (ECL, Amersham Corp.).

Northern Blot Analysis—Total RNA was isolated on freeze-clamped liver tissues according to the RNA-STAT kit (Tel-TEST "B" Inc., Friendswood, TX). The isolated RNA was assessed for purity by the 260/280 ratio absorbance. Twenty μg of total RNA were electrophoresed on a 1.2% formaldehyde-denatured agarose gel in 1 × MOPS running buffer. The RNA was visualized with ethidium bromide and transferred to a Hybond-N membrane (Amersham Corp.). The Glc-6-Pase cDNA was a 1.25-kilobase pair *Eco*RI-*Hin*dIII fragment (kindly provided by Dr. R. Taub, University of Pennsylvania), and tubulin cDNA probes were labeled with ^{32}P using the Megaprime labeling system kit (Amersham Corp.). Prehybridization and hybridization were carried out using the rapid hybridization buffer (Amersham Corp.). The filters were then exposed to Fuji x-ray films for 12–48 h at −80 °C with intensifying screens. Quantification of Glc-6-Pase was done by scanning densitometry, normalized for ribosomal RNA signal to correct for loading irregularities.

Analytical Procedures—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Inc.) and plasma insulin by radioimmunoassay using rat and porcine insulin standards. Plasma non-esterified fatty acid concentrations were determined by an enzymatic method with an automated kit according to the manufacturer's specifications (Waco Pure Chemical Industries, Osaka, Japan). Differences between groups were determined by analysis of variance.

RESULTS AND DISCUSSION

Studies of the physiologic regulation of hepatic Glc-6-Pase have been largely limited to the assessment of its activity in microsomal preparations (4, 10, 21–23). Increased activity has been consistently demonstrated in experimental diabetes mellitus (4, 10, 21–23). This increase correlates with the plasma glucose concentrations in these animals (10, 15) and can be decreased or normalized by insulin treatment (10, 22, 23). The recent cloning of the human, rat, and murine Glc-6-Pase cDNAs (11–14) allows one to examine the physiologic regulation of its gene expression. Although insulin *per se* can decrease Glc-6-Pase mRNA in FAO rat hepatoma cells (13) and in BB diabetic rats (14), the physiologic *in vivo* regulation of Glc-6-Pase gene expression remains to be delineated. In fact, though in two recent reports insulinopenic diabetes was associated with a marked increase in Glc-6-Pase mRNA in the liver (14, 15), the potential regulatory role of prolonged hyperglycemia *per se* on Glc-6-Pase gene expression has not been studied. Thus, we quantitated the hepatic mRNA and protein levels of Glc-6-Pase in diabetic rats following normalization of the plasma glucose concentration, with or without insulin administration.

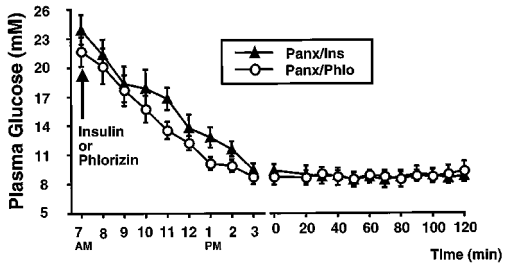

FIG. 1. **Plasma glucose concentrations in diabetic conscious rats treated with insulin (*Ins, open circles*) and phlorizin (*Phlo, closed triangles*).** Diabetic rats (plasma glucose concentration, ~22 mM) were randomly assigned to receive a variable insulin infusion or a subcutaneous injection of phlorizin (0.3 g/kg). The plasma glucose concentrations were slowly decreased to ~9 mM within 8 h. Rats were sacrificed and liver freeze-clamped *in situ* following 2 h at the desired plasma glucose levels (displayed in the *right graph* of the figure and in Table II). All tissue determinations were performed on samples obtained at the completion of these *in vivo* studies. The average rate of insulin infusion was 1.8 ± 0.3 milliunits/kg·min during the first 3 h and 0.9 ± 0.3 milliunits/kg·min during the last 3 h of the *in vivo* study. The plasma glucose concentration in the control (group I) and diabetic (group II) groups averaged 8.2 ± 0.2 and 21.0 ± 1.3 mM, respectively. The mean rate of plasma glucose decline was ~2 mM/h in both groups. *Panx*, pancreatectomized.

