Perturbation of Fuel Homeostasis Caused by Overexpression of the Glucose-6-phosphatase Catalytic Subunit in Liver of Normal Rats*

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The terminal step in hepatic gluconeogenesis is catalyzed by glucose-6-phosphatase, an enzyme activity residing in the endoplasmic reticulum and consisting of a catalytic subunit (glucose-6-phosphatase (G6Pase)) and putative accessory transport proteins. We show that Zucker diabetic fatty rats (fa/ fa), which are known to exhibit impaired suppression of hepatic glucose output, have 2.4-fold more glucose-6-phosphatase activity in liver than lean controls. To define the potential contribution of increased hepatic G6Pase to development of diabetes, we infused recombinant adenoviruses containing the G6Pase cDNA (AdCMV-G6Pase) or the β-galactosidase gene into normal rats. Animals were studied by one of three protocols as follows: protocol 1, fed ad libitum for 7 days; protocol 2, fed ad libitum for 5 days, fasted overnight, and subjected to an oral glucose tolerance test; protocol 3, fed ad libitum for 4 days, fasted for 48 h, subjected to oral glucose tolerance test, and then allowed to refeed overnight. Hepatic glucose-6-phosphatase enzymatic activity was increased by 1.6–3-fold in microsomes isolated from AdCMV-G6Pase-treated animals in all three protocols, and the resultant metabolic profile was similar in each case. AdCMV-G6Pase-treated animals exhibited several of the abnormalities associated with early stage non-insulin-dependent diabetes mellitus, including glucose intolerance, hyperinsulinemia, decreased hepatic glycogen content, and increased peripheral (muscle) triglyceride stores. These animals also exhibited significant decreases in circulating free fatty acids and triglycerides, changes not normally associated with the disease. Our studies show that overexpression of G6Pase in liver is sufficient to perturb whole animal glucose and lipid homeostasis, possibly contributing to the development of metabolic abnormalities associated with diabetes.

The liver has a large capacity for glucose production from gluconeogenesis and glycogenolysis, thereby providing protection against hypoglycemia. Production of free glucose via these pathways requires hydrolysis of glucose 6-phosphate, a step catalyzed by the glucose-6-phosphatase enzyme complex. Genetic and biochemical evidence suggests that the complex consists of the glucose-6-phosphatase catalytic subunit (G6Pase) that resides in the lumen of the endoplasmic reticulum and putative endoplasmic reticulum membrane-localized glucose 6-phosphate, glucose, and inorganic phosphate transporter activities (1–6). Of these, only the G6Pase catalytic subunit and a potential glucose-6-phosphate translocase have been cloned (7–10).

Hepatic expression of G6Pase is increased, whereas levels of the glucose phosphorylating enzyme glucokinase are decreased, when diabetes is induced by partial pancreatectomy or streptozotocin administration (12–16). It has also been shown that metabolic abnormalities associated with NIDDM such as hyperglycemia and hyperlipidemia enhance hepatic expression of G6Pase (14, 16–18). Based on these findings, it has been suggested that perturbation of the balance between glucose phosphorylation and glucose 6-phosphate hydrolysis might contribute to the abnormal regulation of hepatic glucose production that is characteristic of non-insulin-dependent diabetes mellitus (NIDDM).

Although the foregoing observations are important, they do not provide direct evidence for a role of altered hepatic G6Pase expression in regulation of fuel homeostasis, for two main reasons. First, it cannot be assumed that altered expression of the G6Pase catalytic subunit alone will be sufficient to increase flux throughout the entire G6Pase complex. To partially test this point, we have recently used recombinant adenovirus technology to overexpress the G6Pase catalytic subunit in INS-1 insulinoma cells or primary rat hepatocytes, resulting in increased glucose 6-phosphate hydrolysis in both cell preparations (19, 20). In hepatocytes, overexpression of G6Pase resulted in marked lowering of glucose 6-phosphate levels and attendant decreases in glycogen synthesis and glycolytic flux (20). Whereas these data make it clear that flux through the glucose-6-phosphatase enzyme complex can be altered by manipulation of the expression level of the G6Pase catalytic subunit in vivo, it remains to be determined whether the results achieved in cultured cells can be replicated in vivo. The second major concern is that maneuvers that have been used to modulate G6Pase expression in vivo such as partial pancreatectomy, glucose infusion, or intralipid infusion can be expected to influence expression of a wide spectrum of genes encoding metabolic enzymes or other proteins (21), making it difficult to isolate the specific impact of G6Pase overexpression.

