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Maternal Dietary Glucose Modifies Phosphoenolpyruvate Carboxykinase (PEPCK) Gene Expression in the Kidney of Newborn Rats

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The consequence of low maternal dietary glucose on perinatal phosphoenolpyruvate carboxykinase (PEPCK EC 4.1.32) gene expression was investigated. Pregnant rats were fed isoenergetic diets containing graded levels of glucose (0, 12, 24, and 60%) from gestation day 2 to lactation day 15. The postnatal developmental profile of PEPCK mRNA in the neonatal kidney was analysed by Northern blot and presented as PEPCK/GAPDH mRNA ratios. In comparison with the 24 and 60% dietary groups, maternal dietary glucose restriction (0 or 12%) during pregnancy resulted in a significant delay in postnatal renal PEPCK gene expression. In these glucose restricted pups, renal PEPCK mRNA was barely detected at birth and was fully visualized only at 4-6 hr; it peaked 24 hr after birth, which was 12 hr later than pups born to dams fed 24 or 60% glucose diets. These results demonstrate for the first time that maternal dietary glucose can modify postnatal renal PEPCK gene expression during perinatal development when glucose homeostasis via gluconeogenesis is critical for neonatal survival.

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the first step in gluconeogenesis and is the key enzyme in the gluconeogenic pathway in both liver and kidney. PEPCK is not subject to allosteric regulation but is controlled via gene expression (1, 2). Regulation of PEPCK is complex, as its gene expression changes in response to cell types, hormones, environmental factors and development (3, 4, 5, 6). In the adult rat, renal PEPCK mRNA can be induced by starvation (7, 8) and suppressed by oral glucose loading (9). Recently, a study using transgenic mice showed that a high carbohydrate diet suppressed endogenous renal PEPCK mRNA production; in contrast, a high protein diet, induced PEPCK mRNA (10). Although the ontogenic pattern of hepatic PEPCK mRNA (11) and effect of diet on its gene expression during perinatal development (12, 13) have been well characterized, no studies have described the developmental profile of renal PEPCK mRNA. Our study was the first to examine the effect of maternal diet on renal PEPCK gene expression during perinatal development.

Induction of PEPCK and gluconeogenic pathway near term ensures an adequate glucose supply to the newborn when hepatic glycogen stores becomes depleted within 12 hours after birth (14, 15). Therefore, maturational changes in renal PEPCK and renal gluconeogenesis could be critical for the survival of the intrauterine growth retarded neonate, since these newborns have reduced levels of hepatic glycogen reserves (16) and impaired hepatic PEPCK activity (17). Our previous studies had shown that maternal dietary glucose restriction can cause intrauterine growth retardation (IUGR) (18, 19) similar to that in other animal models which perturb glucose delivery to the fetuses and neonates, and such as chronic hypoglycemic and hyperinsulinemic (HH) fetus and the newborn from dams with maternal uterine artery ligation. The potential role of renal PEPCK gene expression in these IUGR pups which experience high perinatal mortality (19), however, has received little, if any, attention. Study of the renal gluconeogenic potential in such animal models is important, as human infants with IUGR are frequently hyoglycemic. Whether renal gluconeogenesis is impaired in these hypoglycemic IUGR pups is unknown. To study the response of perinatal renal PEPCK gene expression to maternal glucose restriction in IUGR pups, pregnant rats were fed graded levels of glucose diets (0%, 12%, 24% and 60%) and PEPCK mRNA levels in the neonatal kidney were evaluated by northern blot analysis . Our novel results showed that maternal dietary glucose restriction delayed postnatal renal PEPCK gene expression during the first 24 hr after birth, which is associated with the previously reported high perinatal mortality (19).

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MATERIAL AND METHODS

Experimental design and diets. Impregnated Sprague-Dawley rats weighting 180-220 g were purchased from Charles River Canada (St-Constant, Quebec), and received within two days of mating. Dams were randomly assigned to one of the experimental groups which combined one of the four levels of dietary glucose (0, 12, 24, 60%) with a time of development (0-2hr, 4-6hr, 12-16hr, 24hr, 3d, 6d, 15d). All the newborn rats were delivered vaginally and killed at the same time of the day. The kidney samples were collected and stored at −80°C until analysed. The details of the diet formulation have been previously described (18,19). Sodium bicarbonate was added to control for metabolic acidosis (18).

