Fetal Programming of Perivenous Glucose Uptake Reveals a Regulatory Mechanism Governing Hepatic Glucose Output During Refeeding

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Increased hepatic gluconeogenesis maintains glycemia during fasting and has been considered responsible for elevated hepatic glucose output in type 2 diabetes. Glucose derived periportally via gluconeogenesis is partially taken up perivenously in perfused liver but not in adult rats whose mothers were protein-restricted during gestation (MLP rats)—an environmental model of fetal programming of adult glucose intolerance exhibiting diminished perivenous glucokinase (GK) activity. We now show that perivenous glucose uptake rises with increasing glucose concentration (0–8 mmol/l) in control but not MLP liver, indicating that GK is flux-generating. The data demonstrate that acute control of hepatic glucose output is principally achieved by increasing perivenous glucose uptake, with rising glucose concentration during refeeding, rather than by down-regulation of gluconeogenesis, which occurs in different hepatocytes. Consistent with these observations, glycogen synthesis in vivo commenced in the perivenous cells during refeeding, MLP livers accumulating less glycogen than controls. GK gene transcription was unchanged in MLP liver, the data supporting a recently proposed posttranscriptional model of GK regulation involving nuclear-cytoplasmic transport. The results are pertinent to impaired regulation of hepatic glucose output in type 2 diabetes, which could arise from diminished GK-mediated glucose uptake rather than increased gluconeogenesis. *Diabetes* 52:1326–1332, 2003

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3MP, 3-mercaptopicolinate; FVR, fractional volume remaining; GK, glucokinase; GNG, gluconeogenesis; G6P; glucose 6-phosphate; GKRP, glucokinase regulatory protein; MLP, maternal low protein; NMR, nuclear magnetic resonance; PAS, periodic acid-Schiff; PVGU, perivenous glucose uptake; TSP, trans-sinusoidal pathway.

The precise mechanism(s) by which the liver normally switches rapidly from glucose production during fasting to glucose uptake and glycogen synthesis after refeeding or a glucose load remain unclear. Net hepatic glycogenolysis is promptly curtailed during glucose infusion, but gluconeogenesis is not decreased (1). These and other observations (2) have contributed to the view that glycogen is mainly synthesized via an "indirect" pathway during the early postprandial phase, in which gluconeogenic glucose-6-phosphate (G6P) is diverted to glycogen (3,4), rather than via the "direct" pathway from glucose to glycogen. Experiments in humans and in rats have demonstrated that if insulin is maintained at constant levels but glycemia is increased by glucose administration, then hepatic glucose output is rapidly suppressed and hepatic glucose uptake rises (1,5–8); postprandial hyperglycemia in type 2 diabetes is partly caused by failure of this mechanism. Hyperglycemia per se has no significant effect on hepatic gluconeogenic flux (1), and refeeding does not suppress rat liver G6P (9) in the time frame in which glucose production is switched off in individuals without diabetes (6,7). These observations raise the problem of how the balance between glycogen synthesis, gluconeogenesis, and glucose output is regulated from a generally assumed common pool of G6P.

However, none of these studies takes account of metabolic heterogeneity along the radius of the hepatic lobule. If glucose output and uptake occurred in different hepatocytes, then a relatively simple explanation as to how hepatic glucose output is controlled could be achieved. We have reexamined the pathways of glycogen synthesis during refeeding in starved rats, mapping glucose metabolism (10) and glycogen formation within the hepatic lobule in intact liver. Using this technique, we have previously shown that in starved rats, gluconeogenesis is confined to periportal cells (70–80% of lobular volume) (10,11), whereas in the perivenous cells, where glucokinase (GK) activity was most concentrated (12,13), uptake of glucose occurs (12,14).