The purpose of this study was to address these issues and to

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¶ The abbreviations used are: G6Pase, glucose-6-phosphatase; AdCMV-G6Pase, recombinant adenovirus containing the cDNA encoding the catalytic subunit of glucose-6-phosphatase; ZDF, Zucker diabetic fatty; NIDDM, non-insulin-dependent diabetes mellitus; β-Gal, β-galactosidase; OGTT, oral glucose tolerance test; FFA, free fatty acids; TG, triglycerides.
evaluate the potential role of hepatic overexpression of the G6Pase catalytic subunit in the development of diabetes. In an initial set of experiments, we provide the first demonstration that hepatic G6Pase enzymatic activity is significantly elevated in a genetic model of obesity and diabetes, the Zucker diabetic fatty (ZDF) rat (fa/), relative to lean controls. To assess the potential contribution of up-regulated G6Pase expression to development of diabetes and obesity, we have used a recombinant adenovirus containing the G6Pase cDNA to overexpress the enzyme in liver of normal rats. Metabolic analyses performed on these animals under a variety of conditions demonstrate that they develop glucose intolerance and hyperinsulinemia, a marked decrease in hepatic glycogen levels and circulating hypolipidemia associated with tissue fat overstorage. These results demonstrate that up-regulated expression of the G6Pase catalytic subunit in liver alters a wide spectrum of metabolic parameters.

MATERIALS AND METHODS

Preparation of Recombinant Adenoviruses—A recombinant adenovirus containing the cDNA encoding the catalytic subunit of glucose-6-phosphatase (G6Pase) was prepared as described previously (19). A virus containing the Escherichia coli β-galactosidase gene (AdCMV-β-Gal; see Ref. 22) was used for control experiments.

Animal Experiments—Male Wistar rats (Charles River), Zucker diabetic fatty (ZDF) rats (fa/), and ZDF lean controls were used in these studies. ZDF obese and lean animals were studied at 9–10 weeks of age. Animals were housed individually and given free access to laboratory chow and water, except where indicated below, and food intake and body weight were recorded on a daily basis. In an initial set of experiments, liver samples were collected from all three rat strains for measurement of hepatic levels of G6Pase protein and enzymatic activity, using methods described below. In a subsequent series of experiments, AdCMV-G6Pase or AdCMV-β-Gal viruses were infused into normal Wistar rats, weighing 250–300 g. To reduce immunologic responses to CMV-infected tissue, liver samples were collected from all three rat strains for measurement of hepatic glucose-6-phosphatase activity of rat, using previously described methods (19). Metabolite and Hormone Assays—Blood samples were collected in tubes or syringes pre-rinsed with 0.1 M EDTA and centrifuged at 15,000 × g for 5 min. Plasma glucose concentrations were measured with a HemoCue glucose analyzer. Insulin radioimmunooassay was carried out with 25 μl of plasma, using the Linco rat insulin kit and rat insulin standards. Plasma triglyceride levels were determined with a Sigma triglyceride-GPO kit, and free fatty acids (FFA) were measured in fresh plasma with the Half-Micro test kit from Boehringer Mannheim. Liver glycogen levels were measured as described (26). To measure muscle triglyceride levels, 1 g of frozen gastrocnemius muscle was homogenized in 2 ml of buffer consisting of 18 mm Tris, pH 7.5, 300 mm mannitol, 50 μM ECTA, and 0.1 mm phenylmethylsulfonyl fluoride. After removal of a small aliquot of the homogenate for determination of total protein, 100 μl of the homogenate was mixed with 4 ml of chloroform/methanol mixture (2:1, v/v) for extraction of total lipids. After insoluble materials were filtered from the chloroform/methanol/lipid solution, 0.8 ml of water was added to separate the organic phase containing tissue lipid from the aqueous phase. A portion of this lipase-containing solution was dried under nitrogen gas, resuspended in 60 μl of n-butanol alcohol, and then mixed with 40 μl of Triton/methanol (2:1, v/v) to completely dissolve the lipid suspension.

