

Development and Regulation of Glucose-6-Phosphatase Gene Expression in Rat Liver, Intestine, and Kidney

In Vivo and In Vitro Studies in Cultured Fetal Hepatocytes

Florence Chatelain, Jean-Paul Pégrier, Carole Minassian, Nathalie Bruni, Sandrine Tarpin, Jean Girard, and Gilles Mithieux

The mRNA and the activity of glucose-6-phosphatase (Glc-6-Pase) were present in the liver, kidney, and small intestine of 15-day-old suckling rats, but were absent from the stomach, colon, lung, white and brown adipose tissues, muscle, heart, brain, and spleen. The mRNA encoding Glc-6-Pase was present in the liver of 21-day-old fetal rats and increased markedly immediately after birth. From 5 days after birth to the end of the suckling period, it returned to 50% of the level found in the liver of 48-h starved adult rats. When rats were weaned at 21 days onto a high-carbohydrate, low-fat (HCLF) diet, the concentration of liver Glc-6-Pase mRNA was markedly increased. In the fetal rat jejunum, the activity and mRNA of Glc-6-Pase were very low. It increased during the 5 days after birth and then declined to reach very low levels. Neither mRNA nor activity of Glc-6-Pase was present in the fetal kidney. They appeared and increased slowly during the suckling period to reach maximal levels 15 days after birth and then remained constant. Weaning onto the HCLF diet did not change the Glc-6-Pase gene expression, neither in the jejunum nor in the kidney. The regulation of Glc-6-Pase gene expression by hormones and nutrients was studied in cultured hepatocytes from 20-day-old rat fetuses. Bt_2cAMP stimulated the Glc-6-Pase gene expression in a dose-dependent manner. This probably resulted from an increased gene transcription since the half-life of the transcript was not affected by dibutyl cAMP (Bt_2cAMP). The Bt_2cAMP -induced Glc-6-Pase mRNA accumulation was antagonized by insulin in a dose-dependent manner. Long-chain fatty acids (LCFAs), but not medium-chain fatty acids, induced the accumulation of Glc-6-Pase mRNA and the stabilization of the transcript. The peroxisome proliferator, clofibrate, induced a threefold increase in Glc-6-Pase mRNA concentration. Both stim-

ulation of Glc-6-Pase mRNA by LCFAs and clofibrate were inhibited by insulin. Increasing concentrations of glucose (from 0 to 20 mmol/l) did not affect the Bt_2cAMP -induced Glc-6-Pase gene expression. By contrast, high glucose concentration (25 mmol/l) markedly induced the Glc-6-Pase gene expression in fed adult rat hepatocytes. The difference in the response to glucose between fetal and adult rat hepatocytes is discussed. We conclude that the rapid increase in hepatic Glc-6-Pase mRNA levels that accompanies the fetal-to-neonatal transition in the rat is triggered by the reciprocal change in circulating insulin and LCFA concentrations, coupled to the rise in liver cAMP concentration. *Diabetes* 47:882-889, 1998

Glucose-6-phosphatase (Glc-6-Pase) is a key enzyme involved in systemic glucose homeostasis. It catalyzes the last biochemical reaction of both gluconeogenesis and glycogenolysis (i.e., the dephosphorylation of glucose-6-phosphate [Glc-6-P] to glucose). It is expressed in substantial amounts only in the liver and the kidney cortex in adult mammals (1,2), conferring to these tissues the capacity to export glucose into the bloodstream. In addition, several other tissues have been reported to possess a low Glc-6-Pase activity (1). However, we failed to detect any Glc-6-Pase mRNA in several of these tissues, either by using Northern blot or by reverse transcriptase-polymerase chain reaction (RT-PCR) approaches (3). This suggested that Glc-6-Pase was less widely expressed than previously assumed.

It is well known that gluconeogenesis is markedly increased in the liver during fasting and diabetes (4) but that also it is increased in the kidney cortex during metabolic acidosis, diabetes, and prolonged fasting (5). The suckling period constitutes another situation associated with an active gluconeogenesis in the liver and the kidney (6). A significant amount of glucose is synthesized from lactate in the intestinal mucosa of suckling rats (7) caused by the presence of all gluconeogenic enzymes (6). Recently, it was reported that the small intestine of adult rodents was able to produce glucose from fructose (8) and from gluconeogenic substrates (9).

We have previously emphasized the regulatory role that Glc-6-Pase gene induction might play in triggering gluconeogenesis in both the liver and kidney during fasting or diabetes (2,3,10). The first aim of the present work was to study the

From the Institut National de la Santé et de la Recherche Médicale (U449) (C.M., N.B., S.T., G.M.), Faculté de Médecine R.T.H. Laënnec, Lyon; and Endocrinologie, Métabolisme et Développement (F.C., J.-P.P., J.G.), CNRS (UPR 1524), Meudon-Bellevue, France.

Address correspondence and reprint requests to J.-P. Pégrier, CNRS (UPR 1524), 9, rue Jules Hetzel, 92190 Meudon-Bellevue, France. E-mail: pegrier@infobiogen.fr.

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BSA, bovine serum albumin; Bt_2cAMP , dibutyl cAMP; DRB, 5,6-dichlorobenzimidazole riboside; Glc-6-P, glucose-6-phosphate; Glc-6-Pase, glucose-6-phosphatase; HCLF, high-carbohydrate, low-fat; IRS, insulin response sequence; LCFA, long-chain fatty acid; MEM, minimum essential medium; PPAR, peroxisome proliferator-activated receptor; RT-PCR, reverse transcriptase-polymerase chain reaction.

development of mRNA concentrations and Glc-6-Pase activity in the three potential sites of glucose production: liver, kidney, and small intestine.

Moreover, all the physiological (fasting, suckling period) or pathological (diabetes) situations associated with an enhanced gluconeogenesis and Glc-6-Pase activity and gene expression were characterized by high plasma glucagon and low plasma insulin concentrations and a high level of circulating fatty acids (6). The second aim of the present work was to investigate the role of hormonal (glucagon via cAMP, insulin) and nutritional (fatty acids) environments in the postnatal induction of liver Glc-6-Pase using primary culture of fetal hepatocytes.

RESEARCH DESIGN AND METHODS

Animals. Female Wistar rats bred in our laboratory were housed at 24°C in individual plastic cages with light from 0700 to 1900. They had free access to water. The stage of pregnancy was determined as described previously (11). Fetuses (20 and 21 days) were delivered by Cesarean section after cervical dislocation of the mother. Newborn pups were allowed to suckle from 2 h after birth onward. Rats were weaned at 21 days onto a semisynthetic high-carbohydrate, low-fat (HCLF) diet: 72% carbohydrate, 1% fat, 27% protein (in terms of energy) (12).

The studies were performed in 21-day-old fetal rats, in 1-, 5-, 10-, 15-, and 21-day-old suckling rats, and in 22- and 30-day-old rats weaned onto a HCLF diet. The tissue-specific expression of Glc-6-Pase and PEPCK genes was performed in 15-day-old suckling rats. Intestines were inverted, washed at 37°C in a phosphate-buffered saline (PBS: NaCl 0.13 mol/l; KCl 2.7 mmol/l; Na₂HPO₄ 22 mmol/l; NaH₂PO₄ 1.45 mmol/l; pH 7.6). All the tissues were frozen in liquid nitrogen and stored at -80°C for subsequent extraction of RNA.

