Gluconeogenesis, Glucose Handling, and Structural Changes in Livers of the Adult Offspring of Rats Partially Deprived of Protein During Pregnancy and Lactation

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Abstract

Maternal protein restriction is a model of fetal programming of adult glucose intolerance. Perfused livers of 48-h–starved adult offspring of rat dams fed 8% protein diets during pregnancy and lactation produced more glucose from 6 mM lactate than did control livers from rats whose dams were fed 20% protein. In control livers, a mean of 24% of the glucose formed from lactate in the periportal region of the lobule was taken up by the most distal perivenous cells; this distal perivenous uptake was greatly diminished in maternal low protein (MLP) livers, accounting for a major fraction of the increased glucose output of MLP livers. In control livers, the distal perivenous cells contained 40% of the total glucokinase of the liver; this perivenous concentration of glucokinase was greatly reduced in MLP livers. Intralobular distribution of phosphenolpyruvate carboxykinase was unaltered, though overall increased activity could have contributed to the elevated glucose output. Hepatic lobular volume in MLP livers was twice that in control livers, indicating that MLP livers had half the normal number of lobules. Fetal programming of adult glucose metabolism may operate partly through structural alterations and changes in glucokinase expression in the immediate perivenous region. (*J. Clin. Invest.* 1997. 100:1768–1774.) Key words: fetal development • glucose intolerance • glucokinase • hepatic gluconeogenesis • phosphenolpyruvate carboxykinase

Introduction

Epidemiological studies have shown reproducibly strong links between indices of poor fetal and infant growth and impaired glucose tolerance, non-insulin-dependent diabetes mellitus, and the insulin resistance syndrome (1). A hypothesis has been proposed to explain these linkages in terms of poor maternal and fetal nutrition (2). It was suggested that poor nutrition, particularly as it relates to dietary protein, could cause growth retardation and permanent changes in key organs responsible for glucose homeostasis in adult life. To test this hypothesis, we have adopted a rat model where rat dams are fed diets in which the protein content has been reduced from 20 to 8% during pregnancy and lactation (3). The offspring of these pregnancies are henceforth designated maternal low protein (MLP),[1] are small at birth, and show more rapid deterioration of glucose tolerance in adult life (4, 5), with increased hepatic phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32)—a flux-regulating enzyme of gluconeogenesis—and decreased activity of glucokinase (GK)—a high K_m hexokinase IV (EC 2.7.1.2)—regulating hepatic glucose uptake (5, 6). GK is expressed particularly in the perivenous region of the hepatic lobule and PEPCK, predominantly periportally, though this zonation is less obvious with starvation (7).

We have developed (8) a new technique in one of our laboratories for detailed mapping of metabolic function, metabolite concentration, and enzyme activity along the radius of the hepatic lobule from the immediate periportal cells to the perivenous region, without the need to prepare isolated hepatocytes. We have used this technique to map glucose handling in the hepatic sinusoid in perfused livers from MLP and control rats. It was shown that the net glucose output from 6 mM lactate in livers from starved MLP rats was greater than that from starved control rat livers. In control rats, part of the glucose produced periportally was removed by perivenous cells; in MLP rats this process is absent or greatly diminished. MLP rats do not appear to possess the substantial amount of glucokinase that exists in cells close to the central vein in control rats. This fact may be a major part of the reason for the increase in net glucose output in livers from MLP rats. We also report initial observations of hepatic morphometric changes in MLP rats.

Methods

Animals. All animals used were female Wistars, of mean age 5.6±0.2 mo (Dunn Nutritional Laboratory, Cambridge, UK). Their dams were fed as previously described (9), receiving ad lib diets containing either 20% protein, or an isocaloric 8% protein during pregnancy and

1. *Abbreviations used in this paper:* FGO, fraction of glucose output remaining; FVR, fractional volume remaining; GK, glucokinase; GKR, glucokinase activity remaining; HK, hexokinase; HKR, hexokinase activity remaining; MLP, maternal low protein; PEPCK, phosphoenolpyruvate carboxykinase; PEPCKR, phosphoenolpyruvate carboxykinase activity remaining.

lactation. Their offspring have been designated as control and MLP, respectively. It has been shown in previous studies that the total caloric consumption of pregnant dams in the two groups are not significantly different (9). All pups were weaned at 21 d onto a standard laboratory chow diet containing 20% protein, and were thereafter housed and fed under identical conditions. We have shown (unpublished data) that MLP offspring have caloric intakes 4–10 wk after birth that are similar to those of control offspring when expressed per unit body wt. The offspring were deprived of food but not water for 48 h before the study. MLP animals at the age used are not glucose intolerant, but are destined to become so (4).