Low birth weight predisposes to increased risk of type 2 diabetes in humans (15). In the maternal low protein (MLP) model of fetal programming of glucose intolerance (16), GK activity is deficient in the perivenous cells (12). From the age of 12 months, glucose tolerance declines in

MLP rats faster than in controls (16). Adult MLP livers exhibited increased hepatic glucose output on perfusion with lactate, primarily caused by decreased perivenous glucose uptake (PVGU) (12). We hypothesized that if PVGU were normally mediated by GK, then it might increase substantially as blood glucose increased, because of the kinetics of this enzyme (half-saturated [$S_{0.5}$] at 8 mmol/l glucose). GK-mediated PVGU would then make a potentially important contribution to net hepatic glucose balance, but this might be diminished in MLP liver. Furthermore, the relevance of PVGU at physiological glucose concentrations needed to be established because in our previous work (12), the concentration of glucose reaching the perivenous cells was <1 mmol/l, derived entirely from periportal gluconeogenesis. We have examined the distribution of glycogen formation during early refeeding, because the most simple consequence of the indirect pathway would be that glycogen synthesis commenced in the gluconeogenic periportal cells. The current and previous studies suggested that failure of PVGU in MLP liver was caused by perivenous depletion of GK, and we have explored the question of whether GK gene expression in MLP liver is attenuated during refeeding.

RESEARCH DESIGN AND METHODS

Animals. The feeding regimens for the control and MLP rats were as previously described (12); briefly, pregnant dams were fed ad libitum 20% (control) or 8% (MLP) protein diets during pregnancy and lactation. After weaning, control and MLP offspring received identical RM1-chow (SDS, Witham, Essex, U.K.). In each of the studies, male and female control and MLP animals were studied as adults in a blocked design such that comparisons between type of animal, age, and sex could be made. The total number of animals studied was 99 controls (age 8.5 ± 0.4 months) and 100 MLPs (age 9.1 ± 0.4 months). No significant differences were found between age or sex, and data were pooled for analysis between control and MLP for each study. All experiments on animals were conducted under ethically reviewed Government License (Scientific Procedures [Animals] Act 1986, U.K.).

Liver perfusion. Rats were studied at a mean age of 7.4 ± 0.4 (n = 24) and 8.0 ± 05 (n = 24) months for control and MLP, respectively (NS). Nonrecirculating isolated liver perfusions were established (14,17) from 48-h fasted animals, using erythrocyte and albumin-free perfusate and a flow rate of 10 ml · min^{-1} · 100 g rat wt^{-1}. The Krebs' perfusate (18) contained 6 mmol/l sodium L(+)-lactate and 0, 2, or 8 mmol/l glucose. From 25 min to 40 min (t = 0, time of cannulation), portal and hepatic venous samples were taken, weighed, and mixed with 4% perchloric acid. All samples were stored at −70°C until analysis. At 45 min, 10–15 ml of digitonin (4 g/l in Tris buffer [pH 7.4]) was perfused retrogradely to destroy the perivenous 20% of hepatic lobular volume; this procedure leaves the remaining 80% histologically intact and functionally stable with respect to gluconeogenesis (10). Twenty-percent retrograde destruction ablates the cells previously shown to exhibit glucose uptake (12). Antegrade perfusion was restored, and additional portal and venous samples were taken at 50–65 min. At 70 min, the liver was perfused-fixed (10% formalin), tissue blocks were removed, sections were cut and stained with hematoxylin and eosin, and the fraction of lobular volume remaining intact (FVR) was determined by semiautomated histomorphometry (10). The mean FVR achieved by digitonin in the control and MLP liver was between 0.79 and 0.82 (range 0.7–0.92; NS for type of animal or perfusion group). Perchloric acid–extracted perfusate samples were neutralized, and glucose concentration was measured with GOD-PAP (10) in quintuplet in quartz cuvettes. Glucose output/uptake was calculated by the Fick principle. Perivenous glucose uptake was calculated by difference from hepatic glucose output before and after digitonin. Gluconeogenesis is the net hepatic glucose production after digitonin.

GK activity. GK activity was measured by the method of Davidson and Arion (19).

Glycogen synthesis. The mean age of rats was 9.7 ± 1.03 (n = 50) and 10.2 ± 1.02 (n = 50) for control and MLPs, respectively (NS). Control and MLP rats were refed for 2 h after a 48-h fast with either RM1 or re-pelleted RM1 reconstituted with 99% [1-^{13}C]glucose as 10% by weight. Animals were killed by cervical dislocation and livers were immediately excised and freeze-clamped in liquid N_2. Some livers were perfused-fixed, and adjacent sections were stained with periodic acid-Schiff (PAS) and diastase-PAS. Glycogen synthesis was measured by two methods: *1*) amyloglucosidase digestion and spectrophotometry for total glycogen and glycogen mapping or *2*) H_2SO_4 digestion, $Ba(OH)_2$ neutralization, and estimation by nuclear magnetic resonance (NMR). ^{1}H spectra were obtained on a Bruker AMX 600 MHz spectrometer, the α and βC1 resonances were used to quantify glycogen, along with their [1-^{13}C] satellites for labeling in glycogen. Two-dimensional heteronuclear single quantum coherence was used for detection of labeling in glucose carbons other than C1.