Isolation and primary culture of fetal and fed adult rat hepatocytes. Fetal hepatocytes were isolated as described previously (13) and plated in 75-cm² Petri dishes (3–5 × 10⁶ cells/dish) in a minimum essential medium (MEM) containing penicillin (10 UI/ml), streptomycin (100 µg/ml), and kanamycin (50 µg/ml). During cell attachment (4 h), a substitute of fetal calf serum (Ultraser G, 2%; IBF, Villeeneuve la Garenne, France) was present. After removal of most of the nonadherent hematopoietic cells, the medium was replaced by Ultraser G and arginine-free MEM containing different effectors.

Adult rat hepatocytes were isolated as described previously (14) and cultured in the same conditions as fetal hepatocytes. Duplicate dishes were used for all experiments. The cultures were maintained at 37°C in an incubator equilibrated with O₂/CO₂ (95/5%).

Effects of dibutyryl cAMP and dexamethasone on Glc-6-Pase mRNA concentrations. The effect of dibutyryl cAMP (Bt₂cAMP; 10⁻⁴ mol/l) was tested over 24 and 48 h of culture after cell attachment. The half-maximum effect of Bt₂cAMP was assessed by using a concentration range of 10⁻⁸ to 10⁻³ mol/l. The effect of dexamethasone (10⁻⁷ mol/l) was tested over 48 h of culture either alone or in association with half-maximal concentration of Bt₂cAMP (10⁻⁵ mol/l).

Effects of glucose on Glc-6-Pase mRNA accumulation. The effect of glucose (from 0 to 20 mmol/l) was tested in fetal hepatocytes cultured for 48 h either in the absence or in the presence of Bt₂cAMP (10⁻⁴ mol/l). The effect of glucose (25 mmol/l) was also assayed in hepatocytes from fed adult rats cultured for 24 h. The possible effect of hyperosmolarity was tested by culturing hepatocytes in the presence of L-glucose at the same concentration.

Effects of fatty acids and clofibrate on Glc-6-Pase mRNA levels. The effects of medium-chain fatty acids (octanoate [C8:0]) and long-chain fatty acids (LCFAs) (*cis*-9-oleate [C18:1], *cis*-9,12-linoleate [C18:2]) were tested at a concentration of 0.5 mmol/l, during 48 h of culture in a glucose-free medium containing a mixture of lactate:pyruvate (10:1 mmol/l) as energy substrates. Fatty acids were added immediately after cell attachment. To avoid the detergent effect of free fatty acids, they were bound to bovine serum albumin (BSA, final concentration 0.2%). Finally, the effect of clofibrate, a peroxisomal proliferator, was tested at a concentration of 0.5 mmol/l after 48 h of culture. In some experiments, the effect of insulin (10⁻⁷ mol/l) was studied in fetal hepatocytes cultured in the presence of either linoleate (0.5 mmol/l) or clofibrate (0.5 mmol/l).

Effects of insulin on Bt₂cAMP-induced Glc-6-Pase mRNA concentration. Hepatocytes were cultured for 48 h in the presence of Bt₂cAMP (10⁻⁴ mol/l) and different concentrations (10⁻¹¹ to 10⁻⁷ mol/l) of insulin. Bt₂cAMP and insulin were added immediately after cell attachment in a glucose-free medium supplemented with a lactate:pyruvate ratio.

Extraction and Northern blot analysis of total RNA. Total RNA from frozen tissues and from hepatocytes of two Petri dishes were extracted with guanidium thiocyanate followed by a purification through a CsCl cushion gradient accord-

ing to Chirgwin et al. (15). RNA was quantified by ultraviolet absorbance at 260 nm (260:280 ratio >1.8), and 1 µg was submitted to electrophoresis in 1% agarose gel to check the quality of the RNA preparation. Northern blot analysis of total RNA (20 µg) was performed after 1% agarose gel electrophoresis in 2.2 mol/l formaldehyde as previously described (3,13). Hybridization of the blots with an excess of [³²P]ATP-labeled synthetic oligonucleotide specific for the 18S rRNA subunit (16) allowed us to correct for possible variations in the amount of RNA transferred onto the membranes. The hybridization probe was the 2.6-kb *Pst*I fragment from phosphoenol carboxy kinase (PCK) 10 and the 1.1-kb *Pst*I fragment for Glc-6-Pase as described previously (3,13). Probes were radiolabeled using the multiprime DNA labeling system (Amersham). Quantifications were performed by scanning densitometry of the autoradiographs.

Measurements of Glc-6-Pase mRNA stability. Fetal hepatocytes were first cultured for 48 h in the presence of Bt₂cAMP (10⁻⁴ mol/l) or linoleate (0.5 mmol/l). Then the medium was replaced by a fresh medium containing 5,6-dichlorobenzimidazole riboside (DRB; 25 µg/ml), a specific inhibitor of RNA polymerase II (17), and the culture was continued for 0.5–24 h in the absence or in the presence of either Bt₂cAMP (10⁻⁴ mol/l) or linoleate (0.5 mmol/l). Cells were scraped off and lysed every 30 min, and total RNA was extracted and treated as described above. **Measurements of Glc-6-Pase activity.** Frozen tissues were powdered in liquid nitrogen and homogenized in 10 mmol/l HEPES, 0.25 mol/l sucrose, pH 7.4 (9 vol/g tissue) by 10 × 1 s ultrasonic pulses. The homogenates were treated by 0.5% (mass/vol) 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS) for 20 min at 4°C to free Glc-6-Pase from membranous constraints (3,10,18). Treated homogenates were diluted 10 times before Glc-6-Pase activity was assayed at maximal velocity (20 mmol/l Glc-6-P) at 30°C, on complexometry of inorganic phosphate (P_i) produced from Glc-6-P. The phosphohydrolyzing activity toward β-glycerophosphate (20 mmol/l) was determined and subtracted from the total Glc-6-Pase activity in all cases to clear the specific Glc-6-Pase activity from the contribution of nonspecific phosphatases. The results are expressed as micromoles of substrate hydrolyzed per minute per gram of wet tissue.

Analytical methods. Proteins were determined by the method of Lowry et al. (19) with BSA as standard.

Statistical analysis. Results are expressed as means ± SE, and statistical analysis was performed using the rank-order test (20).

RESULTS

Tissue-specific expression of Glc-6-Pase in 15-day-old suckling rats. In 15-day-old suckling rats, Glc-6-Pase mRNA and activity were found in the liver, small intestine (from duodenum to ileum), and kidney (Fig. 1). A low Glc-6-Pase activity was also found in the colon and heart, but mRNA and activity were virtually absent from other tissues analyzed (Fig. 1). It is noteworthy that the level of Glc-6-Pase mRNA in the liver and kidney of suckling rats represents 50% of the concentrations found in the liver of fasted adult rats (Fig. 1). Although the small intestine had a much lower Glc-6-Pase mRNA concentration than the liver and kidney, Glc-6-Pase mRNA concentrations in this tissue were higher than the PEPCK mRNA concentrations (Fig. 1). Indeed, the PEPCK mRNA levels (expressed as % of 48-h starved adult rat liver) were 81 ± 5 and 12 ± 1%, respectively, for the liver and the ileum, thus leading to a liver:ileum ratio for PEPCK mRNA of ~6, whereas for Glc-6-Pase mRNA this ratio was ~3.