Isolated liver perfusions were set up as previously described (10). The erythrocyte and albumin-free perfusate at 37°C consisted of Krebs bicarbonate buffer (11) gassed with $95\%O_2/5\%CO_2$ to give pH 7.4, with 6 mM sodium L(+)-lactate as the sole substrate. The flow rate was 11 ml min^{-1} per 100 g rat wt. Perfusion was switched to non-recirculation at 10 min (t = 0 defined as the time of cannulation) and then allowed to stabilize for a further 20 min. 4-ml perfusate samples were taken at 35, 40, and 45 min from the portal and hepatic venous perfusion lines, the perfusate being mixed immediately with 1 ml aqueous 20% perchloric acid (PCA, vol/vol) (final [PCA] = 4%) and frozen at −70°C until glucose assays were performed. Flow rate was measured at the same time as perfusate sampling. Hepatic venous PO_2 was always > 20 kPa.

Mapping of intralobular glucose production and enzyme activity. This mapping was carried out as previously described in detail (8). In brief, baseline portal and hepatic venous samples were obtained as above for estimation of net glucose output by the Fick principle. At 50 min, digitonin (4 mg ml^{-1} in Tris buffer, pH 7.4) was then perfused retrogradely at ∼ 30 ml·min^{-1} for times that varied from 10 s to 2 min in different livers. Digitonin avidly binds plasma membrane cholesterol on a first-pass basis, the digitonin front thus moving retrogradely along the sinusoid at a slower rate than the medium. Digitonin-affected cells are permeabilized and lose their cytosolic contents, unlike mitochondria, which are grossly swollen. Cells which have not been reached by the digitonin front are histologically (12, 13), electron-microscopically (13), and functionally (8) normal in terms of gluconeogenesis from lactate. Demarcation of digitonin-destroyed from digitonin-unaffected cells is sharp (8, 12, 13). Flow was resumed in the normal antegrade direction, and portal and hepatic vein sample pairs were taken at 60, 70, and 80 min for estimation of net glucose production. The liver was then fixed by perfusion at 10 ml·min^{-1} with 10% formalin, and blocks were removed for preparation of haematoxylin and eosin–stained sections. The proportion of destruction by area was then determined in each liver by automated histomorphometry (8).

For study of enzyme content, the posterior-superior lobe was removed before digitonin perfusion. Two blocks of liver (∼ 400 mg each) were stored as follows: (*a*) For GK and hexokinase (HK, EC 2.7.1.1) assay, blocks were placed in 4 ml 0.9% NaCl (wt/vol) at 4°C; (*b*) for PEPCK assay, in a 5-ml Universal tube and rapidly frozen in liquid nitrogen.

The flow rate was reduced by 15% after lobe excision, the digitonin procedure was instituted and the anterior lobe was removed for a second determination of enzyme activities after the glucose output samples had been taken, and before formalin fixation. Primary plots were then made of the fractional change of net glucose output or enzyme activity (post- compared with predigitonin) against the mean fractional volume of lobule (and therefore, fractional volume of liver) remaining unaffected by digitonin. Fractional volume remaining (FVR) was determined by raising the fractional area destroyed as measured by histomorphometry to the power of 3/2, and subtracting from unity. Curves were then fitted to these primary plots (see statistical methods).

Analytical procedures. For glucose, perfusate samples were thawed and neutralized to pH 7.4, and glucose concentration was measured with the GOD-PAP method (14). Hepatic glucose output was then obtained using the Fick principle. Lobes excised for enzyme activities were processed for glucokinase and hexokinase assay, essentially as

described by Arion and Davidson (15). All samples were collected and stored within 2 min, and were analyzed within 8 h.

A 33.3% (wt/vol) liver homogenate was prepared at 4°C in a medium containing Hepes (sodium salt, 50 mM), KCl (0.1 M), of EDTA (sodium salt, 1 mM), $MgCl_2$ (5 mM), and dithiothreitol (2.5 mM) at a final pH of 7.4. The supernatant was obtained for the assay by centrifugation at 100 000 *g* for 60 min at 4°C (Sorvall ultracentrifuge OTD 65B, 12 × 12 rotor), which was then performed at 37°C using two different glucose concentrations, 100 mM for total glucokinase and hexokinase, and 0.5 mM for hexokinase only, allowing glucokinase activity determination via subtraction. The assay was performed in the presence of glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*, using NAD as cofactor). The activity was expressed as μmol NADH formed per min. The conditions of the estimation were such as to include both free glucokinase and that bound to the glucokinase-binding protein (16). For PEPCK (17), the liver was homogenized at 4°C in 0.25 M sucrose, 5.0 mM Tris/HCl, and 1.0 mM mercaptoethanol at pH 7.4. The homogenate concentration was 33.3% (wt/vol), and the supernatant was obtained by centrifugation as described above for GK. The activity was assayed at 37°C in the presence of NADH and malate dehydrogenase by measuring the rate of [^{14}C]bicarbonate incorporation into malate. 1 U of activity is that amount of enzyme that catalyzes fixation of 1 μmol [^{14}C]bicarbonate per min. All reagents used were of analytical quality.