Basal Metabolic Parameters—There were no significant differences in the mean body weights between the control and the diabetic groups (Table I). The diabetic animals were carefully matched for their metabolic parameters prior to assignment to the three experimental protocols. In fact, the non-fasting plasma glucose concentrations during the 2-week period prior to the *in vivo* studies were significantly and similarly elevated in the diabetic groups, compared with the control group ($p < 0.01$). The non-fasting plasma insulin concentrations were significantly decreased in all diabetic rats ($p < 0.01$), while the plasma glucagon concentrations were unchanged.

Effect of Short-term Correction of Hyperglycemia on Hormo-

TABLE II

Steady state plasma glucose, FFA, insulin, and glucagon concentrations during the in vivo studies

Plasma samples for the determination of plasma FFA, insulin, and glucagon concentrations were sampled at 30-min intervals during the last 2 h of the *in vivo* studies. Plasma samples for the determination of plasma glucose concentrations were sampled at 10-min intervals during the last 2 h of the *in vivo* studies. Values displayed in this table represent the average levels during this time period. The plasma glucose concentration in group 4 refers to that measured in the 10 rats not infused with glucose (see "Materials and Methods" and Fig. 3*B*).

Group	Glucose	FFA	Insulin	Glucagon
	mM	*mM*	*microunits/ml*	*pg/ml*
1. Control	8.2 ± 0.2	0.79 ± 0.09	41 ± 5	158 ± 11
2. Pancreatectomized	21.0 ± 1.3[a]	1.18 ± 0.12[a]	25 ± 6[a]	175 ± 18
3. Pancreatectomized/insulin	9.1 ± 0.5	0.75 ± 0.06	58 ± 9	166 ± 16
4. Pancreatectomized/phlorizin	9.1 ± 0.7	1.26 ± 0.19[a]	21 ± 7[a]	219 ± 19[a]

[a] $p < 0.01$ *versus* control.

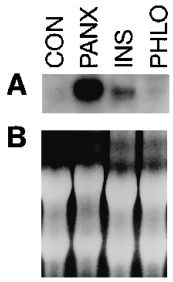

FIG. 2. **Effect of diabetes and of the normalization of plasma glucose concentrations with either insulin or phlorizin on hepatic Glc-6-Pase mRNA.** *A*, Northern analysis of Glc-6-Pase mRNA in liver freeze-clamped *in situ* at the completion of the *in vivo* studies. Northern blots were probed with ^{32}P-labeled Glc-6-Pase cDNA. 20 μg of each liver RNA sample were used. *B*, equal loading of RNA per *lane* as confirmed by ethidium-stained ribosomal RNA bands. The figure depicts Glc-6-Pase mRNA in groups ($n = 3$) of samples obtained from control rats (*CON*), untreated diabetic rats (*PANX*), insulin-treated diabetic rats (*INS*), and phlorizin-treated diabetic rats (*PHLO*). Rats were sacrificed and liver freeze-clamped *in situ* following 2 h at the desired plasma glucose levels (as displayed in Table II). All tissue determinations were performed on samples obtained at the completion of these *in vivo* studies. Analysis was performed several times for all rats included in Tables I and II with similar results.