Developmental changes of Glc-6-Pase gene expression and activity in liver, jejunum, and kidney. As shown in Fig. 2, the mRNAs coding for Glc-6-Pase were already present in the liver of 21-day-old rat fetuses at a concentration half of that found in adult rat liver, whereas the activity of the enzyme was 80% lower than in fed adult rat liver (6.7 ± 0.5 U/g, *n* = 5) (Fig. 3). Immediately after birth, the mRNA concentration and the activity of Glc-6-Pase increased markedly in the liver, returned to 50% of the adult value 5 days after birth, and remained at this level during the suckling period (Figs. 2 and 3). When rats were weaned onto the HCLF diet, the concentration of Glc-6-Pase mRNA was markedly increased in the liver of 22- and 30-day-old rats (Fig. 2). By contrast, the activ-

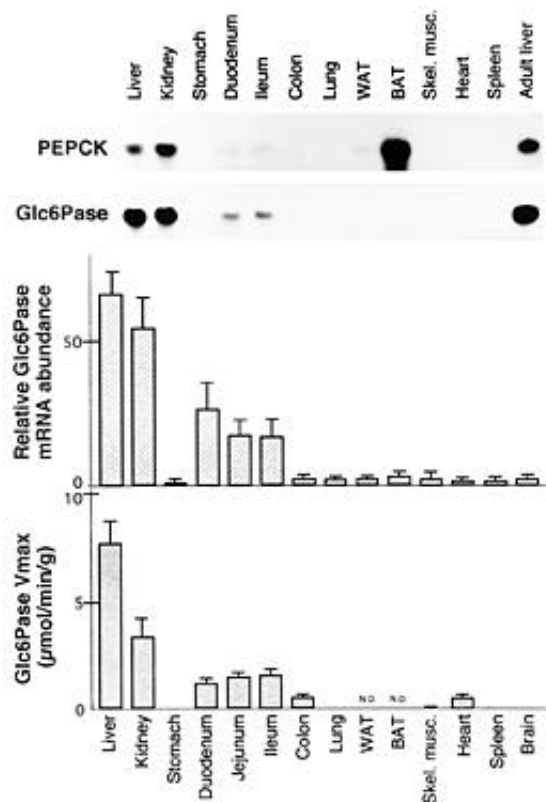


FIG. 1. Tissue-specific expression of Glc-6-Pase in 15-day-old suckling rats. Total RNA was extracted from frozen tissues and analyzed by Northern blot technique as described in METHODS. PEPCK mRNA determination was used as control. Densitometric quantification of four different Northern blots was performed, and the results are expressed as percentage of Glc-6-Pase mRNA concentration found in the liver of 48-h starved adult rats. The Glc-6-Pase activity has been measured in each tissue, and the result is expressed as means \pm SE of five different animals.

ity did not change significantly in the liver of 22- and 30-day-old rats weaned onto the HCLF diet (Fig. 3).

In the fetal rat jejunum, the concentration of Glc-6-Pase mRNA was detectable but very low (10% of adult rat liver value, Fig. 2). By contrast, the activity of Glc-6-Pase in the fetal jejunum (Fig. 3) was similar to that found in fed adult rat jejunum (0.63 ± 0.03 U/g, $n = 5$) and only slightly lower than in 48-h-starved adult rat jejunum (1.3 ± 0.2 U/g, $n = 5$). The Glc-6-Pase mRNA concentration increased markedly during the 10 days after birth to reach almost the level observed in the liver of starved adult rats. Then it declined progressively during the suckling and the post-weaning periods to reach <10% of the level found in the liver of starved adult rats (Fig. 2). During the first 10 days after birth, the activity of the enzyme was also increased to reach levels higher by twofold (Fig. 3) than in the 48-h starved adult rat jejunum (1.3 ± 0.2 U/g, $n = 5$) and then declined until the end of the suckling period. In the jejunum of the HCLF-weaned rats, the Glc-6-Pase activity was similar to that found in fed adult rat jejunum (Fig. 3).

In the fetal kidney, the mRNA concentration and the activity of Glc-6-Pase were undetectable (Figs. 2 and 3). The mRNA concentration and the activity of Glc-6-Pase increased slowly during the 10 days after birth and then rapidly increased

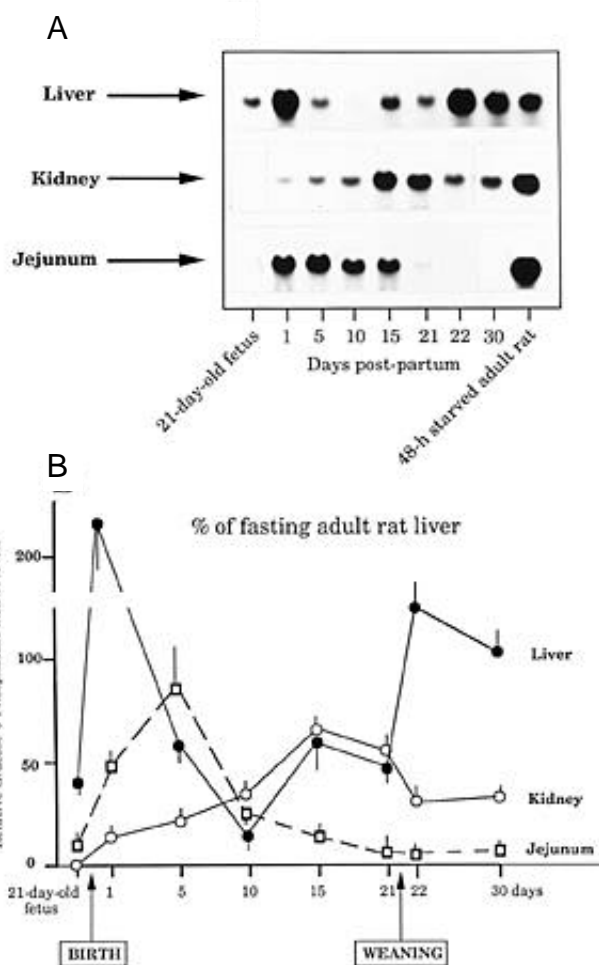


FIG. 2. Developmental changes in Glc-6-Pase mRNA concentration in liver, kidney, and jejunum. **A:** Representative Northern blot of Glc-6-Pase mRNA concentrations in liver, kidney, and jejunum during the suckling period and in rats weaned at 21 days onto a high-carbohydrate diet. **B:** Densitometric quantification of four different Northern blots. The results are expressed as percentage of Glc-6-Pase mRNA concentration found in the liver of 48-h starved adult rats.

between 10 and 21 days. In 21-day-old suckling rats, the Glc-6-Pase mRNA concentration was $\sim 85\%$ of that found in the 48-h starved adult rat liver (Fig. 2), whereas the activity (Fig. 3) was 50% to that observed in the 48-h starved adult rat kidney (9.8 ± 1.2 U/g, $n = 5$). When rats were weaned onto the HCLF diet, the concentration of Glc-6-Pase mRNA was decreased (Fig. 2), whereas the activity remained constant (Fig. 3).

Regulation of Glc-6-Pase gene expression in cultured fetal rat hepatocytes: effects of dexamethasone and Bt_2cAMP . The fall in plasma insulin levels and the rise in plasma glucagon levels that occur at birth result in a rapid increase in liver cAMP concentration (6). Thus, we investigated the effect of Bt_2cAMP on Glc-6-Pase mRNA concentrations in cultured fetal hepatocytes. Glc-6-Pase mRNA concentration was low in freshly isolated hepatocytes and fell during cell attachment to remain at this low concentration during the following 48 h of culture under basal conditions (Fig. 4A).