Glycogen was quantitatively mapped within the liver lobule using a modified mapping technique (10); briefly, isolated perfused livers (see above) from 2-h refed rats were perfused with 10 mmol/l glucose and 1 nmol/l insulin (human recombinant' Roche, Lewes, U.K.). After 2 min, the inferior right lateral lobe was tied off, excised, and freeze-clamped in liquid N_2 for glycogen estimation. Each liver was then perfused retrogradely with 5–60 ml of digitonin (4 g/l) to obtain 5–80% destruction of lobules. Antegrade perfusion was restored for 10 min, and an additional liver sample was obtained (right lobe). In control studies, using the same procedure with digitonin-free buffer, the fraction of liver glycogen in the second biopsy compared with the first was 0.92 ± 0.12 (mean ± SE; n = 7; $P > 0.3$), demonstrating stability of glycogen during mapping.

GK gene expression. Rats were studied in two age groups for both Northern and in situ hybridization, 4–6 months and 12–17 months of age. No differences were found between age groups, and the data were pooled for comparison between control and MLP. For Northerns, mean age was 11.9 ± 2.3 (n = 6) and 10.6 ± 2.1 (n = 6) months (control and MLPs, respectively; NS). Total RNA was prepared from rat liver tissues using QIAGEN RNeasy Protect kit according to the manufacturer's protocol. Five micrograms of total RNA/lane was denatured with glyoxal/DMSO, run on 1% agarose gel, and transferred to a nylon membrane as described in Northern Max-Gly protocol (Ambion). Blots were probed with a GK exon nine probe generated by PCR and labeled with [^{32}P]dCTP (Amersham) by random priming using NEBlot kit (BioLabs). The probe was purified by gel filtration on Micro Bio-Spin P-30 column (BioRad). GK transcript levels were determined by comparison with the signals obtained with a β-actin–specific probe using a Kodak 1D 3.5 with Kodak ID Image Analysis Software.

For in situ hybridization studies, rats were studied at a mean age of 5.7 ± 0.6 (n = 19) and 6.8 ± 0.8 (n = 20) months for control and MLPs, respectively (NS). Liver and heart were removed, frozen in liquid nitrogen, and stored at −70°C. Ten-micrometer sections were mounted on poly-L-lysine–coated microscope slides and stored at −70°C. A 420-bp fragment of PB-GK-2 (2,326 nucleotides in a plasmid vector Bluescript KS+, provided by Dr. P. B. Iynedjian) was subcloned and transcribed in vitro in the presence of 200 μCi [α-^{35}S]UTP (Amersham International). Sections were fixed in 4% paraformaldehyde and acetylated in 0.25% (vol/vol) acetic anhydride in 0.1 mol/l triethanolamine buffer (pH 8.0). After prehybridization, sections were hybridized overnight (45°C) with sense and antisense riboprobe (5 × 106 cpm/μl). After washing and incubation with 30 μg/ml RNase A (Roche) for 1 h (37°C), sections were rewashed (final wash 0.1 × SSC, 1 h at 60°C) and dehydrated, air dried, and dipped in photographic NTB 2 emulsion (Kodak, Rochester, NY). Slides were kept at 4°C for 14 days before developing and counterstaining with hematoxylin and eosin. The hybridization signal was quantified using Visilog by automatic "counting" of silver grains *1*) adjacent to the central venule (perivenous region), *2*) adjacent to a portal tract (periportal region), *3*) midway between the two regions, and *4*) on areas of the slide not occupied by tissue (background counts). Three to five lobules were assessed in each section. "Counts" were also made on heart (non-GK expressing) and liver sections incubated with sense probe as further controls; signal was not significantly different from background in such sections.

Insulin assay. One milliliter of blood was taken from the inferior vena cava, plasma isolated, and stored at −70°C for insulin assay (Linco, Rat Insulin kit, Biogenesis, Poole, U.K.).