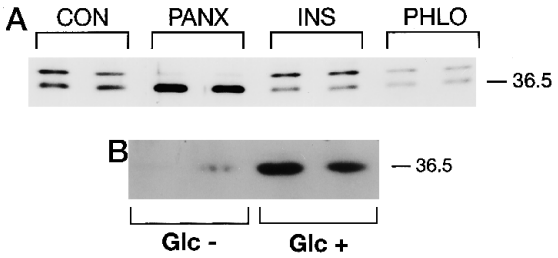

FIG. 3. **Effect of diabetes and of the normalization of plasma glucose concentrations with either insulin or phlorizin on hepatic Glc-6-Pase protein.** *A*, immunoblot of liver microsomal fraction of control rats (*CON*), untreated diabetic rats (*PANX*), insulin-treated diabetic rats (*INS*), and phlorizin-treated diabetic rats (*PHLO*). *B*, immunoblot of liver microsomal fraction of phlorizin-treated diabetic rats in which the plasma glucose concentration is maintained at *diabetic* levels of ~21 mM (*Glc +*) or decreased and maintained at normoglycemic levels (*Glc −*). Rats were sacrificed and liver freeze-clamped *in situ* following 2 h at the desired plasma glucose levels (as displayed in Table II). All tissue determinations were performed on samples obtained at the completion of these *in vivo* studies. Analysis was performed several times for all rats included in Tables I and II with similar results.

nal and Biochemical Parameters—As depicted in Fig. 1, the infusion of insulin and the injection of phlorizin resulted in a progressive and similar lowering of the plasma glucose concentration in diabetic rats in groups III and IV, respectively. Table II shows plasma glucose, FFA, insulin, and glucagon concentrations after ~8 h of vehicle (saline, insulin, or phlorizin) administration. The mean plasma glucose concentration, significantly higher in the diabetic rats as compared with the normal rats, was restored to normal values after acute insulin and phlorizin treatments (Table II). In groups III and IV, the steady state plasma glucose concentration remained at normoglycemic levels (~9 mM) throughout the study protocol (Fig. 1). The administration of insulin, but not of phlorizin, normalized the plasma insulin levels. Plasma glucagon was not affected by the insulin infusion; however, the acute normalization of the plasma glucose concentration with phlorizin moderately increased the plasma glucagon concentration (Table II). The circulating FFA levels were increased in the diabetic rats compared with control rats. Normalization of glycemia with insulin, but not with phlorizin treatment, restored the plasma FFA concentrations to the normal values (Table II). Thus, this *in vivo* experimental manipulation allows us to examine diabetic rats in the presence of high glucose and low insulin (group II), low glucose and high insulin (group III), and low glucose and low insulin (group IV). To investigate the potential direct effect of phlorizin administration on Glc-6-Pase mRNA and protein, a subgroup of diabetic rats received phlorizin injection and variable glucose infusions designed to maintain the plasma glucose concentration at the diabetic level. In this group an average rate of 30.9 ± 2.5 mg/kg·min was required to maintain the plasma glucose concentration at 20.4 ± 2.2 mM. The latter value was similar to the plasma glucose concentrations at base line (Table I) and during the *in vivo* studies (Table II) in the untreated diabetic rats (group II).

Effect of Short-term Correction of Hyperglycemia on Hepatic Glc-6-Pase mRNA Levels—The relative abundance of Glc-6-Pase mRNA was determined by Northern blot analysis of total RNA obtained from liver samples at the end of the *in vivo* studies. We used a 1.25-kilobase pair cDNA probe that recognized the region encoding the catalytic portion of the Glc-6-Pase. Glc-6-Pase mRNA concentrations in liver of non-diabetic rats were used as control (set at 100%). Multiple densitometric scanning of different Northern blots (example is shown in Fig. 2) shows that the diabetic rats manifested a >5-fold increase in Glc-6-Pase mRNA concentrations as compared with non-diabetic rats. As expected, low dose insulin administration almost completely reversed this marked increase in the Glc-6-Pase messenger RNA. However, since the infusion of insulin concomitantly decreases the plasma glucose and FFA concentrations and increases the plasma insulin concentration, we wished to examine the effect of the normalization of blood glucose concentration *per se* on hepatic Glc-6-Pase gene expression. Thus, we induced a similar gradual decline in the plasma glucose concentration with the glycosuric agent, phlorizin, which normalized the plasma glucose but not the plasma insulin and FFA levels in diabetic rats. Phlorizin treatment causes the same marked reduction in Glc-6-Pase mRNA as seen with insulin. When glucose was infused to prevent the decline in the plasma glucose concentration, phlorizin administration did not alter the Glc-6-Pase mRNA in diabetic liver (not shown). These results suggest that correction of hyperglycemia *per se* is able

to suppress the marked diabetes-induced increase in Glc-6-Pase mRNA.