RESULTS

Increased Expression of the G6Pase Catalytic Subunit in Liver of Zucker Diabetic Fatty (ZDF) Rats—Defective suppression of hepatic glucose output is a hallmark of human NIDDM. Like human NIDDM subjects, ZDF rats exhibit profound hyperglycemia, hyperinsulinemia, and hyperlipidemia, and impaired control of hepatic glucose production (27–31). The potential role of hepatic glucose-6-phosphatase expression in development of diabetes in ZDF rats has never been investigated, although increased expression of the mRNA encoding the G6Pase catalytic subunit in the islets of Langerhans of these animals has been reported (32). We therefore evaluated expression of the G6Pase catalytic subunit by immunoblot analysis and measurement of enzymatic activity in liver microsomes isolated from obese ZDF diabetic males, lean non-diabetic ZDF males of the same age (9 weeks), and weight-matched Wistar male rats. We observed a 2.4-fold increase in hepatic glucose-6-phosphatase enzymatic activity in ZDF diabetic animals compared with either control group, and this increase was accompanied by a similar increase in the expression of immunodetectable G6Pase catalytic subunit (Fig. 1). Glucose-6-phosphatase enzymatic activity was not detectable in skeletal muscle of ZDF diabetic males, ZDF lean controls, or Wistar rats, providing evidence that expression of G6Pase was not activated in other important homeostatic tissues in diabetic animals. These data suggest that up-regulation of hepatic G6Pase expression could contribute to development of the diabetic phenotype in ZDF rats and led us to investigate the specific metabolic impact of overexpression of the enzyme in liver of normal rats.

Effects of G6Pase Overexpression in Wistar Rats Fed Ad Libitum—Normal Wistar rats were infused with AdCMV-G6Pase or AdCMV-β-Gal and allowed access to food and water ad libitum. Seven days after viral infusion, blood samples were collected for measurement of circulating metabolites and hormone levels prior to sacrificing the animals for tissue collection. At this time point, circulating levels of aspartate aminotransferase, alkaline phosphatase, and creatinine were all within the normal range in both the AdCMV-G6Pase and AdCMV-β-Gal-treated animals, indicating no substantial liver or kidney dysfunction. Ad libitum—
damage in response to viral infusion (data not shown). Liver microsome samples were used for analysis of levels of the G6Pase catalytic subunit by immunoblotting and measurement of enzymatic activity. A representative immunoblot showing the clear increase in hepatic expression of the G6Pase catalytic subunit protein in AdCMV-G6Pase compared with AdCMV-β-Gal-treated animals is shown in Fig. 2. This increase in protein corresponded to a 2.9-fold increase in G6Pase enzymatic activity measured in freshly isolated microsomes (Table I).

Previous studies in mouse have demonstrated that systemic administration of recombinant adenovirus results in gene transfer primarily to liver (22). To ensure that this was also the case in the current studies performed in rats, we measured glucose-6-phosphatase enzymatic activity in skeletal muscle, abdominal fat, and pancreas samples from the same AdCMV-G6Pase- and AdCMV-β-Gal-treated animals used for the hepatic measurements described above. We found no detectable activity in muscle or pancreas of either AdCMV-G6Pase- or AdCMV-β-Gal-treated animals. G6Pase activity was found at very low levels in abdominal fat but with no significant differences between the two experimental groups (0.107 ± 0.016 units/mg protein compared with 0.096 ± 0.015 units/mg in fat tissues of AdCMV-G6Pase- and AdCMV-β-Gal-infused animals, respectively, n = 6 for both groups). Based on these data, we conclude that the G6Pase gene was not targeted to these important extrahepatic homeostatic tissues by the adenovirus vector.