Statistics. Means are given ± SE. For comparison of means, paired or unpaired t tests were used as appropriate. Fitting of linear regression and assessment of significance of slope and intercept were done by Spearman's rank test.

RESULTS

Effect of glucose concentration on perivenous glucose uptake and net hepatic glucose output. Net hepatic glucose output (Fig. 1*A*) was progressively suppressed as glucose concentration in the perfusate increased (0–8 mmol/l) in control liver. This direct effect of glucose on regulation of hepatic glucose output in control

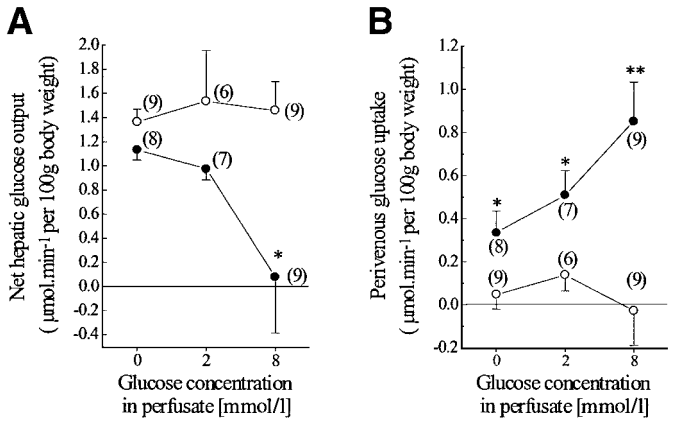

FIG. 1. Hepatic glucose output is controlled by PVGU. *A*: Dependence of hepatic glucose output (HGO) on glucose concentration in perfused livers of 48-h–fasted control (●) and programmed (MLP) (○) rats. *B*: Dependence of PVGU on glucose concentration in control and MLP liver (same convention as *A*). Mean glucose concentrations reaching the perivenous zones (equal to postdigitonin effluent glucose) were 0.15, 2.15, and 8.1 mmol/l (control) and 0.14, 2.17, and 8.14 mmol/l (MLP) when the perfusate concentrations were 0, 2, and 8 mmol/l, respectively. Unpaired *t* tests, control versus MLP: *$P < 0.05$, **$P < 0.01$. In *A* and *B*, data are means ± SE, and the numbers in parentheses refer to the number of animals studied.

liver was absent in MLP liver (Fig. 1*A*). The intralobular mapping technique (10) allowed us to determine which cells within the hepatic lobule were responsible for suppressing hepatic glucose output and why this failed in MLP liver. Figure 1*B* shows glucose uptake of the perivenous zone (~20% of the lobular volume), which increased progressively with glucose concentration in the perfusate in controls (0.06 μmol · min^{-1} · 100 g rat wt^{-1} per mmol/l increment). The increase in perivenous glucose uptake accounts for most of the decline in net hepatic glucose output in control liver. In contrast, in MLP liver, increase in glucose concentration produced no rise in perivenous glucose uptake. Net glucose release from the periportal cells (~80% of the lobular volume), as a result of gluconeogenesis from lactate, was not significantly affected by glucose concentration in either control or MLP liver, although a trend to decreased gluconeogenesis was apparent at 8 mmol/l glucose in controls. In controls, gluconeogenesis was 1.47 ± 0.07 ($n = 8$), 1.49 ± 0.12 ($n = 7$), and 0.94 ± 0.38 ($n = 9$) and in MLP liver was 1.41 ± 0.08 ($n = 9$), 1.68 ± 0.44 ($n = 6$), and 1.43 ± 0.26 ($n = 9$) μmol · min^{-1} · per 100 g rat wt^{-1} when perfusate glucose was 0, 2, and 8 mmol/l, respectively. The main effect of glucose per se on hepatic glucose output was therefore the result of increased glucose uptake by the perivenous cells as glucose concentration rises, rather than downregulation of gluconeogenesis or periportal glucose release. GK activity was significantly decreased in MLP liver (control 2.16 ± 0.16, $n = 38$; MLP, 1.54 ± 0.13 units/g liver wet wt, $n = 39$; $P < 0.005$).