The level of Glc-6-Pase mRNA was increased 2.8 ± 0.3 ($n = 3$) and 4.8 ± 0.6 fold ($n = 10$) after 24 and 48 h of culture in the presence of 10^{-4} mol/l Bt_2cAMP (Fig. 4). Since the maximal accumulation of Glc-6-Pase mRNA was observed 48 h

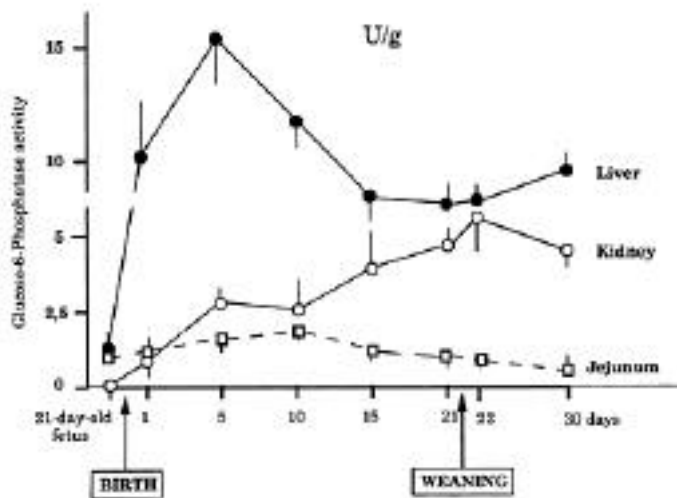


FIG. 3. Developmental changes in Glc-6-Pase activity in liver, kidney, and jejunum. Results are means \pm SE of five different animals. Rats were weaned at 21 days onto a high-carbohydrate diet.

after the addition of Bt_2cAMP , subsequent experiments were performed after 48 h of culture.

The dose-response curve of Glc-6-Pase mRNA accumulation in response to Bt_2cAMP showed that the half-maximal effect was achieved at a concentration of 3×10^{-5} mol/l (Fig. 4B).

Glucocorticoids are generally required for hepatocyte adherence onto plastic dishes (21). They also have a direct or permissive effect on hormone-induced gene expression, as shown for Glc-6-Pase in cultured adult rat hepatocytes (22). To know whether glucocorticoids had a direct effect or a permissive effect on Bt_2cAMP -induced Glc-6-Pase mRNA accumulation, fetal hepatocytes were cultured for 48 h in the presence of 10^{-7} mol/l dexamethasone alone or in association with 10^{-5} mol/l Bt_2cAMP (half-maximal concentration). Dexamethasone alone had no effect on Glc-6-Pase gene expression (1.0 ± 0.1 , $n = 4$) and did not potentiate the Bt_2cAMP -induced Glc-6-Pase mRNA accumulation (2.1 ± 0.5 , $n = 3$; compare with Fig. 4B).

Effects of glucose on Bt_2cAMP -induced Glc-6-Pase gene expression. The supply of carbohydrate from the diet is reduced after birth, and the concentration of glucose in the portal vein is low. It was thus of interest to know whether a change in the glucose concentration in the culture medium could modify the accumulation of Glc-6-Pase mRNA. Indeed, when hepatocytes were cultured for 48 h in the absence of glucose, the Glc-6-Pase mRNA concentration was $67 \pm 16\%$ ($n = 6$) higher when compared with fetal hepatocytes cultured in the presence of 5 mmol/l glucose. These results suggest that glucose could have an inhibitory effect on Glc-6-Pase gene expression in fetal rat hepatocytes. However, because basal expression of Glc-6-Pase was low in fetal rat hepatocytes (Fig. 4A), it was difficult to know whether glucose had a specific effect on Glc-6-Pase gene expression. Thus the effect of glucose was tested on the Bt_2cAMP -induced Glc-6-Pase gene expression. The accumulation of Glc-6-Pase mRNA in response to 10^{-4} mol/l Bt_2cAMP was not affected by large changes in glucose concentration (0–20 mmol/l) in the culture medium (Fig. 5).

By contrast, when hepatocytes from fed adult rats were cultured for 24 h in the presence of 25 mmol/l glucose, the con-

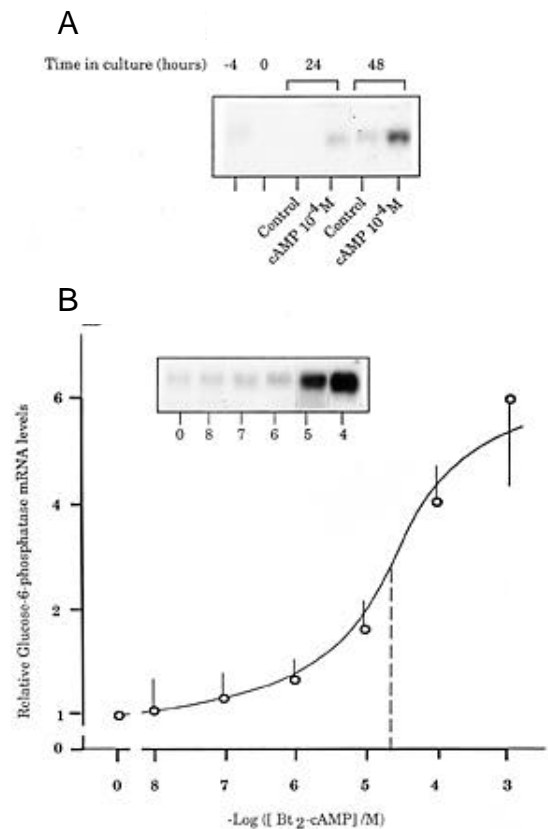


FIG. 4. Time course and dose-response curve of the effects of Bt_2cAMP on Glc-6-Pase mRNA expression in 20-day-old rat fetuses. **A:** Hepatocytes were cultured for 24 or 48 h after cell attachment (time 0) in the absence or in the presence of Bt_2cAMP (10^{-4} mol/l). This blot is representative of four different cultures. **B:** Hepatocytes were cultured for 48 h in the presence of Bt_2cAMP at the indicated concentrations. Results are expressed as arbitrary units, and the reference value (1) is the concentration of Glc-6-Pase mRNA in hepatocytes cultured in control conditions (absence of Bt_2cAMP). Results are means \pm SE of four different experiments. A representative Northern blot is shown.

centration of Glc-6-Pase mRNA was increased 7.1 ± 0.6 -fold ($n = 4$; $P < 0.01$ when compared with cultures in the absence of glucose; Fig. 5). This effect is specific since L-glucose used at the same concentration did not affect the Glc-6-Pase mRNA levels (data not shown).

Effects of various fatty acids and clofibrate on Glc-6-Pase gene expression. Because rat milk is rich in triglycerides, 30% of which contains medium-chain fatty acids (6), we investigated the role of different fatty acids on Glc-6-Pase gene expression. When hepatocytes were cultured in the presence of 0.5 mmol/l octanoate (a medium-chain fatty acid), no significant accumulation of Glc-6-Pase mRNA was observed (Table 1). By contrast, oleate and linoleate (LCFAs) increased the concentration of Glc-6-Pase mRNA by twofold (Table 1). Since it has been suggested that the effect of LCFAs on gene expression was mediated by the peroxisome proliferator-activated receptors (PPARs) (23), we tested the effect of clofibrate, a peroxisome proliferator, on Glc-6-Pase gene expression in fetal hepatocytes cultured for 48 h. Clofibrate induced a threefold increase in Glc-6-Pase mRNA concentrations (Table 1).