Table I provides a summary of a group of metabolic parameters assayed in the AdCMV-G6Pase and AdCMV-β-Gal animals fed ad libitum. Circulating glucose levels were raised by 14% and insulin levels by 47% in AdCMV-G6Pase-treated animals relative to controls. Despite the mild hyperglycemia and hyperinsulinemia, liver glycogen levels were reduced by 57% in AdCMV-G6Pase-treated animals. In contrast, lipid metabolism was regulated in a manner consistent with the elevated insulin levels. Circulating FFA and triglycerides (TG) were decreased by 37 and 29%, respectively, in AdCMV-G6Pase-treated animals (Table I), whereas triglyceride levels in isolated muscle samples were increased by 59% (Fig. 3).

Effects of G6Pase Overexpression in Fasted Rats Subjected to an Oral Glucose Tolerance Test—The increases in circulating glucose and insulin levels in AdCMV-G6Pase-treated rats fed ad libitum was suggestive of a state of glucose intolerance. To evaluate this possibility in more detail, animals were infused with AdCMV-G6Pase or AdCMV-β-Gal viruses, allowed free access to food for the ensuing 5 days, and were then fasted overnight. After the fast, both groups were subjected to an oral glucose tolerance test. At the end of this test, animals were sacrificed for tissue collection. AdCMV-G6Pase-infused animals had 2.3-fold more hepatic G6Pase activity than AdCMV-β-Gal-infused controls (Table I). As shown in Fig. 4, circulating glucose levels were not different in AdCMV-G6Pase-treated compared with AdCMV-β-Gal-infused animals after the overnight fast and prior to initiation of the OGTT (0 time point).

**FIG. 2.** Immunoblot analysis of G6Pase catalytic subunit expression in liver of AdCMV-G6Pase-treated and control Wistar rats. Rats were infused with AdCMV-G6Pase (lanes 1 and 2) or AdCMV-β-Gal (lanes 3 and 4) and allowed access to food ad libitum for 7 days prior to tissue collection (“Materials and Methods,” see protocol 1). Each lane contains 40 μg of protein from liver microsome homogenates, blotted with an antibody against the catalytic subunit of G6Pase (25). Data are representative of results in all three protocols (see Tables I—III for enzymatic activity measurements for all animals in the study).

**FIG. 3.** Muscle triglyceride levels in AdCMV-G6Pase- and AdCMV-β-Gal-infused animals. Muscle triglyceride levels were measured as described under “Materials and Methods.” Data represent the mean ± S.E. for six animals per condition (see Table I for other metabolic parameters for this same set of animals). The * indicates that muscle TG levels are elevated in AdCMV-β-Gal-infused animals relative to controls, at a level of significance of p < 0.02.

**TABLE I**

Metabolic parameters of rats fed ad libitum after infusion of AdCMV-G6Pase or AdCMV-β-Gal adenoviruses

Blood and liver samples were collected from rats fed ad libitum for 7 days after virus infusion, as described under “Materials and Methods,” protocol 1. Results represent the mean ± S.E. for assays performed in duplicate on samples taken from the number of animals shown in parentheses in the left hand column.

| Viral treatment       | G6Pase activity units/mg protein | Plasma glucose mg/dl | Plasma insulin ng/ml | Plasma FFA mmol/l | Plasma triglycerides mg/dl | Liver glycogen μg/mg protein |
|-----------------------|---------------------------------|----------------------|----------------------|-------------------|---------------------------|-------------------------------|
| AdCMV-G6Pase (n = 6)  | 1.47 ± 0.6                      | 184 ± 12             | 2.2 ± 0.9            | 0.72 ± 0.3        | 124 ± 29                  | 240 ± 55                       |
| AdCMV-β-Gal (n = 6)   | 0.51 ± 0.1                      | 161 ± 21             | 1.5 ± 0.7            | 1.14 ± 0.5        | 174 ± 62                  | 563 ± 167                      |
| Statistical significance | p < 0.01                       | p < 0.05             | p < 0.03             | p < 0.05          | p < 0.04                  | p < 0.005                      |
Metabolic abnormalities were similar in all three experimental

defined. The hepatic expression of the catalytic subunit of G6Pase, or a combination of both changes could result in reduced capacity for glucose utilization and storage in liver. In this study, we provide the first evidence that both hepatic expression of the catalytic subunit of G6Pase and glucose-6-phosphatase enzymatic activity are significantly increased in a genetic model of obesity and NIDDM, the ZDF rat (fa/fa). However, this finding does not establish a causal link between G6Pase expression and the diabetic phenotype. Furthermore, linkage between mutations in the gene encoding the G6Pase catalytic subunit and human NIDDM have not yet been established. While the search for such genetic mutations continues, further insight into the potential role of G6Pase in the development of NIDDM may be gained by studies in which recombinant adenovirus, a technique that provides near exclusive delivery of transgenes to liver (see Ref. 22 and this study), to investigate this issue.