Glycogen synthesis and intralobular distribution in control and MLP liver. After 2 h of refeeding, hepatic glycogen content was significantly decreased in MLP liver (control 13.6 ± 1.2, $n = 50$; MLP, 9.8 ± 0.7, $n = 50$, mg/g liver wet wt; $P < 0.01$). Figure 2 shows the effect of the degree of retrograde digitonin-based destruction on the glycogen content of the remaining viable liver in control and MLP liver, respectively, expressed as a fraction of the predigitonin content. The dotted diagonal is the theoretical locus of data if glycogen distribution were uniform. It can be seen that in control liver when half of the lobule is ablated (FVR = 0.5), glycogen content is decreased by 80%. After 2 h of refeeding, 80% of liver glycogen is therefore contained within the perivenous zone, confirming the perivenous preponderance of glycogen obtained by PAS staining (Fig. 3) (20).

Labeling of glycogen during refeeding with [1-^{13}C]glucose. There was a strong correlation ($r = 0.95$; $P < 0.001$) and virtually identical relationships in control and MLP livers between glycogen concentration at 2 h and the [1-^{13}C] abundance in glucosyl moieties of glycogen (Fig. 4), despite widely varying rates of glycogen synthesis (from 3 to 45 and 1 to 17 mg/g wet wt, respectively, in control and MLP liver). The intercept on the ordinate is significant, although small, accounted for by residual glycogen present after 48 h of starvation. The mean percentage of [1-^{13}C]glucosyl in glycogen in control and MLP liver was 6.69 and 6.35, respectively. No label was detected in the C2, C5, or C6 in liver glycogen using NMR.

Concentration of GK mRNA and distribution within the liver lobule of refed control and MLP rats. Northern analysis showed no significant difference in GK mRNA in livers from control and MLP animals 2 h after refeeding from a 48-h fast (Fig. 5*A*). However, because measurements in whole liver could mask zonal changes, the time course and distribution of GK mRNA appearance during refeeding were examined by in situ hybridization. Figure 5*b* shows GK mRNA in control and MLP liver after 1, 2, and 4 h of refeeding. In accordance with previous findings (21), GK mRNA was almost undetectable in liver of fasted rats (control or MLP), and lobular distribution of GK mRNA in refed control liver was virtually identical to that reported by Moorman et al. (21). By 1 h, GK mRNA is present in all zones in both control and MLP liver. In controls, a signif-

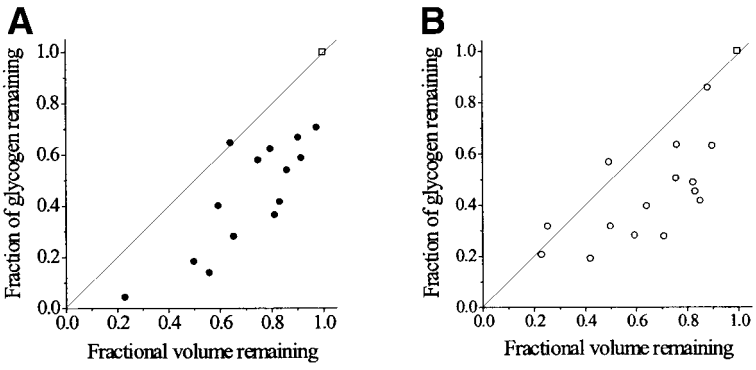

FIG. 2. Glycogen synthesis predominates perivenously in vivo during the early postprandial period. *A*: Fraction of glycogen remaining in control livers after retrograde digitonin perfusion (10) plotted against the fraction of viable lobular volume remaining. Each point represents data from a single liver. The square symbol indicates that glycogen content is unchanged when lobules are intact. Diagonal represents theoretical locus of data if glycogen per unit volume was uniform throughout liver. *B*: Same as *A*, except in MLP liver.