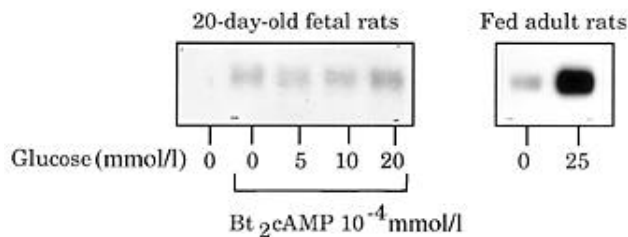


FIG. 5. Effects of glucose on Bt_2cAMP -induced Glc-6-Pase mRNA accumulation. Hepatocytes were cultured for 48 h in the presence of Bt_2cAMP (10^{-4} mol/l) and the indicated concentrations of glucose. This Northern blot is representative of three different cultures.

Antagonistic effect of insulin on Bt_2cAMP -, linoleate, and clofibrate-induced Glc-6-Pase gene expression.

To know whether the postnatal fall in plasma insulin concentration could be important for the induction of Glc-6-Pase gene expression, hepatocytes were cultured for 48 h in the presence of a maximal concentration of Bt_2cAMP (10^{-4} mol/l) and increasing concentrations of insulin (10^{-11} to 10^{-7} mol/l). The half-maximal inhibitory effect of insulin was achieved at a concentration of 10^{-9} mol/l (Fig. 6).

The increase in Glc-6-Pase mRNA after exposure to 0.5 mmol/l linoleate or clofibrate was also suppressed by 100 and 80%, respectively, in the presence of 10^{-7} mol/l insulin (Table 1). **Effects of Bt_2cAMP and linoleate on Glc-6-Pase mRNA half-life.** The decay in Glc-6-Pase mRNA concentration after a 48-h exposure to Bt_2cAMP or linoleate was calculated from experiments performed in the presence of DRB, an inhibitor of RNA transcription. The Glc-6-Pase mRNA half-life (67 ± 7 min, $n = 5$; Fig. 7) was not modified by the presence of Bt_2cAMP (82 ± 10 min, $n = 5$). In contrast, the Glc-6-Pase mRNA half-life was 60% longer in the presence of linoleate (107 ± 12 min versus 67 ± 7 min, $n = 5$, $P < 0.05$; Fig. 7).

DISCUSSION

The present work shows that Glc-6-Pase mRNA accumulates in three tissues capable of performing an active gluconeogenesis during the suckling period. The changes are more marked in the liver and the small intestine than in the kidney, and the time courses are different in the three tissues. It is noteworthy that the abundance of Glc-6-Pase mRNA in the jejunum of 5-day-old suckling rats is transiently as high as that in the liver of the 48-h starved adult rats.

In the kidney, Glc-6-Pase mRNA concentration and activity increase more slowly than in the liver and small intestine. These results are in agreement with the developmental pattern observed for other gluconeogenic enzymes (6). The suckling period is characterized by a high level of circulating fuels (lactic acid, free fatty acids, ketone bodies) that could lead to an activation of renal gluconeogenesis through a change in the acid-base status. Indeed, kidney PEPCK gene expression was increased by metabolic acidosis in adult rats (24,25), whereas renal Glc-6-Pase activity was not increased by metabolic acidosis in adult rats (26). By contrast, the increase in renal Glc-6-Pase mRNA and activity in starvation or diabetes is totally reversed by insulin (3). Moreover, glu-

TABLE 1

Effects of various fatty acids and clofibrate on Glc-6-Pase gene expression in cultured fetal hepatocytes

Additions	Glc-6-Pase mRNA levels (arbitrary units)
Control	1
Octanoate (0.5 mmol/l)	0.92 ± 0.05 (3)
Oleate (0.5 mmol/l)	2.0 ± 0.4 (4)*
Linoleate	
0.1 mmol/l	1.3 ± 0.4 (3)
0.5 mmol/l	1.9 ± 0.2 (11)*
0.5 mmol/l + insulin	0.9 ± 0.2 (6)
Clofibrate (0.5 mmol/l)	3.0 ± 0.4 (4)†
0.5 mmol/l + insulin	1.4 ± 0.3 (4)

Data are means \pm SE (n). Hepatocytes from 20-day-old fetuses were cultured for 48 h in a glucose-free medium (control) or in the presence of various fatty acids or clofibrate at the indicated concentrations. Before addition, fatty acids were bound to fat-free albumin (0.2% final concentration). In some experiments, the effect of insulin (10^{-7} mol/l) was tested on the linoleate- or clofibrate-induced Glc-6-Pase gene expression. Results are expressed as arbitrary units, the reference value (1) being the level of Glc-6-Pase mRNAs in control conditions. * $P < 0.05$ and † $P < 0.01$ when compared with controls.

cocorticoids and triiodothyronine have been shown to be involved in the regulation of renal energy metabolism during development (27,28). It is noteworthy that the time course of increase in glucocorticoids and triiodothyronine concentrations in the plasma (6) parallels that of renal Glc-6-Pase mRNA levels. Whether or not the changes in plasma insulin, glucocorticoids, or triiodothyronine concentrations, which occur during the suckling period, are involved in the increase in renal Glc-6-Pase gene expression remains to be determined.

The present work also demonstrates that the small intestine expresses both Glc-6-Pase mRNA and activity at a significant level during the suckling period. These results are in agreement with the presence of an active gluconeogenesis in intestinal mucosa of suckling rats (7). An active glucose production and a Glc-6-Pase activity have also been reported in the small intestine of suckling pigs (29), mice (30), and guinea pigs (9). It is noteworthy that the small intestine from suckling rats expressed not only genes encoding gluconeogenic enzymes, but also genes encoding ketogenic enzymes (31). The gluconeogenic and ketogenic capacities of the small intestinal mucosa disappear after weaning (7). This is due to the fall in gene expression and activity of key enzymes of these metabolic pathways (31), especially Glc-6-Pase (30; present work). It is noteworthy that Glc-6-Pase is expressed to a small extent in the small intestine of rat and human adults (32,33). The factors involved in the expression of these genes and in the regulation of the metabolic fluxes in the small intestine during development are still unknown. However, it could be emphasized that the hormonal environment (low-insulin, high-glucagon concentrations), which prevails during the suckling period, could exert a control on gluconeogenic enzymes gene expression. For instance, incubation of scraped intestinal mucosa from 12-day-old suckling rats with Bt_2cAMP enhanced the rates of glucose production from lactate (7). Conversely, insulin treatment of the diabetic