Measurement of lobular dimensions. Only postdigitonin liver samples from livers given a marking pulse with relatively small volumes of digitonin were used to make measurements of lobular size (mean fraction destroyed by volume = 0.28 and 0.24 for control and MLP, respectively). The cells immediately surrounding a central hepatic venule were thus permeabilized and stained poorly, allowing confident identification of centrilobular venules and portal tracts. Linear lobular dimensions were measured from the center of the hepatic venule to the center of three related portal vein radicals identified on the basis of clear sinusoidal relationships between portal vein and hepatic vein. The mean radius of each lobule was then calculated. Five lobules in each liver were thus assessed.

Statistical methods. Means were expressed±SEM. Two-tailed paired or unpaired *t* tests were used as appropriate. Net glucose output were the means of the three predigitonin estimations and three postdigitonin measurements. Curves were fitted to the primary plots of fractional glucose output remaining (or fractional enzyme activity remaining) against FVR using the Levenburg-Marquandt algorithm (18). The curves were chosen by goodness of fit (minimum residual sum of squares) and to pass through the (1,1) point on this plot; this latter constraint has been justified for several variables, including glucose output (8). Where curvilinear fits were used, they were always significantly better than straight lines. The equations of the fitted curves were then transformed, assuming that the lobules are on average spherical as described (8), to give plots (with their confidence limits) of relative glucose output or uptake or enzyme activity at different points along the radius of the hepatic lobule. Metabolic rates or enzyme activities are expressed relative to those in the most proximal periportal region studied. The corresponding FVR at which this point was chosen is given in the figure legends.

Results

Hepatic glucose production in nondigitonized perfused livers. The design of the liver perfusion protocol precluded weighing the livers. In previous studies we have shown (9) that the weight of livers of MLP rats form the same percentage of body wt as in controls. Therefore, in calculating hepatic glucose output we have expressed results in relation to body wt. The hepatic glucose output with 6 mM L-lactate as substrate in perfused livers from starved control rats was 1.18±0.10 μmol. min^{-1}·100 g^{-1} rat (*n* = 9); that by livers from starved MLP rats

(1.59 ± 0.14 μmol.min^{-1}·100 g^{-1} rat [$n = 9$]) was significantly greater ($P < 0.03$).

Intralobular glucose handling. Fig. 1 shows the effect of varying retrograde destruction by digitonin on glucose output. Two models were fitted to all 60 data points. The first model (M1) was the best-fitting two-parameter model (fraction of glucose output remaining [FGO] = 2.42 [FVR] $-$ 1.478 [FVR]$^{0.5}$), chosen from among several empirical two-parameter models, all of which had the necessary property that y = 1 when FVR = 1. The second model (M2) had four parameters; it consisted of two models like M1, one fitted to the control group, and the other fitted to the MLP group. The equations for M2 are given in the legend to Fig. 1 for the control group and the MLP group, respectively. For M1, the residual sum of squares was 1.88547 on 58 degrees of freedom, while for M2 it was 1.54259 on 56 degrees of freedom. Hence, the mean square for the extra two parameters in M2 was 0.17144 on two degrees of freedom, giving an F-statistic of 6.22 on 2 and 56 degrees of freedom. Thus, there is strong evidence ($P = 0.005$) that model M1 is not a satisfactory fit to the data compared to model M2.

In control animals, small perivenous destructions (FVR > 0.7) resulted in an increase (mean 24±4%; $n = 14$; $P = 0.0002$) in glucose output over the predigitonin value. In livers from MLP rats, small perivenous destructions (FVR > 0.7) produced no significant change in glucose output (mean increase 7±5%; $n = 15$; $P = 0.32$). With progressively larger destructions, mean glucose output fell similarly in both groups. These data suggest that glucose formed from lactate in the more periportal zones was partially taken up in the distal perivenous cells in the control livers, but that this effect was not significant in MLP livers.

Each data point in Fig. 1 refers to the function of the whole of the remaining viable liver, i.e., periportal to the demarcation from digitonin-affected cells. To determine the function (or enzyme activity) at each point along the lobular radius relative to the value at the most periportal region studied, the data has to be transformed as described in Methods and, in more detail, in appendix 1 of reference 8. For this purpose, curves have been fitted (18) to the data points of Fig. 1, and their equations are given in the legends to Fig. 1. These equations do not represent any preconceived model of glucose handling, but have been developed to allow the mathematical differentiation involved in the transformation. The assumptions implicit in this procedure (8) are that the hepatic lobules are, on average, spherical, and that from the standpoint of metabolism of substrates entering via the portal vein and tracts, blood enters the periphery of the lobule and traverses the sinusoids centripetally to the hepatic venules.