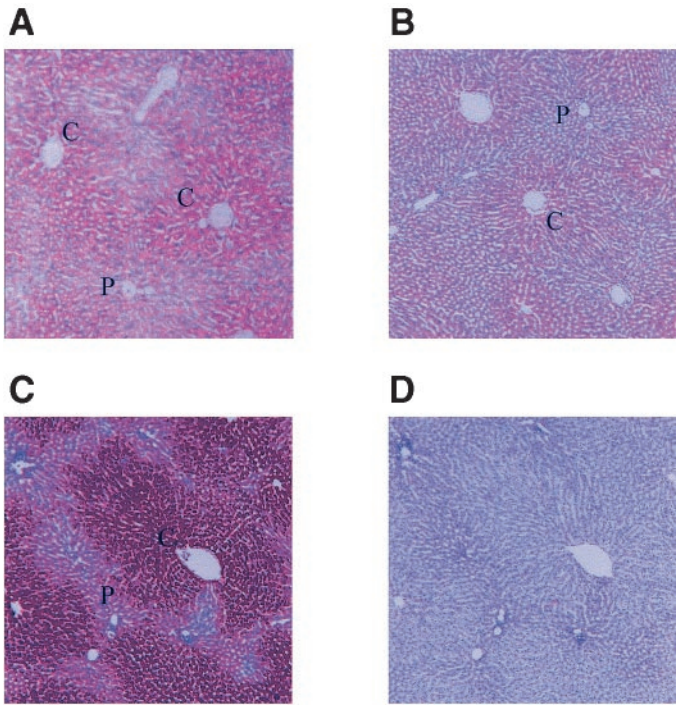

FIG. 3. Liver sections stain for glycogen predominantly in the perivenous zone after 2 h of refeeding fasted rats. *A*: PAS stain for glycogen in control animals fasted for 48 h and then refed for 2 h. *B*: Same as *A*, except in MLP liver. *C*: PAS-stained liver of control refed for 24 h after a 48-h fast. *D*: PAS-diastase glycogen digestion in situ of adjacent section to *C* results in absence of staining of lobular regions, used as a control for *C* (similar adjacent control sections for *A* and *B* gave qualitatively similar results). P, periportal zone; C, perivenous (centrilobular) zone.

icant periportal-perivenous gradient of increasing GK mRNA was apparent at all time points, and although qualitatively similar in MLP liver, lobular gradients were not significant. Mean plasma insulin (all refeeding times) was 381 ± 59 and 366 ± 127 pmol · l^{-1} · l^{-1} for control and MLP rats, respectively (NS).

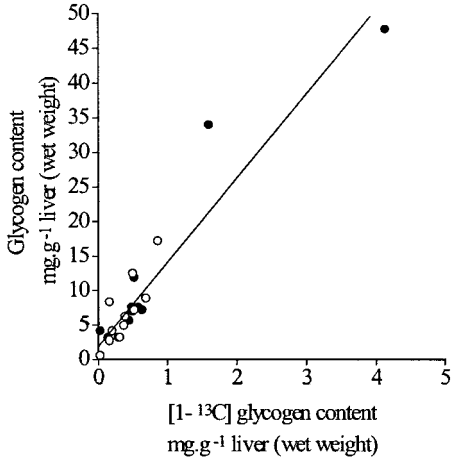

FIG. 4. Relationship of glycogen concentration and accumulation of labeled glucose in the diet. Linear relationship between glycogen content and [1-^{13}C]glucosyl labeling in glycogen in control and MLP liver 2 h after start of refeeding with chow containing 10% by weight 99% [1-^{13}C]glucose. ●, control liver; ○, MLP liver.

DISCUSSION

Key findings of the present study are *1*) glucose concentration per se can regulate the net output of glucose from the perfused livers of normal animals within the physiological range of glucose concentration; *2*) suppression of glucose output with rising perfusate glucose is principally achieved by increased PVGU, controlled by GK; *3*) glycogen synthesis during refeeding commences in the perivenous cells, which are deficient in gluconeogenic function but which readily take up glucose; and *4*) decreased GK activity in MLP liver is not the result of impaired GK gene transcription during refeeding.