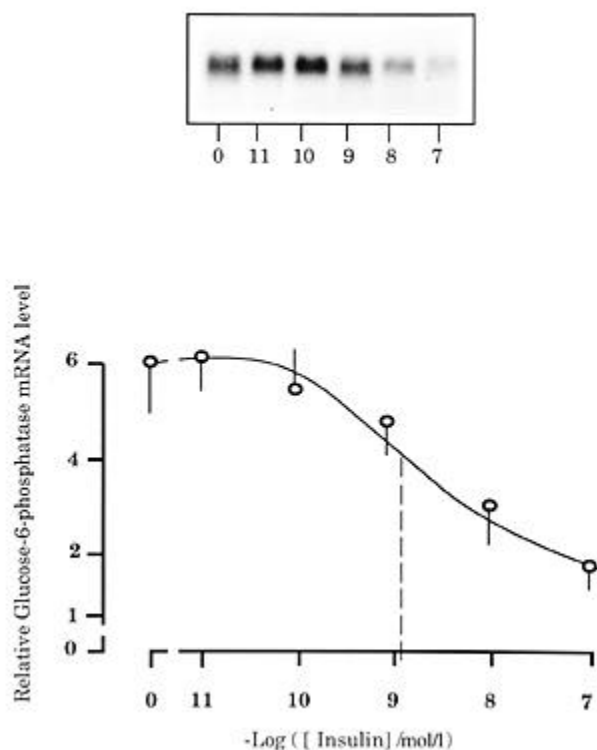


FIG. 6. Dose-response curve of the effect of insulin on Bt_2cAMP -induced Glc-6-Pase mRNA accumulation. Hepatocytes were cultured for 48 h in the presence of Bt_2cAMP (10^{-4} mol/l) and the indicated concentrations of insulin. Results are expressed as arbitrary units, and the reference value (1) is the concentration of Glc-6-Pase mRNA in hepatocytes cultured under control conditions (absence of Bt_2cAMP and insulin). Results are means \pm SE of four different experiments. A representative Northern blot is shown.

rat reverses the diabetes-induced Glc-6-Pase gene expression in the small intestine of adult rats (33). Whether or not cAMP and/or insulin regulate Glc-6-Pase and/or other gluconeogenic enzyme gene expression in small intestinal mucosa of suckling rats remains to be determined. Finally, the presence of large amounts of LCFA triglycerides in the lumen of the small intestine of suckling rats would promote both the increased rates of intestinal gluconeogenesis, as shown in the liver (6), and increased gene expression of key gluconeogenic enzymes such as Glc-6-Pase. Indeed, if the contribution of LCFA in the regulation of gene expression is now well documented in the liver and adipose tissue (34), then a recent study showed that liver Glc-6-Pase mRNA concentration was increased in adult rats by infusion of an emulsion rich in triglycerides (35). However, since primary culture of enterocytes is not feasible, the question of whether pancreatic hormones and/or fatty acid regulate Glc-6-Pase gene expression during the prenatal period was addressed in the only available system, the primary culture of hepatocytes.

In the liver, Glc-6-Pase mRNA, which is already present toward the end of gestation (36; present work), markedly accumulates immediately after birth to reach a level twice that found in the fasting adult rat liver. It is noteworthy that the concentration of Glc-6-Pase mRNA markedly decreases between 1 and 10 days after birth, whereas the activity is less

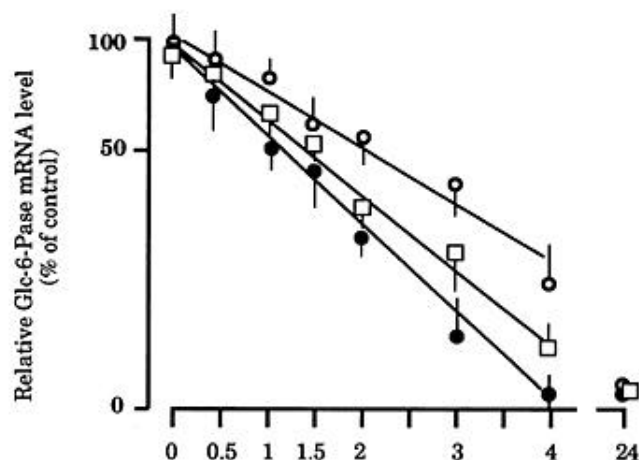


FIG. 7. Effects of cAMP and linoleate on Glc-6-Pase mRNA stability. Hepatocytes were cultured for 48 h in the absence (control) or in the presence of either Bt_2cAMP (10^{-4} mol/l) or linoleate (0.5 mmol/l). Then the medium was replaced by a fresh medium containing DRB (25 μ g/ml), and the culture was continued for the indicated times in the absence (●) or in the presence of either Bt_2cAMP (□) or linoleate (○) at the same concentration. This plot represents five different cultures.

affected. Similar observations were previously reported for the developmental pattern of PEPCK gene expression (37) and were attributed to an increase in the half-life of the protein and/or to a post-transcriptional regulation. Whether or not this explains the discrepancies between mRNA levels and Glc-6-Pase activity during development remains to be determined. At least one of the following three major events occurs within the first hours of extra-uterine life that could be involved in the regulation of hepatic Glc-6-Pase gene expression: 1) an increase in cyclic AMP concentration as the result of the fall in plasma insulin and the rise in plasma glucagon concentrations (6), 2) a low portal glucose concentration caused by the consumption of a low-carbohydrate diet (6), and 3) an increased supply of plasma fatty acids to the liver arising from the hydrolysis of milk triglycerides (6). The contribution of each of these factors in the regulation of Glc-6-Pase gene expression has been studied in cultured hepatocytes from 20-day-old rat fetuses.

It was reported that glucagon or thyroxine injection to fetal rats induced the premature development of hepatic Glc-6-Pase activity (38,39). The present work shows that Bt_2cAMP induces, in a dose-dependent manner, the accumulation of Glc-6-Pase mRNA in fetal rat hepatocytes. This increase probably results from a stimulation of Glc-6-Pase gene transcription since Bt_2cAMP does not affect the stability of the Glc-6-Pase transcript. The stimulation of Glc-6-Pase gene expression by cAMP has been previously reported in cultured adult rat hepatocytes (22). However, the time course is different since maximal stimulation is achieved after 24 h of culture in adult cells, whereas it required 48 h of culture in fetal hepatocytes. Such a delay in the response to cAMP in fetal hepatocytes has been previously observed for carnitine palmitoyltransferase-I gene expression (40). By contrast, cAMP-induced PEPCK or mitochondrial HMG-CoA synthase gene expression is much faster in fetal cells (13,31). This has been interpreted as a delay necessary for DNase-I hypersensitive sites to be in an open configuration in genes that have never been transcribed previously. The cAMP-induced Glc-6-Pase mRNA accumulation

is markedly potentiated by the presence of a relative or an absolute insulin deficiency, suggesting that the postnatal fall in plasma insulin concentration is required for the maximal induction of Glc-6-Pase gene expression at birth. An inhibitory effect of insulin on Glc-6-Pase gene expression has been previously reported in insulin-treated diabetic rats (3), in rats recovering from partial hepatectomy (36), or in cultured hepatocytes from adult rats (22). This inhibitory effect of insulin is mediated via an insulin response sequence (IRS) in the promoter region of the Glc-6-Pase gene (22,41). As previously reported in cultured hepatocytes from adult rats (22), dexamethasone has no direct effect on Glc-6-Pase gene expression in fetal cells. However, whereas dexamethasone slightly potentiated the effect of cAMP in adult hepatocytes (22), it did not in fetal cells. This could result from the concentration of dexamethasone used in adult cells, which is tenfold higher than the one used in the present study (10^{-7} mol/l). At the concentration of 10^{-7} mol/l, dexamethasone has no effect in adult hepatocytes (22). Thus the contribution of dexamethasone to the regulation of Glc-6-Pase gene expression in hepatic cells remains questionable if it required such pharmacological concentrations of glucocorticoids.