Our experiments establish that hepatic overexpression of the catalytic subunit of G6Pase, even to a relatively modest extent (1.6–3-fold increases in enzyme activity), is sufficient to cause significant perturbation of fuel homeostasis. The pattern of metabolic abnormalities was similar in all three experimental
TABLE III

| Viral treatment | G6Pase activity | Plasma glucose | Plasma insulin | Plasma FFA | Plasma triglycerides | Liver glycogen |
|-----------------|-----------------|----------------|---------------|-------------|---------------------|---------------|
|                 | units/mg protein| mg/dl           | ng/ml         | mU          | mg/dl               | µg/mg protein  |
| AdCMV-G6Pase (n = 11) | 0.94 ± 0.2 | 183 ± 19 | 2.1 ± 1.1 | 0.58 ± 0.2 | 59.2 ± 17.1 | 948 ± 289 |
| AdCMV-β-Gal (n = 11) | 0.56 ± 0.1 | 148 ± 17 | 1.1 ± 1.0 | 1.35 ± 0.4 | 87.7 ± 20.1 | 2090 ± 208 |
| Statistical significance | p < 0.002 | p < 0.01 | p < 0.03 | p < 0.05 | p < 0.05 | p < 0.001 |

Fig. 5. Oral glucose tolerance test in AdCMV-G6Pase- and AdCMV-β-Gal-infused animals after a 48-h fast. Animals were treated as described under “Materials and Methods,” protocol 3. Data for AdCMV-G6Pase-treated animals (black line, filled diamonds) and AdCMV-β-Gal-treated controls (gray line, filled squares) are shown and represent the mean ± S.E. for 11 animals per group. Glucose levels were significantly higher in the AdCMV-G6Pase-treated rats at levels of significance indicated by the following symbols: *, p < 0.01; ** p < 0.001.

Despite the mild hyperglycemia and hyperinsulinemia, glycogen levels were decreased by 53–58% in the three groups of AdCMV-G6Pase-treated animals relative to controls (Tables I–III). Interestingly, liver glycogen levels are also reduced in humans with NIDDM (27). Our findings in intact rats are consistent with our earlier study in isolated hepatocytes, in which a 50% decrease in glycogen accumulation was noted in AdCMV-G6Pase-treated cells compared with controls (20).

Some of the alterations in fuel metabolism in response to G6Pase overexpression reported in this study are observed in NIDDM, including glucose intolerance, hyperinsulinemia, decreased hepatic glycogen content, and increased peripheral lipid storage. Other features, most notably hyperlipidemia, are not commonly found in NIDDM. These findings raise the question of whether up-regulation of G6Pase can make a significant contribution to the development of the disease. We propose two models that represent opposite extremes for explaining how this could occur. The first assumes that increased expression of G6Pase is the primary genetic lesion associated with the disease. In this scenario, a gradual rise of G6Pase overexpression will result in development of glucose intolerance and hyperinsulinemia, resulting in turn in increased lipid storage in peripheral tissues, as demonstrated in this study. Peripheral overstorage of fat is strongly correlated with development of insulin resistance (reviewed in Ref. 34). If a causal role for lipid overstorage is correct, lipid-induced insulin resistance will cause the hormone to lose its capacity to inhibit peripheral lipolysis, resulting in a gradual rise in circulating FFA and TG. It might also be anticipated that peripheral glucose disposal would be decreased as insulin resistance develops. This model predicts further that overexpression of G6Pase in the liver for...
periods of weeks to months (as opposed to the 7-day time course of the current study) might result in gradual development of hyperlipidemia and hyperglycemia, complementing hyperinsulinemia, and reduced hepatic glycogen levels to produce the entire syndrome of NIDDM. This hypothesis can be tested directly in future experiments.