The positive dependence in control rats of PVGU on glucose concentrations in the range 0.15–8.1 mmol/l strongly suggests that GK ($S_{0.5}$ ~8 mmol/l) is the key enzyme in this process. This conclusion is reinforced by the failure of PVGU in MLP liver throughout the perfusate glucose concentrations used, because the perivenous cells in MLP liver are GK-deficient (12). It is noteworthy that mice with one disrupted GK allele exhibit markedly decreased GK activity and failure of hyperglycemic suppression of net hepatic glucose production (22). Rossetti et al. (1) demonstrated that in normal 6-h fasted rats, acute hyperglycemia (but low insulin) caused marked inhibition of net hepatic glucose production through increased uptake of plasma glucose and inhibition of glycogenolysis but without significant effect on gluconeogenesis. PVGU mediated by GK could be responsible for such observations of glucose-dependent glucose uptake in vivo, reinforcing the importance of hepatic GK to glucoregulation (23). The suppression of net hepatic glucose output by rising perfusate glucose is analogous to the phenomenon of "glucose effectiveness," whereby increments in glycemia per se increase glucose disposal and decrease net hepatic glucose output in nondiabetic subjects (6,8). Hyperglycemia in type 2 diabetes is partly caused by failure of glucose effectiveness (8). The present observations suggest a mechanism for glucose effectiveness and that the postprandial pathophysiology of type 2 diabetes may be based principally on failure of PVGU rather than on increased gluconeogenesis, although a minor component of the latter cannot be excluded.

One fate of glucose taken up by perivenous cells in fasted rats was glycolysis (14), and we speculated that during refeeding an additional fate might be glycogen. Because PVGU in perfused MLP liver was markedly diminished, we anticipated that glycogen synthesis would also be decreased, and indeed the glycogen content was 28% less in MLP liver after 2 h of refeeding. The quantitative mapping of glycogen distribution using direct chemical analysis confirms the predominantly perivenous distribution of PAS staining at 2 h. Corroboration was necessary because of the differing morphology of glycogen deposits perivenously (diffuse) and periportally (granular) (20). The glycogen mapping and PAS staining show that the perivenous zone, which lacks gluconeogenic capability (10), accumulates glycogen approximately four times faster than that of the remainder of the lobule in the first 2 h of refeeding.

Whether hepatic glycogen is synthesized primarily via the direct (G6P via glucose) or indirect pathway (G6P via gluconeogenesis) is pertinent to understanding the bio-

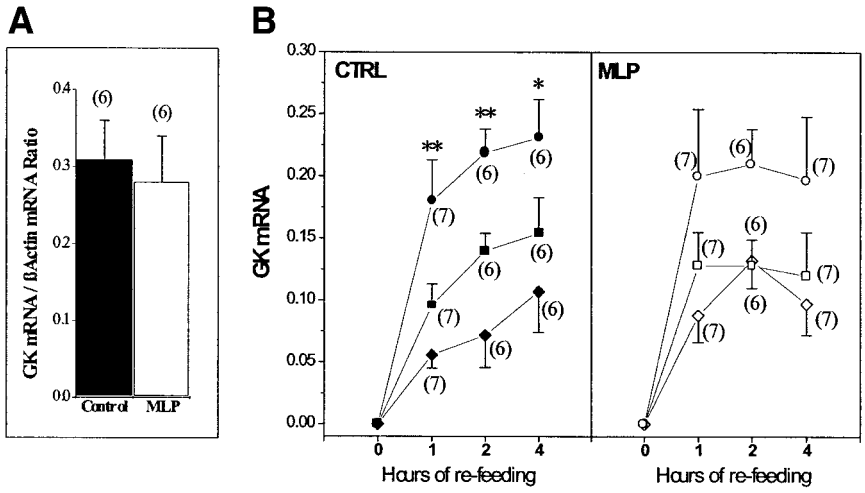

FIG. 5. GK gene transcription is not attenuated in MLP liver. *A*: Northern analysis of GK mRNA present after 2 h of refeeding in control and MLP liver. *B*: Expression of GK mRNA (background subtracted) by hepatic lobular zones, measured by quantitative in situ hybridization in livers of control and MLP rats after 1, 2, and 4 h of refeeding after a 48-h fast. Before refeeding, GK mRNA is not detectable above background. The ordinate represents the fraction of the area of interest occupied by bright granules (means ± SE). Upper curve is from perivenous region, intermediate curve from mid-zone, and lower curve from periportal region. Numbers in parentheses refer to number of animals studied. *$P < 0.05$, **$P < 0.01$, perivenous vs. periportal at the same time point.