The low concentration of glucose in the portal blood immediately after birth and during the suckling period could provide a suitable nutritional environment for the induction of a gluconeogenic enzyme such as Glc-6-Pase. However, the present study shows that glucose has no inhibitory effect on Bt₂cAMP-induced Glc-6-Pase. This is in marked contrast with the observations reported recently in adult rats and in hepatoma cells. The increased Glc-6-Pase gene expression in diabetic rats was normalized after correction of hyperglycemia by phlorizin treatment (42). This was interpreted as the result of the decrease in blood glucose concentration. However, as plasma glucagon levels were decreased in such experimental conditions (43), the decrease in plasma glucagon could be responsible for the decrease in Glc-6-Pase gene expression rather than the decrease in blood glucose concentration. Indeed, when rats are weaned onto a HCLF diet, the concentration of plasma insulin increased, whereas plasma glucagon levels markedly decreased (44). However, despite the rise in insulin concentration in high-carbohydrate-weaned rats, the levels of hepatic Glc-6-Pase mRNA are markedly increased in the liver, whereas they are decreased in the kidney. This would suggest that the regulation of Glc-6-Pase gene expression is different in these two organs as previously reported in diabetic or starved adult rats (3). In addition, the effect of glucose could overcome the inhibitory effect of insulin in the liver. Indeed, the high concentration of glucose (25 mmol/l) markedly increased the Glc-6-Pase gene expression in cultured adult rat hepatocytes (45; present work) and in hepatoma cells (45,46). The difference between fetal and adult rat hepatocytes or hepatoma cells could result from a difference in hepatic glucose metabolism. Indeed, phosphorylation of glucose seems to be essential for glucose-induced gene regulation (47). Recently, the inhibition of PEPCK gene expression in hepatoma cells has been shown to be dependent of the expression of glucokinase (48), and the stimulation of Glc-6-Pase gene expression in vivo in response to glucose has been suggested to result from an increase in xylulose-5-phosphate concentration (49), a metabolite of the pentose phosphate pathway that is very active in hepatoma cells. The low level of glucokinase and of the enzymes of the

pentose phosphate pathway in newborn and suckling rat hepatocytes (47) could explain the absence of the effect of glucose on Glc-6-Pase gene expression. In keeping with this, it was recently shown that overexpression of glucokinase in Fao cells via means of recombinant adenovirus vectors results in a marked increase in Glc-6-Pase gene expression in response to high glucose concentrations (45).

This work also provides direct evidence that in an hormone-free environment, LCFAs stimulate hepatic Glc-6-Pase gene expression. Similar conclusions were reached from in vivo experiments (35). Indeed, in a well-controlled hormonal milieu, intralipid infusion increases liver Glc-6-Pase mRNA concentration, whereas inhibition of lipolysis by nicotinic acid infusion has the opposite effect (35). Although most of the data agree that the regulation of gene expression by LCFAs occurs at a transcriptional level (50), this remains to be determined for Glc-6-Pase. The present work provides evidence that LCFAs also have a stabilizing effect on Glc-6-Pase mRNA. Thus, the molecular mechanism involved in gene regulation by LCFAs remains unclear. It has been suggested that LCFAs act via PPARs, nuclear receptors of the steroid/thyroid hormone superfamily (34). In the liver, the activation of the PPARs by fatty acids has been reported for genes encoding peroxisomal and mitochondrial enzymes involved in fatty acid metabolism (34). What is not yet clear is whether peroxisome proliferators (fibrates) and fatty acids act through the same or distinct mechanisms. Taking into account the effect of clofibrate, the current work would suggest that LCFAs and peroxisome proliferators could regulate Glc-6-Pase gene expression through a common signaling pathway. Although similarities in the effects of LCFAs and peroxisome proliferators on hepatic and/or adipocyte gene expression have led many authors to conclude that LCFAs regulate gene transcription via a PPAR-dependent pathway, further investigations are required to conclude whether this is also the case for Glc-6-Pase gene. Finally, the LCFA- and clofibrate-induced Glc-6-Pase mRNA accumulation in fetal rat hepatocytes is totally impaired when insulin is present in the culture medium, confirming that the fall in plasma insulin concentration at birth is a necessary event for the induction of postnatal Glc-6-Pase gene expression.

REFERENCES

1. Nordlie RC: *Glucose-6-Phosphatase Phosphotransferase: Roles and Regulation in Relation to Gluconeogenesis*. New York, John Wiley, 1976, p. 53-152
2. Mithieux G: New knowledge regarding glucose-6-phosphatase gene and protein and their roles in glucose metabolism. *Eur J Endocr* 136:137-145, 1997
3. Mithieux G, Vidal H, Zitoun C, Bruni N, Daniele N, Minassian C: Glucose-6-phosphatase mRNA and activity are increased to the same extent in kidney and liver of diabetic rats. *Diabetes* 45:891-896, 1996
4. Pilkis SJ, El-Maghrabi MR, Claus TH: Hormonal regulation of hepatic gluconeogenesis and glycolysis. *Ann Rev Biochem* 57:755-783, 1988
5. Adroque HJ: Glucose homeostasis and the kidney. *Kidney Int* 42:1266-1282, 1992
6. Girard J, Ferré P, Pégrier JP, Duée PH: Adaptations of glucose and fatty acid metabolism during the perinatal period and the suckling-weaning transition. *Physiol Rev* 72:507-562, 1992
7. Hahn P, Wei-Ning H: Gluconeogenesis from lactate in the small intestine mucosa of suckling rats. *Pediatr Res* 20:1321-1323, 1986
8. Bismut H, Hers G, Van Schaftingen E: Conversion of fructose to glucose in the rabbit small intestine: a reappraisal of the direct pathway. *Eur J Biochem* 213:721-726, 1993
9. Anderson JW, Rosendall AF: Gluconeogenesis in jejunal mucosa of guinea pig. *Biochim Biophys Acta* 304:384-388, 1973
10. Minassian C, Zitoun C, Mithieux G: Differential time course of liver and kidney glucose-6-phosphatase activity during fasting correlates with differential