Fig. 2 shows the result of transforming the curves. It may be noted that periportally there was net glucose output by hepatocytes that ceased in both control and MLP rats at $\sim$ 50% of the way down the radius of the lobule from the portal tracts. More perivenously, there was increasing net glucose uptake in the control livers, whereas in MLP livers the confidence bands include zero. It should be noted that the derived curves in Fig. 2 do not extend to fractional radii less than 0.3. Such small fractional radii encompass the radius of the centrilobular vein, and consist of fractional cell volumes of 0.027 or less.

Enzyme activities. Table I shows the activities of GK, HK, and PEPCK in livers from MLP and control rats before digitonin. PEPCK activity was significantly greater, and glucokinase
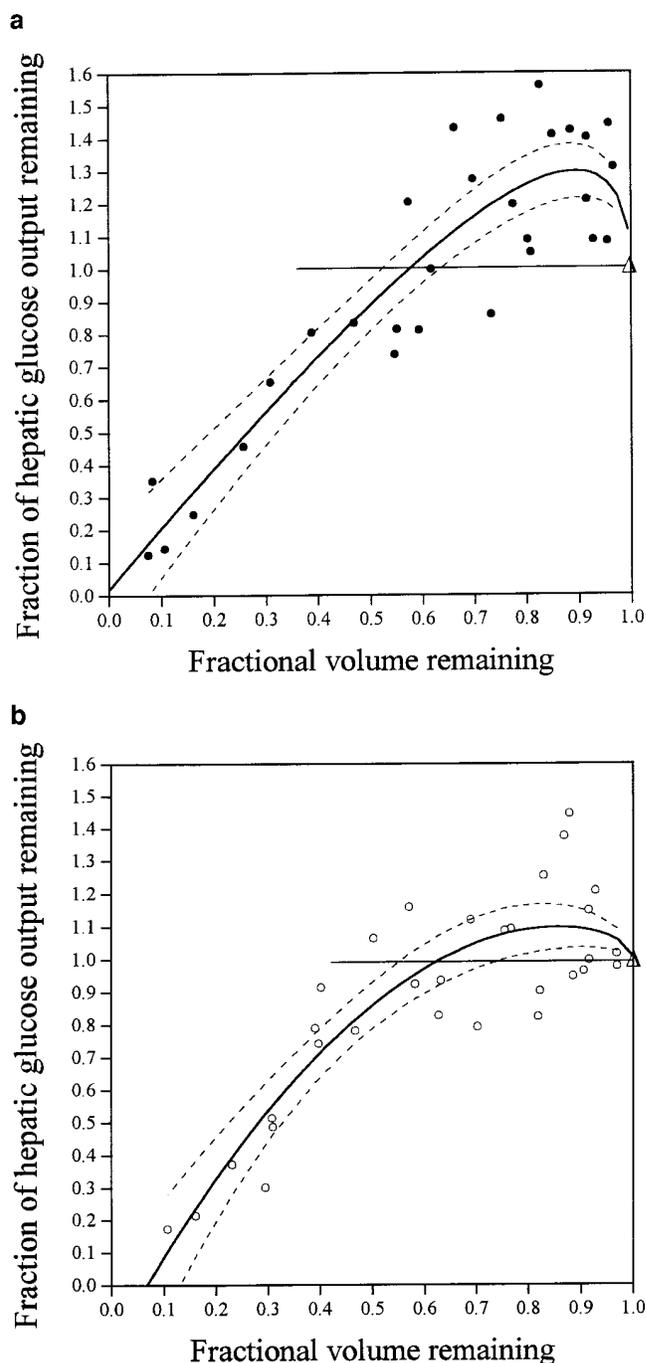
Figure 1. FGO remaining after retrograde digitonin perfusion of varying duration, plotted against average FVR. 6 mM lactate was the sole substrate. Each point is derived from a single liver (*filled circles*, livers from control rats; *open circles*, livers from MLP rats). Points above the horizontal line denote that net glucose output was increased after digitonin. The solid curve in (*a*) represents the equation FGO = 1 $-$ 2.81(1-FVR) + 1.83(1-FVR)$^{0.5}$, fitted to the control data. The solid curve in (*b*) represents the equation FGO = 1 $-$ 1.528(1-FVR)^2 + 0.343(1-FVR)$^{0.5}$, fitted to the MLP data. Both fitted curves pass through the point (1,1), denoted by Δ (see Methods). The 95% confidence limits of the curves are also shown.
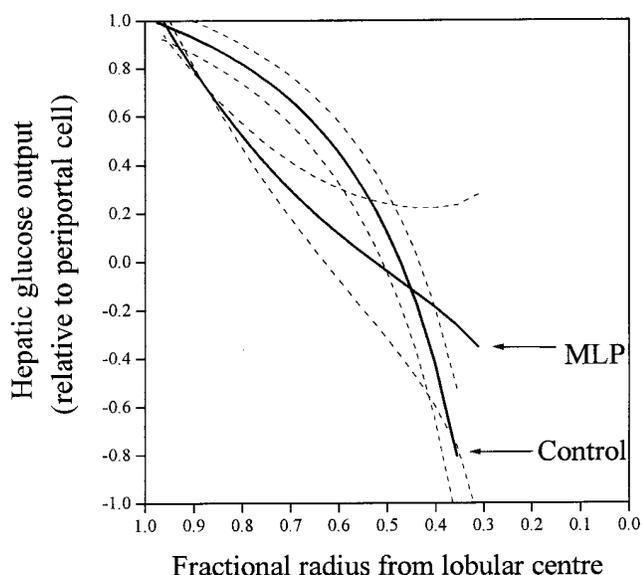
Figure 2. Effect of mean fractional radius from lobular centre on relative cellular net glucose output. These curves are derived from the primary fitted curves of Fig. 1 as described in Methods and in reference 8. The 95% confidence limits of each curve are shown. For each curve, the value at the most periportal location studied (the minimum FVR) was taken as unity. These minimum FVRs were as follows: control livers, 0.074; MLP livers, 0.107.