RNA isolation and Northern blot. Specific PEPCK mRNA levels from each dietary group were measured by Northern blot and standardised by GAPDH mRNA. Total RNA was isolated from newborn rat kidney by the single step guanidinium thiocyanate-phenol-chloroform method according to the procedure described by Chomczynski and Sacchi (20). Fifteen ug of total RNA was electrophorsed on a 1.0% agarose gel in the presence of 6.6% formaldehyde. The RNA was transferred to the Hybond-N^+ nylon membrane (Amersham, Oakville, ON) in the presence of 10×SSC by using a Bio-Rad 785 vacuum transfer system. The filter was cross-linked by UV cross-linker Stratalinker 2400. Prehybridization was performed at 42°C in 35% formamide, 5×Denhardt's solution, 5×SSPE, 0.5% SDS and 100 ug/ml salmon sperm DNA. PEPCK cDNA was a 1.5 kb *Pst*I fragment of the plasmid pPCK10 and kindly provided by Dr. Richard W. Hanson Case Western Reserve University. The cDNA was labelled with (α-^{32}P) dCTP by random priming. After hybridization the filters were washed 3 times in 2×SSC, 0.1% SDS at 42°C for 15 min and once in 0.1×SSC, 0.1% SDS at 68°C for 15 min and then exposed to Kodak S°AFETY x-ray film for 2 days at −80°C with a DuPont Cronex intensifying screen. Blots were then stripped and rehybridized with a radiolabelled DNA probe corresponding to rat glyceraldehyde-3-phosphate dehydrogenase (GADPH) which was kindly provided by Dr. R. E. Mackenzie, McGill University. Quantitation of PEPCK mRNA was accomplished by densitometric scanning of the autoradiograms (Macintosh Photoshop and NIH 1.60 software). The ratio of PEPCK to GAPDH density was calculated for each sample.

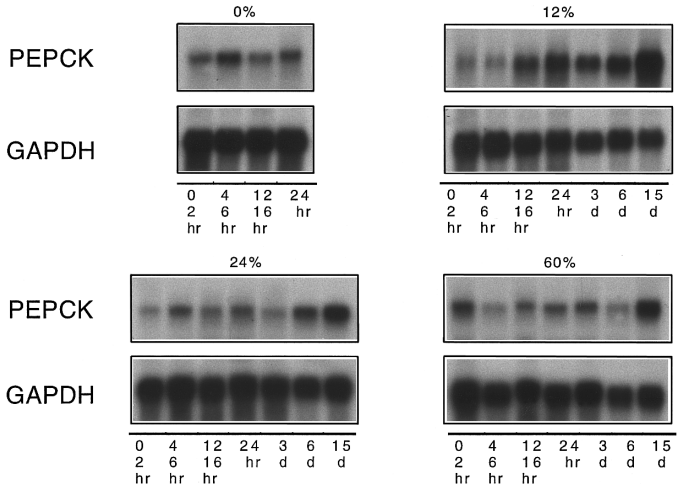

FIG. 1. Representative autoradiogram of Northern blot assay, comparing the PEPCK mRNA developmental profiles in the newborn pups derived from graded levels of maternal glucose groups. RNA was isolated from different kidney samples obtained from different time periods after birth. Each lane contained 15 μg of total RNA from 1 representative animal of each treatment group (n=3) at each time. RNA was size fractionated by electrophoresis on a 1.0% agarose gel, transferred to a nylon membrane, and hybridized with a ^{32}P-labelled PEPCK cDNA probe. After exposure to the film at −80°C for 2 days, the membrane was stripped and rehybridized with a ^{32}P-labelled GAPDH cDNA probe.

TABLE 1

Analysis of PEPCK mRNA in Newborn Rats Following the Feeding of Graded Levels of Maternal Dietary Glucose throughout Pregnancy and Lactation

	Maternal dietary glucose		
Time	0–12%	24–60%	P value
0–2 hr	0.02 ± 0.01 (n = 6)	0.24 ± 0.11 (n = 6)	p = 0.05*
4–6 hr	0.29 ± 0.07 (n = 6)	0.22 ± 0.08 (n = 6)	p = 0.55
12–16 hr	0.29 ± 0.08 (n = 6)	0.62 ± 0.13 (n = 6)	p = 0.05*
24 hr	0.69 ± 0.08 (n = 6)	0.24 ± 0.07 (n = 6)	p = 0.002*
3 d	0.27 ± 0.04 (n = 3)	0.56 ± 0.15 (n = 6)	p = 0.25
6 d	0.63 ± 0.08 (n = 3)	0.63 ± 0.15 (n = 6)	p = 0.99
15 d	1.67 ± 0.31 (n = 3)	1.42 ± 0.05 (n = 6)	p = 0.28

Quantification of PEPCK mRNA by densitometric scan of 3 autoradiograms in each dietary group at each time period, and the results were presented as PEPCK/GAPDH ratios (means ± SEM). Data from either 0 and 12% or 24 and 60% were pooled respectively due to no significant difference between the combined groups. Possibilities of significant differences relative to control group at each time period are shown using LSD post hoc test. An asterisk (*) indicates a significant difference between the comparison groups ($p < 0.05$ vs. control).

Statistical analysis. Significance of developmental and dietary effects on PEPCK mRNA level were tested by general linear models (GLM). To compare the data from different dietary groups at the same time period, a least square difference (LSD) post hoc test was performed. Values were considered significant if $p<0.05$. Since no significant difference in PEPCK/GAPDH mRNA ratios were found between 0% and 12% or 24% and 60% dietary groups, the data were pooled and defined as the glucose restriction group (0% and 12% glucose) and control group (24% and 60% glucose).