- time course of messenger RNA level. *Mol Cell Biochem* 155:37–41, 1996
11. Girard JR, Cuendet GS, Marliss EB, Kervran A, Rieutort M, Assan R: Fuels, hormones and liver metabolism at term and during the early postnatal period in the rat. *J Clin Invest* 52:3190–3200, 1973
 12. Coupé C, Perdereau D, Ferré P, Hitier Y, Narkewicz M, Girard J: Lipogenic enzyme activities and mRNA in rat adipose tissue at weaning. *Am J Physiol* 258:E126–E133, 1990
 13. Pégrier JP, Salvado J, Forestier M, Girard J: Dominant role of glucagon in the initial induction of phosphoenolpyruvate carboxykinase (PEPCK) mRNA in cultured hepatocytes from fetal rats. *Eur J Biochem* 210:1053–1059, 1992
 14. Pégrier JP, Duée PH, Herbin C, Laulan PY, Bladé C, Peret J, Girard J: Fatty acid metabolism in hepatocytes isolated from rats adapted to high-fat diets containing long- or medium-chain triacylglycerols. *Biochem J* 249:801–806, 1988
 15. Chirgwin JM, Przybyla AE, McDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5299, 1979
 16. Chan YL, Gutell R, Noller HF, Wool IG: The nucleotide sequence of a rat 18 S ribosomal ribonucleic acid gene and a proposal for the secondary structure of 18 S ribosomal ribonucleic acid. *J Biol Chem* 259:224–230, 1984
 17. Zandomeni R, Mittleman B, Bunick D, Ackerman S, Weinmann R: Mechanism of action of dichloro- β -ribofuranosylbenzimidazole: effect on in vitro transcription. *Proc Natl Acad Sci USA* 79:3167–3170, 1982
 18. Minassian C, Ajzanny A, Riou JP, Mithieux G: Investigation of the mechanism of glycogen rebound in the liver of 72-h fasted rats. *J Biol Chem* 265:16585–16588, 1994
 19. Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
 20. Wilcoxon F: Probability tables for individual comparisons by ranking methods. *Biometrics* 3:119–122, 1947
 21. Laishes BA, Williams GM: Conditions affecting primary cell cultures of functional adult rat hepatocytes. II. Dexamethasone enhanced longevity and maintenance of morphology. *In Vitro* 12:821–832, 1976
 22. Argaud D, Zhang Q, Pan W, Maitra S, Pilks SJ, Lange AJ: Regulation of rat liver glucose-6-phosphatase gene expression in different nutritional and hormonal states: gene structure and 5'-flanking sequence. *Diabetes* 45: 1563–1571, 1996
 23. Keller H, Wahli W: Peroxisome proliferator-activated receptors: a link between endocrinology and nutrition? *Trends Endocrinol Metab* 4:291–296, 1993
 24. Iynedjian PB, Hanson RW: Messenger RNA for renal phosphoenolpyruvate carboxykinase (GTP): its translation in a heterologous cell-free system and its regulation by glucocorticoids and by changes in acid-base balance. *J Biol Chem* 252:8398–8403, 1977
 25. Hwang JJ, Curthoys NP: Effect of acute alteration in acid-base balance on rat renal glutaminase and phosphoenolpyruvate carboxykinase gene expression. *J Biol Chem* 266:9392–9396, 1991
 26. Burch HB, Narins RG, Chu C, Fagioli S, Choi S, McCarthy W, Lowry OH: Distribution along the rat nephron of three enzymes of gluconeogenesis in acidosis and starvation. *Am J Physiol* 235:F246–F253, 1978
 27. Djouadi F, Bastin J, Gilbert A, Röttig P, Merlet-Benichou C: Mitochondrial biogenesis and development of respiratory chain enzymes in kidney cells: role of glucocorticoids. *Am J Physiol* 267:C245–C254, 1994
 28. Wijkhuisen A, Djouadi F, Vilar J, Merlet-Benichou C, Bastin J: Thyroid hormones regulate development of energy metabolism enzymes in rat proximal convoluted tubule. *Am J Physiol* 268:F634–F642, 1995
 29. Darcy-Brillon B, Posho L, Morel MT, Bernard F, Blachier F, Meslin JC, Duée PH: Glucose, galactose and glutamine metabolism in pig isolated enterocytes during development. *Pediatr Res* 36:175–181, 1994
 30. Menard D, Malo C: Glucose-6-phosphatase activity in mouse small intestine during postnatal development. *Develop Biol* 65:508–514, 1978
 31. Thumelin S, Forestier M, Girard J, Pégrier JP: Developmental changes in mitochondrial 3-hydroxy-3-methyl-glutaryl-CoA synthase gene expression in rat liver, intestine and kidney. *Biochem J* 292:493–496, 1993
 32. Ockerman PA: Glucose-6-phosphatase in human jejunal mucosa: properties demonstrating the specific character of the enzyme activity. *Biochim Biophys Acta* 105:22–33, 1965
 33. Mithieux G, Bruni N, Tarpin S, Zitoun N: Control of glucose-6-phosphatase mRNA and activity in small intestine of fasted and diabetic rats (Abstract). *Diabetologia* 40 (Suppl. 1):A24, 1997
 34. Wahli W, Braissant O, Desvergne B: Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more. *Chem Biol* 2:261–266, 1995
 35. Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L: Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. *Diabetes* 46:153–157, 1997
 36. Haber BA, Chin S, Chuang E, Buikhuisen W, Naji A, Taub R: High levels of glucose-6-phosphatase gene and protein expression reflect an adaptive response in proliferating liver and diabetes. *J Clin Invest* 95:832–841, 1995
 37. Lyonnet S, Coupe C, Girard J, Kahn K, Munnich A: In vivo regulation of glycolytic and gluconeogenic enzyme gene expression in newborn rat liver. *J Clin Invest* 81:1682–1689, 1988
 38. Greengard O, Dewey HK: Initiation by glucagon of the premature development of tyrosine aminotransferase, serine dehydratase and glucose-6-phosphatase in fetal rat liver. *J Biol Chem* 242:2986–2991, 1967
 39. Greengard O, Dewey HK: The developmental formation of liver glucose-6-phosphatase and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase in fetal rats treated with thyroxine. *J Biol Chem* 243:2745–2749, 1968
 40. Chatelain F, Kohl C, Esser V, McGarry JD, Girard J, Pégrier JP: Cyclic AMP and fatty acids increase carnitine palmitoyltransferase I transcription in cultured fetal rat hepatocytes. *Eur J Biochem* 235:789–798, 1996
 41. Streeper RS, Svitek CA, Chapman S, Greenbaum LE, Taub R, O'Brien RM: A multicomponent insulin response sequence mediates a strong repression of mouse glucose-6-phosphatase gene transcription by insulin. *J Biol Chem* 272:11698–11701, 1997
 42. Massillon D, Barzilai N, Chen W, Hu M, Rossetti L: Glucose regulates in vivo glucose-6-phosphatase gene expression in the liver of diabetic rats. *J Biol Chem* 271:9871–9874, 1996
 43. Brichard SM, Henquin JC, Girard J: Phlorizin treatment of diabetic rats partially reverses the abnormal expression of genes involved in hepatic glucose metabolism. *Diabetologia* 36:292–298, 1993
 44. Decaux JF, Ferré P, Girard J: Effects of weaning on different diets on hepatic gluconeogenesis in the rat. *Biol Neonate* 50:331–336, 1986
 45. Argaud D, Kirby TL, Newgard CB, Lange AJ: Stimulation of glucose-6-phosphatase gene expression by glucose and fructose-2,6-bisphosphate. *J Biol Chem* 272:12854–12861, 1997
 46. Lange AJ, Argaud D, El-Maghrabi MR, Pan W, Maitra SR, Pilks SJ: Isolation of a cDNA for the catalytic subunit of rat liver glucose-6-phosphatase: regulation of gene expression in FAO hepatoma cells by insulin, dexamethasone and cAMP. *Biochem Biophys Res Comm* 201:302–309, 1994
 47. Girard J, Chatelain F, Boileau J, Prip-Buus C, Thumelin S, Pégrier JP, Fougelle F, Ferré P: Nutrient regulation of gene expression. *J Anim Sci* 75: 46–57, 1997
 48. Scott D, O'Doherty R, Newgard C, Granner D: Repression of hormone-activated PEPCK gene transcription by glucose is dependent on glucose metabolism (Abstract). *Diabetes* 46 (Suppl. 1):21A, 1997
 49. Massillon D, Prus-Wertheimer D, Hu M, Chen W, Liu R, Rossetti L: Carbon flux via the pentose phosphate shunt regulates hepatic gene expression of glucose-6-phosphatase (Glc-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) (Abstract). *Diabetes* 46 (Suppl. 1):49A, 1997
 50. Clarke SD, Jump DB: Polyunsaturated fatty acid regulation of hepatic gene transcription. *J Nutr* 126:1105S–1109S, 1996