activity was significantly less in MLP animals, showing that the previous findings (5, 6) made in livers from fed rats remain true during starvation. There were no significant differences in HK activity.

Fig. 3 shows the primary data using the digitonin technique to map the distribution of GK activity. In the control livers, very small perivenous destructions caused 40–45% loss in GK activity. As the FVR decreases (destruction increases), there was little further change in GK activity until FVR reaches 0.7; at lower FVRs there was a gradual loss of GK activity until it virtually disappeared at small FVR values. In contrast, in MLP rats there was no large loss of GK at small destructions; instead there was a gradual loss of activity. At FVR values < 0.7 the pattern of loss is similar to that in control livers, and the fitted curve is a straight line. Using the same cutoff as for glucose, there is a highly significant difference ($P = 0.002$) between the mean fraction of glucokinase activity remaining at

Table I. Enzyme Activities in Intact Liver from Control and MLP Rats

	Control livers	MLP livers
	U/g liver	*U/g liver*
Phosphoenolpyruvate carboxykinase	1.14 (0.046)	1.996 (0.085)*
	n = 15	*n* = 15
Glucokinase	1.922 (0.178)	1.179 (0.098)‡
	n = 16	*n* = 19
Hexokinase	0.284 (0.018)	0.274 (0.013)§
	n = 16	*n* = 19

Values are mean (SEM). *$P < 0.001$; ‡$P = 0.0012$: §$P = 0.66$.

FVR > 0.7 in the MLP (0.85 ± 0.033, $n = 10$) and control livers (0.62 ± 0.050, $n = 8$).

The inference from these observations is, as shown in Fig. 4 (which maps GK activity on a point-by point basis along the lobular radius), that in control livers the immediate perivenous cells contained a large concentration of GK (substantially greater than in the immediate periportal cells, and much greater than in the cells of the midzone of the lobule where the activity of GK is in any case probably overestimated due to difficulty of curve-fitting to the abrupt changes in this region). This distribution has similarities with that shown by immunochemical methods to be established by 30 d after birth in normal rats (19). In control livers, there are ~ 15 hepatocytes along the lobular radius. The enhanced concentration of GK activity apparently occupies the perivenous one-third of the radius, i.e., approximately five cells. It is necessary, however, to adjust for the contribution of the hepatic venule to the radius; when this is done, perivenous enhancement of GK activity encompasses only the most immediately perivenous 2–3 cells. This was borne out by histological examination of livers from control animals that received a digitonin pulse of only short duration, and only exhibited destruction of the 2–3 most perivenous cells. Nevertheless, these livers characteristically showed a 40% reduction in GK activity. In contrast, in MLP rats there was a uniform distribution of GK activity along the lobular radius.