RESULT AND DISCUSSION

Gluconeogenesis occurs to a significant extent only in liver and kidney. Previous studies have concluded that liver is more important source of glucose under most physiological conditions, whereas the kidney will contribute as much as 50% of blood glucose production under stressed conditions in rats and humans (21, 22) that may include dietary restriction (7-10). In our study, maternal dietary glucose restriction in utero produced barely detectable PEPCK mRNA at birth (Figure 1 and Table 1). A small but significant difference of PEPCK mRNA level ($p=0.05$) was quantified with significantly lower amounts measured in offspring from the glucose-restricted (0% and 12%) compared to glucose-adequate (24% and 60%) dams. However, within 4-6 hrs postpartum, a rapid accumulation of renal PEPCK mRNA occurred in glucose-restricted pups. Little change was observed from 4-6 hr to 12-16

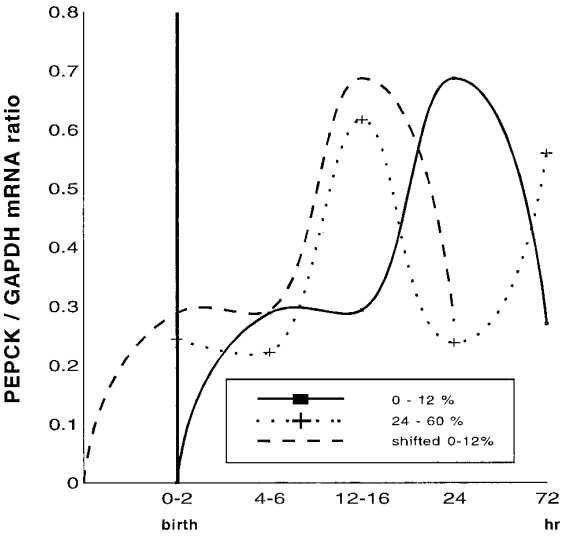

FIG. 2. Effects of maternal dietary glucose on PEPCK mRNA in newborn rat kidney during the first 72 hours after birth. Tissues were removed and analyzed for PEPCK mRNA as depicted in Fig. 1. Data are expressed as PEPCK/GAPDH ratio. The PEPCK mRNA developmental pattern of 0-12% diet was shifted one time period earlier (leftward) and represented by the dashed line.

hr after birth in the glucose-restricted pups. These pups reached their peak level 24 hrs after birth in contrast to the earlier peak (12-16 postpartum hour) in the pups born to dams with the higher (24% and 60%) glucose diets (Figure 2). The decrease in normalized steady state level of PEPCK mRNA in the 24% and 60% dietary glucose groups at 24 hrs approached 38% whereas the 0% and 12% dietary treatment groups were associated with a 2.3 fold increase in steady-state mRNA levels (Table 1 and Figure 2). On postnatal day 3 (72 hr), mRNA level was still 50% lower in the glucose-restricted pups, suggesting a continuing delay at this critical time point. This developmental rise in PEPCK mRNA levels at day 3 had, in earlier dietary studies (19), been associated with high perinatal mortality. By days 6 and 15, values between the glucose-restricted and glucose-sufficient were comparable in surviving pups. In summary, our results show for the first time that maternal dietary glucose restriction impairs PEPCK mRNA accumulation in newborn kidney, and we conclude that the developmental pattern is delayed by at least 12 hr.

Using transgenic mice, Short and colleagues (10) had shown that renal PEPCK gene in adults responded to the dietary carbohydrate level. In their study, with a reduction of the dietary carbohydrate content from 70% to 10%, renal PEPCK mRNA level significantly increased. Pollock (9) also indicated that glucose loading is a negative modulator of renal PEPCK expression, which is independent of the effect of insulin and metabolic acidosis. The results from this study show that the renal PEPCK in the neonates is also regulated by dietary glucose. However, in contrast with renal PEPCK gene expression in the adult, renal PEPCK gene expression in the neonate was suppressed, not stimulated, by maternal glucose restriction. The delay of developmental pattern of PEPCK mRNA in glucose-restricted pups indicates that renal gluconeogenesis can not maintain the glucose homeostasis under dietary stress as we see in the adult. Thus, our results strongly suggest that the regulation of renal PEPCK gene expression by glucose during the perinatal period is more complicated and different from the adult. However, the mechanism of the delayed response of PEPCK gene expression is currently unclear. It might result from immature intracellular transduction pathway or the blunted response to hormones, as a previous study (17) has shown that IUGR pups are not sensitive to physiological levels of glucagon, which normally induce PEPCK gene expression in the adult. The absence of renal PEPCK mRNA at birth also suggests that 0% and 12% maternal glucose feeding compromised in utero fetal kidney development, which contributes to the high perinatal mortality of these pups. More work is required in the fetus and neonate before the underlying relationship between adequate dietary glucose and prenatal renal PEPCK gene expression is fully understood.

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