The second model assumes that there are no genetic abnormalities in the G6Pase gene or its regulatory factors in individuals with NIDDM. In this scenario, overexpression of G6Pase in liver could instead be secondary to metabolic variations that occur in response to genetic lesions in insulin signaling or β-cell function. For example, expression of the G6Pase catalytic subunit has been shown to be increased by elevations in FFA or glucose in both in vivo and in vitro rodent experiments (13–18). Thus, G6Pase overexpression may be induced by rising FFA levels and glucose intolerance at an intermediate stage of NIDDM development. Induction of the gene could then contribute to failure of normal suppression of hepatic glucose production in the fed state, resulting in exacerbation of glucose intolerance and reduced glycogen storage. If up-regulation of G6Pase occurs at a fairly late stage of development of NIDDM, insulin resistance and β-cell failure may already be present, thus negating the role of hyperinsulinemia.

It should be noted that intermediate models, in which G6Pase interacts in different ways with other candidate genes that predispose to insulin resistance or β-cell failure, can also be contemplated. For example, it was initially surprising that mice homozygous for knockout of insulin receptor substrate 1 were found to be modestly insulin-resistant but with no abject hyperglycemia (35, 36). It has since become apparent that individuals with NIDDM. In this scenario, overexpression of G6Pase could instead be secondary to metabolic variations that occur in response to genetic lesions in insulin signal-