Fig. 5 shows that increasing digitonin destruction produces similar decrements in fractional PEPCK activity in control and MLP rats, implying a similar distribution along the lobular radius in the two groups. Nevertheless, in view of the overall increase in PEPCK activity in MLP rats, the absolute activity of
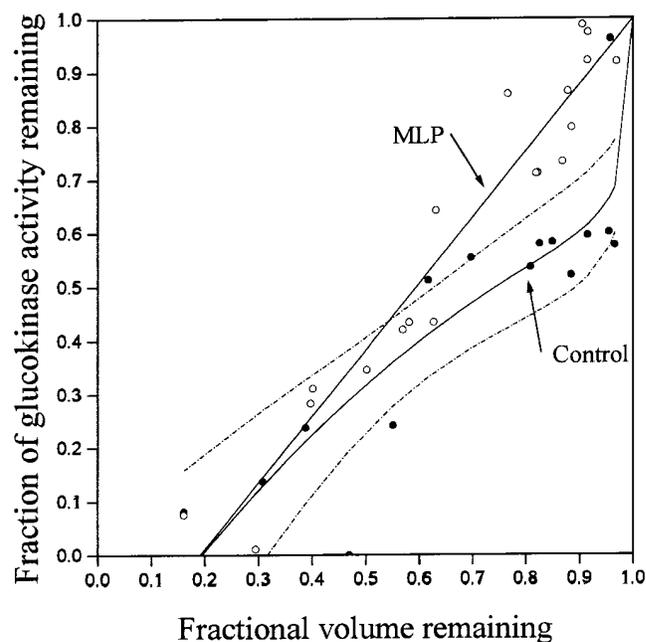

Figure 3. Fraction of glucokinase activity remaining (GKR) plotted against FVR, in control and MLP livers. Studies, style and symbols are as described in Fig. 1. The equation fitted to the control data is GKR = $1 - 1.25(1\text{-FVR})^{1.5} + 0.493(1\text{-FVR})^{0.5} \cdot \ln(1\text{-FVR})$. The data from MLP livers is best fitted by the straight line GKR = $0.993 - 1.219(1\text{-FVR})$. Confidence limits for MLP omitted for clarity.
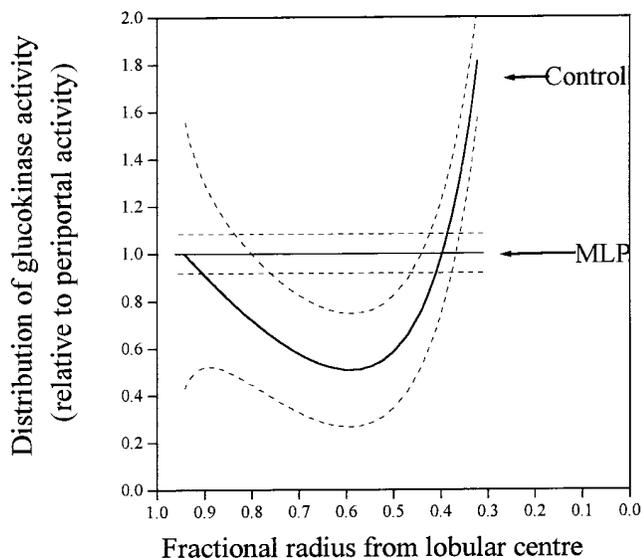
Figure 4. Effect of mean fractional radius from lobular centre on relative glucokinase activity. These curves are derived from the primary fitted curves of Fig. 3 as described in Methods and in reference 8. The 95% confidence limits of each curve are shown. For each curve, the value at the most periportal location studied (the minimum FVR) was taken as unity. These minimum FVRs were as follows: control, 0.161; MLP, 0.161.

PEPCK must be greater than in control livers at any point along the lobular radius. The lack of zonation of PEPCK in starved, as opposed to fed rats, has been previously reported (7). Fig. 6 shows no differences in the uniform distribution of HK between the two groups.

Figs. 2, 4, and 5 demonstrate that as the level of digitonin destruction increased to high levels, the activities of GK, HK, and PEPCK all tended towards zero, indicating that no enzyme activity remained in the digitonin-affected zones.

Lobular dimensions. The mean radius of hepatic lobules was significantly (25%) greater in MLP than in control livers; MLP, 430±28.3 μm ($n = 11$); control, 342±11.1 μm, $P < 0.0001$. If the shape of hepatic lobules is on average spherical, this implies that lobules from MLP rats had double the mean volume of lobules in control livers. Previous studies have shown that the mean liver wt/body wt ratio in the two groups of rats was not significantly different (9), and implies that MLP livers contained fewer, but larger, lobules.