Effect of Short-term Correction of Hyperglycemia on Hepatic Glc-6-Pase Protein Levels—We next examined whether this marked changes in mRNA levels were reflected in a comparable decrease in the protein concentration (Fig. 3, *A* and *B*). The amount of Glc-6-Pase enzyme in liver microsomes was assessed using polyclonal antibodies against the catalytic portion of Glc-6-Pase. Diabetic rats show larger than a 3-fold increase in Glc-6-Pase protein levels, as compared with the control rats. Normalization of the plasma glucose concentration with either insulin or phlorizin similarly decreases the hepatic Glc-6-Pase protein levels. However, maintenance of hyperglycemia following phlorizin administration completely prevented the marked decrease in the hepatic Glc-6-Pase protein level.

Thus, although the changes in hepatic Glc-6-Pase mRNA observed with diabetes and following normalization of the plasma glucose levels tend to be more dramatic than those observed in the protein levels, there is an excellent correspondence of mRNA and protein levels in all experimental conditions. The finding of such a marked effect of extracellular glucose on the mRNA and protein levels of this key hepatic enzyme should not be interpreted as contradictory to the previously demonstrated regulation by insulin (13, 14). In fact, it is likely that both hyperinsulinemia and the restoration of normoglycemia may independently contribute to the physiologic regulation of Glc-6-Pase gene expression in the diabetic liver. Regarding the mechanism by which changes in the plasma glucose concentration may regulate gene expression, it is noteworthy that several key enzymes in carbohydrate and lipid metabolic pathways are transcriptionally regulated by extracellular glucose (24–27). However, it is likely that such regulation requires changes in the pool of phosphorylated glucose and may better correlate with the concentrations of glucose 6-phosphate or of other intracellular metabolites rather than with the extracellular glucose levels *per se* (25–27). Finally, it should be emphasized that the Glc-6-Pase complex is regulated at multiple levels (10, 28–31), which include, but are not limited to, the gene expression of its catalytic subunit.

In conclusion, we provide evidence for a stimulatory effect of hyperglycemia *per se* on hepatic Glc-6-Pase gene expression in diabetic rats. This may represent a molecular mechanism by which prolonged hyperglycemia favors the persistence of excessive hepatic glucose output in diabetes mellitus.

Acknowledgments—We thank Dr. Rebecca Taub for providing key reagents for Glc-6-Pase Western and Northern blot analysis, Dr. Shimon Efrat for critical reading of the manuscript, and Rong Liu and Gary Sebel for excellent technical assistance.