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REFERENCES
1. Nordlie, R. C. (1974) Curr. Top. Cell. Regul. 8, 33–117
2. Arion, W. J., Lange, A. J., Walls, E. H., and Ballas, I. M. (1980) J. Biol. Chem. 255, 10396–10406
3. Lange, A. J., Arion, W. J., and Beaudet, A. L. (1980) J. Biol. Chem. 255, 8381–8384
4. Lei, K. J., Shelly, L. L., Lin, B., Sidbury, J. B., Chen, Y. T., Nordlie, R. C., Chou, J. Y. (1995) J. Clin. Invest. 95, 234–240
5. Lei, K.-J., Chen, H., Pan, C.-J., Ward, J. M., Mosinger, B., Lee, E. J., Westphal, H., Mansfield, B. C., and Chou, J.-Y. (1996) Nat. Genet. 13, 203–209
6. Annabi, B., Hiraiwa, H., Mansfield, B. C., Lei, K. J., Ubegu, T., Polymenopoulos, M. H., Moses, S. W., Parvari, R., Hershkovitz, E., Modell, H., Fryman, M., and Chou, J. Y. (1998) Am. J. Hum. Genet. 62, 400–405
7. Shelly, L. L., Lei, K. J., Pan, C.-J., Sakata, S. F., Ruppert, S., Schutz, G., and Chou, J. Y. (1996) J. Biol. Chem. 271, 21482–21485
8. Lei, K. J., Shelly, L. L., Pan, C. J., Sidbury, J. B., and Chou, J. Y. (1993) Science 262, 580–583
9. Lange, A. J., Argaud, D., El Maghrabi, M. R., Pan, W., Maitra, S. R., and Pilikis, S. J. (1994) Biochem. Biophys. Res. Commun. 201, 302–309
10. Gerin, I., Veiga-da-Cunha, M., Achouri, Y., Collet, J.-F., and Van Schaftingen, E. (1997) FEBS Lett. 419, 235–238
11. Burchell, A., and Cain, D. I. (1980) Diabetes 29, 852–856
12. Barzilai, N., and Rossetti, L. (1993) J. Biol. Chem. 268, 25019–25025
13. Liu, Z., Barrett, E. J., Dalkin, A. C., Zwart, A. D., Chou, J. Y. (1994) Biochem. Biophys. Res. Commun. 202, 680–686
14. Massillon, D., Barzilai, N., Chen, W., Ho, M., and Rossetti, L. (1996) J. Biol. Chem. 271, 9871–9874
15. Milieux, G., Vidal, H., Zitoun, C., Bruni, N., Daniele, N., and Minassian, C. (1996) Diabetes 45, 891–896
16. Argaud, D., Zhang, Q., Pan, W., Maitra, S., Pilikis, S. J., and Lange, A. J. (1996) Diabetes 45, 1563–1571
17. Massillon, D., Barzilai, N., Hawkins, M., Pruzswertherheimer, D., and Rossetti, L. (1997) Diabetes 46, 153–157
18. Argaud, D., Kirby, T. L., Newgard, C. B., and Lange, A. J. (1997) J. Biol. Chem. 272, 12854–12861
19. Trinh, K., Minassian, C., Lange, A. J., O’Doherty, R. M., and Newgard, C. B. (1997) J. Biol. Chem. 272, 24837–24842
20. Seoane, J., Trinh, K., O’Doherty, R. M., Gomez-Fox, A. M., Lange, A. J., Newgard, C. B., and Guinovart, J. J. (1997) J. Biol. Chem. 272, 26972–26977
21. Pilikis, S. J., and Granner, D. K. (1992) Annu. Rev. Physiol. 54, 885–909
22. Herz, J., and Gerard, R. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2812–2816
23. Chen, G., Koyama, K., Yuan, X., Lee, Y., Zhou, Y.-T., O’Doherty, R., Newgard, C. B., and Unger, R. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14795–14799
24. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
25. Havel, R. A., Chiu, S., Chuang, E., Buxkhaus, W., Naji, A., and Taub, R. (1995) J. Clin. Invest. 95, 832–841
26. Newgard, C. B., Hirsch, L. J., Foster, D. W., and McGarry, J. D. (1983) J. Biol. Chem. 258, 8046–8052
27. Magnusson, I., Rothman, D. L., Katz, L. D., Shulman, R. G., and Shulman, G. I. (1992) J. Clin. Invest. 90, 1233–1237
28. Triscari, J., Stern, J. S., Johnson, P. R., and Sullivan, A. C. (1979) Metabolism 28, 163–189
29. Bowen, L., Stein, P. P., Stevenson, R., and Shulman, G. I. (1991) Metabolism 40, 1025–1030
30. Sanchez-Gutierrez, J. C., Sanchez-Aria, J. A., Lechuga, C. G., Valle, J. C., Samper, B., and Feliu, J. E. (1994) Endocrinology 134, 1868–1873
31. Clark, J. B., Palmer, C. J., and Shaw, W. N. (1983) Proc. Soc. Exp. Biol. Med. 173, 68–75
32. Tokuyama, Y., Sturis, J., DePaoli, A. M., Takeda, J., Stoffel, M., Tang, J., Sun, X., Polonsky, K. S., and Bell, G. I. (1995) Diabetes 44, 1447–1457
33. Chang, M. L., and Johnson, M. A. (1976) J. Nutr. 106, 136–141
34. McGarry, J. D. (1994) J. Cell. Biochem. 55, 29–38
35. TAMEMOTO, H., KADOWAKI, T., Tobe, K., YAGI, T., SAKURA, H., HAYAKAWA, T., TERAUCHI, Y., UEKI, K., KABURAGI, Y., SATOH, S., SEKIHARA, H., YOSHIKOA, S., HORIKOSHI, H., FURUTA, Y., IKAWA, Y., KASUGA, M., YAZAKI, Y., and AIZAWA, S. (1994) Nature 372, 182–186
36. ARAKI, E., LIPES, M. A., PATTI, M. E., BRUNING, J. C., HAAG, B., JOHNSON, R. S., and KAHN, C. R. (1994) Nature 372, 186–190
37. BRUNING, J. C., WINNAY, J., BOONER-WIESE, S., TAYLOR, L. J., AICEL, D., and KAHN, C. R. (1997) Cell 88, 561–572
38. TERAUCHI, Y., IWAMOTO, K., TAMEMOTO, H., KOMEDA, K., ISHI, C., KANAZAWA, Y., ASANUMA, N., AIZAWA, T., AKANAUMA, Y., YASUDA, K., KODAMA, T., TOBE, K., YAZAKI, Y., and KADODAWA, T. (1997) J. Clin. Invest. 99, 861–866