Discussion

The first novel finding from these experiments is that in control perfused livers, some glucose formed periportally was taken up by distal perivenous cells. Thus, a clear advantage of the mapping technique used is that it allowed such interzonal relationships to be detected. The observation implies that previous estimates (20) of the gluconeogenic capacity of the perfused liver from starved rats may have been too low. The fate of the glucose taken up in the most perivenous cells is as of yet uncertain. Some possibilities are glycogen and lipid synthesis (unlikely in starved animals), or energy generation by glycolysis, which may be most prominent in the perivenous region (21). Another possibility is glutamine synthesis, which is confined to the most perivenous cells (22), but glucose is a poor
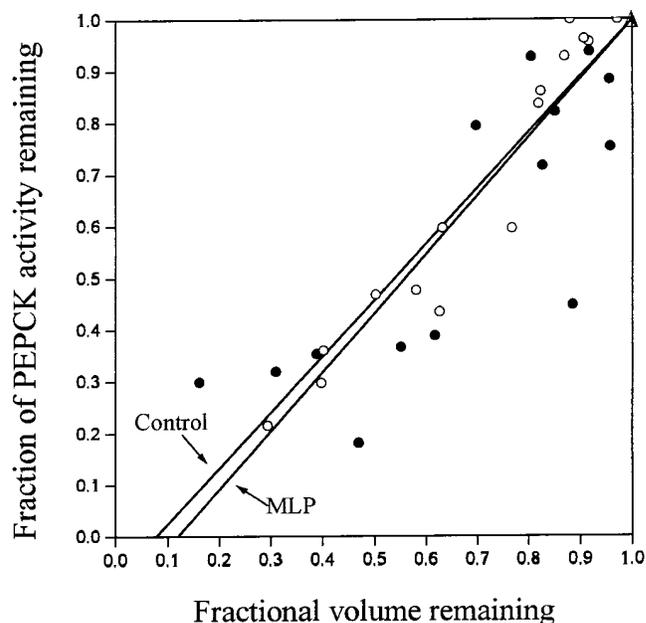

Figure 5. Fraction of phosphoenolpyruvate carboxykinase activity remaining (PEPCKR), plotted against FVR, in control and MLP livers. Studies, style, and symbols are as described in Figs. 1 and 2. Both data sets are best fitted by straight lines (controls, PEPCKR = 1 − 1.084[1 − FVR]; and MLP, PEPCKR = 1 − 1.135 [1 − FVR]). Slopes are not significantly different.

source for the carbon skeleton of glutamine compared with lactate (23). It is also of interest that in our previous work (8), the increase in glucose output at small levels of destruction was not detected in similar studies to those reported here. The dif-
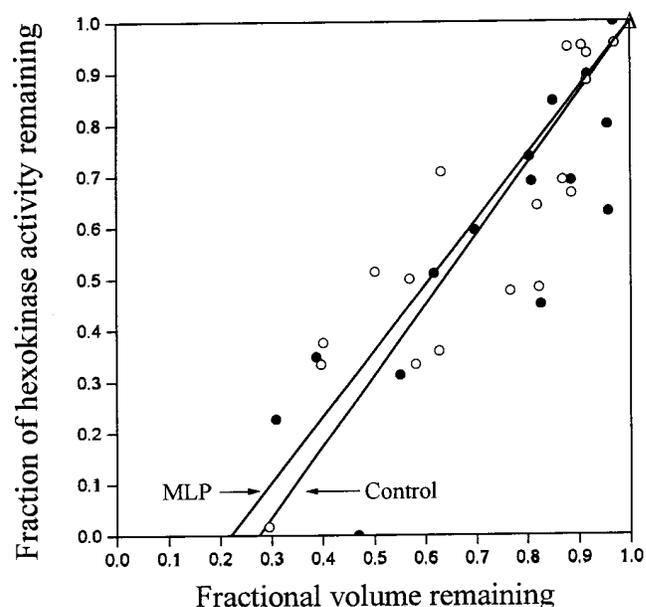

Figure 6. Fraction of hexokinase activity remaining (HKR), plotted against FVR, in control and MLP livers. Studies, style, and symbols are as described in Figs. 1 and 2. Both data sets are best fitted by straight lines (HKR = 1 − 1.379[1 − FVR] for controls; HKR = 1 − 1.2819[1 − FVR] for MLP). Slopes are not significantly different.

ference may be related to the use of male rather than female animals in that study, or to the fact that the perivenous region was studied in greater detail in this study. There was a suggestion of the phenomenon in studies using 1.5 mM rather than 6 mM lactate (8).

The second principal finding of this study is the increased net glucose output from lactate in MLP rats, which is related to the difference in hepatic glucose handling between the two groups of rats. Livers from starved MLP rats perfused with 6 mM lactate as sole substrate did not show the distal perivenous uptake of glucose previously formed in the periportal region that is demonstrated in control livers. Fig. 2 suggests that this effect in control livers takes place in the most distal few perivenous cells. The increment of glucose output on destruction of these cells in control livers averaged 24%; this was of the same order, but somewhat less, than the mean percentage elevation of glucose output (34.5%) seen in MLP when compared with control livers. The discrepancy could be accounted for by increased gluconeogenesis related to the greater absolute PEPCK activity found in MLP livers.