REFERENCES

1. Bell, P. M., Firth, R. G., and Rizza, R. A. (1986) *J. Clin. Invest.* **78,** 1479–1486
2. DeFronzo, R. A. (1988) *Diabetes* **37,** 667–687
3. Chan, T. M., Young, K. M., Hutson, N. J., Brumley, F. T., and Exton, J. H. (1975) *Am. J. Physiol.* **229,** 1702–1712
4. Rossetti, L., Giaccari, A., Barzilai, N., Howard, K., Sebel, G., and Hu, M. (1993) *J. Clin. Invest.* **92,** 1126–1134
5. Diamond, M. P., Rollins, R. C., Steiner, K. E., Williams, P. E., Lacy, W. W., and Cherrington, A. D. (1988) *Metabolism* **37,** 28–33
6. Jennsen, T., Nurjhan, N., Consoli, A., and Gerich, J. E. (1990) *J. Clin. Invest.* **86,** 489–497
7. Jahoor, F., Peters, E. J., and Wolfe, R. R. (1990) *Am. J. Physiol.* **258,** E288–E296
8. Wolfe, R. B., Jahoor, F., and Shaw, J. H. F. (1986) *J. Parenter. Enteral Nutr.* **11,** 109–111
9. Puhakainen, I., Koivisto, V. A., and Yki-Jarvinen, H. (1991) *Diabetes* **40,** 1319–1327
10. Barzilai, N., and Rossetti, L. (1993) *J. Biol. Chem.* **268,** 25019–25025
11. Lei, K-J., Shelly, L. L., Pan, C-J., Sidbury, J. B., and Chou, J. Y. (1993) *Science* **262,** 580–583
12. Shelly, L. L., Lei, K-J., Pan, C-J., Sakata, S. F., Ruppert, S., Schutz, G., and Chou, J. Y. (1993) *J. Biol. Chem.* **268,** 482–485
13. Lange, A. J., Argaud, D., El-Maghrabi, M. R., Pan, W., Maitra, S. R., and Pilkis, S. J. (1994) *Biochem. Biophys. Res. Commun.* **201,** 302–309
14. Haber, B. A., Chin, S., Chuang, E., Buikhuisen, W., Naji, A., and Taub, R. (1995) *J. Clin Invest.* **95,** 832–841
15. Liu, Z., Barrett, E. J., Dalkin, A. C., Zwart, A. D., and Chou, J. Y. (1994) *Biochem. Biophys. Res. Commun.* **205,** 680–686
16. Foglia, V. G. (1944) *Rev. Soc. Argent. Biol.* **20,** 21–37
17. Bonner-Weir, S., Trent, D. F., and Weir, G. C. (1983) *J. Clin. Invest.* **71,** 1544–1553
18. Rossetti, L., Smith, D., Shulman, G. I., Papachristou, D., and DeFronzo, R. A. (1987) *J. Clin. Invest.* **79,** 1510–1515
19. Rossetti, L., and Giaccari, A. (1990) *J. Clin. Invest.* **85,** 1785–1792
20. Lange, A. J., Arion, W. J., Burchell, A., and Burchell, B. (1986) *J. Biol. Chem.* **261,** 101–107
21. Ashmore, J., Hastings, A., and Nesbeth, F. (1954) *Proc. Natl. Acad. Sci. U. S. A.* **40,** 673–678
22. Hanson, T. L., and Nordlie, R. C. (1970) *Biochim. Biophys. Acta* **198,** 66–75
23. Gardner, L. B., Liu, Z., and Barrett, E. J. (1993) *Diabetes* **42,** 1614–1620
24. Muller, S., Boles, E., May, M., and Zimmermann, F. K. (1995) *J. Bacteriol.* **177,** 4517–4519
25. LeFrancois-Martinez, A. M., Diaz-Guerra, M. J., Vallet, V., Kahn, A., and Antoine, B. (1994) *FASEB J.* **8,** 89–96
26. Prip-Buus, C., Perdereau, D., Foufelle, F., Maury, J., Ferre, P., and Girard, J. (1995) *Eur. J. Biochem.* **230,** 309–315
27. Foufelle, F., Gouhot, B., Pegorier, J. P., Perdereau, D., Girard, J., and Ferre, P. (1992) *J. Biol. Chem.* **267,** 20543–20546
28. Burchell, A. (1990) *FASEB J.* **4,** 2978–2988
29. Minassian, C., Ajzannay, A., Riou, J-P., and Mithiex, G. (1994) *J. Biol. Chem.* **269,** 16585–16588
30. Bode, A. M., Foster, J. D., and Nordlie, R. C. (1992) *J. Biol. Chem.* **267,** 2860–2863
31. Fulceri, R., Gamberucci, A., Scott, H. M., Giunti, R., Burchell, A., and Benedetti, A. (1995) *Biochem. J.* **307,** 391–397