The third and potentially most important finding is the virtual absence in MLP livers of the enhanced glucokinase activity observed in the distal perivenous region in control livers. In control livers, small perivenous destructions resulted in loss of ∼ 40% of the total liver glucokinase, comparable with the difference in overall glucokinase activity between whole control and MLP livers; no such large loss occurred in MLP livers subject to similar small perivenous destructions. The question arises as to whether failure of distal perivenous glucose uptake in MLP livers can be attributed to the apparent lack of distal perivenous glucokinase. This question has to be considered in the light of quantitative information available on the kinetics of glucose uptake and phosphorylation in the liver. The perfusate reaching the perivenous cells in these studies had a glucose concentration of < 0.5 mM, derived entirely by gluconeogenesis from lactate. This is above the K_m for hexokinase (∼ 0.1 mM) but below that for glucokinase (10–20 mM), so in control livers glucose phosphorylation should be related to the glucose concentration. One hypothesis is that the capacity of perivenous hexokinase is too low to cope with glucose phosphorylation, which is undertaken by glucokinase in control livers, a route much diminished in MLP rats because they lack the normal concentration of glucokinase in this region. Another possibility is that in both control and MLP livers, glucose uptake was limited by some other step, such as glucose transport into the hepatocyte, which might have differed in its activity in the two groups. The facilitative glucose transporter GLUT2 is expressed in the plasma membranes of hepatocytes in all zones of the lobule, and has a high K_m for glucose (10–15 mM); the GLUT1 transporter (K_m for glucose 1–2 mM) is expressed in the plasma membrane only in the most perivenous cells, though the anatomical extent of its expression is somewhat increased by starvation (24). An argument against either transporter being rate-limiting in control livers is the observation that transfection of rat hepatoma cells in primary culture with an adenovirus containing the glucokinase gene resulted in a striking increase in glucose uptake (25); furthermore, it has been shown (26) that disruption of the glucokinase gene in mice, resulting in +/− heterozygotes, causes a lower liver GK activity (by 37%), and significantly reduced liver glucose phosphorylation flux. These observations are consistent with the hypothesis that it is glucokinase rather than glucose transport

that is rate-determining, and that the loss of perivenous glucokinase in MLP livers is a major factor in the failure of perivenous glucose uptake.

The final major finding from these experiments was the increase in lobule size in MLP rats, though the liver weights, as a proportion of body wt, are unchanged. It is unknown what effect this would have per se on glucose metabolism and other metabolic processes. Increase in lobular size has been found in two other circumstances of which we are aware: after regeneration from partial hepatectomy (27) and during pregnancy (28). It is known that enzyme zonation as determined by immunohistochemistry is altered as lobular size increases after partial hepatectomy, a smaller proportion of the centrilobular cells containing glutamine synthetase than before hepatectomy. Whether similar changes occur during pregnancy is unknown. Studies similar to the present ones in these situations would be of interest. At present, the precise mechanism of the change in hepatic lobule size and enzyme content is unknown, though it is inviting to consider that they may be associated.

Since protein deprivation solely during the period of pregnancy is sufficient to induce these changes (5), it is clear that the mechanism of programming has operated during fetal life. Neither GK or PEPCK, however, is zonated at birth, and their expression is very low until weaning so that the eventual metabolic consequences of the processes taking place in fetal life may not be fully apparent until some time after birth. It is not known whether any intervention is capable of reversing the changes postnatally.

The extent to which these observations can account for increased glucose intolerance with age of MLP rats (4), or can be applied to clinical non-insulin-dependent diabetes, needs to be established. There is clear evidence that low birth weight babies are more likely to develop diabetes in later life. Evidence that the smallness is partly related to nutritional factors in fetal life is accumulating (29); whether the protein deficiency model used in this study is the most relevant is uncertain. The results presented here, however, show that profound and persistent structural and functional changes can be induced in a key organ that serves major metabolic and other functions simply by a relatively modest alteration in the maternal diet during pregnancy and lactation.

Similar processes could occur in man, and further studies of the effects of maternal nutrition should be worthwhile. Patients with the common type of non-insulin-dependent diabetes also show decreased hepatic glucokinase activity (30), though the lobular location of this abnormality is unknown. These observations may also be of interest in relation to the discovery that mutations in the glucokinase gene, or in genes for transcription factors involved in the control of glucokinase expression, are responsible for some cases of maturity onset diabetes of the young (31–33). It has been further shown (34) that insulin resistance contributes to the hyperglycemia of diabetes due to glucokinase gene mutations, and that patients with this disorder exhibit raised hepatic glucose output.

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