chemical mechanism(s) underlying hepatic glucose output (24). In reviewing the evidence in favor of the indirect pathway, McGarry et al. (2) noted that the consequences of hepatocyte heterogeneity were unexplored. Much of the evidence supporting the indirect pathway is based on *1*) dilution of specific activity of ^{3}H label in glycogen glycosyls when glycogen is formed from [U-^{14}C, 3-^{3}H]glucose and *2*) the randomization of label from [1-^{14}C]glucose among C2, 5, and 6 in the glycosyl moieties of glycogen (2–4). The latter occurs only when the pathway traverses the lactate/pyruvate stage before gluconeogenic conversion into G6P (randomization arising during oxaloacetate ↔ fumarate equilibration). In addition, the administration of 3-mercaptopicolinate (3MP), an inhibitor of phosphoenolpyruvate carboxykinase, a gluconeogenic enzyme, decreases both glycogen synthesis and randomization of label (2,4). We find that over a wide range of hepatic glycogen formation, the specific labeling of glycogen glycosyls after feeding [1-^{13}C]glucose remains constant in both control and MLP rats. If two pathways had contributed substantially to glycogen synthesis, then this result could have been obtained only if the ratio of the two pathways had not varied with the rate of glycogen synthesis or type of animal, an unlikely situation. The NMR technique detects labeling in C2, 5, and 6 of glucose, which reaches 10% of that in C1; as we did not detect C2, 5, and 6 labeling, a minimum direct pathway contribution of 69% may be calculated, or 64% if randomization is only 80% complete.

These data (glycogen deposition in nongluconeogenic cells and uniformity of labeling) are consistent with glycogen formation during early refeeding principally via the direct pathway. However, they also indicate an alternative interpretation of the data supporting the indirect pathway (2,4) as follows. Gluconeogenically derived periportal G6P could contribute to glycogen formation perivenously by hydrolysis to glucose, exit from the periportal cells, transport down the sinusoid, and reuptake via GK to form perivenous G6P (Fig. 6). An important difference between the trans-sinusoidal pathway (TSP) and indirect pathways is that in the former, glucose itself is a necessary intermediate. The TSP could account for the labeling observed in C2, 5, and 6 of glycogen glucosyl moieties after administration of C1-labeled glucose (2,4). The effect of 3MP on randomization of label may be accounted for by deletion of the TSP, and inhibition of glycogen synthesis could be due to incomplete correction of the profound hypoglycemia induced by 3MP (4) (correction was based on arterial rather than portal vein glucose).

Both the proposed TSP and direct pathways require GK. Mean GK activity (1 $\mu mol \cdot min^{-1} \cdot g^{-1}$ at $S_{0.5}$ = 8 mmol/l) can produce ~10 mg of glycogen per gram of liver (wet weight) per hour. This calculation requires only that glucose concentration at the perivenous cells is 8 mmol/l during refeeding, justifiable by published data (25). Thus, GK activity is sufficient to account for mean rates of rat liver glycogen synthesis in the present study and those published by other workers (19,25). In fully refed animals, glycogen is present throughout the lobule (PAS staining remaining weak in the immediate periportal zone), raising the question of the source of G6P for periportal glycogen synthesis. We suggest (Fig. 6) that periportal glycogen is synthesized from G6P derived from cytosolic (active) GK, which is present throughout the lobule in fully refed animals (13) and not from gluconeogenically derived G6P. Several lines of evidence support this contention. Recent work has established that G6P produced by GK but not hexokinase is readily converted to glycogen (26,27), and furthermore, only the GK-derived G6P pool activates glycogen synthase (26). These data suggest the presence of two noncommunicating intracellular pools of G6P in hepatocytes and help to explain why GK exhibits high metabolic control over glycogen synthesis (28,29). Glycogen synthesis does not occur during fasting, despite high rates of G6P production via gluconeogenesis, raising the crucial question of why gluconeogenic G6P fails to activate glycogen synthase. We propose that gluconeogenic G6P enters the hexokinase-derived pool (Fig. 6).

The Northern and in situ hybridization studies suggest a posttranscriptional basis to decreased GK in MLP liver, either failure of translation or decreased GK-protein half-life. Refeeding rapidly stimulates GK gene transcription, but very little new enzyme is formed until 4 h of refeeding (30). (The half-lives of GK activity and GK mRNA are 30 h and 45 min respectively [30,31].) During fasting, GK is tightly bound to the inhibitory GK regulatory protein (GKRP) (32) in the nucleus (33) (except perivenously [13]). Farrelly et al. (34) and Grimsby et al. (35) demonstrated that knockout of the GKRP in mice resulted in decreased hepatic GK activity, suggesting that an impor-

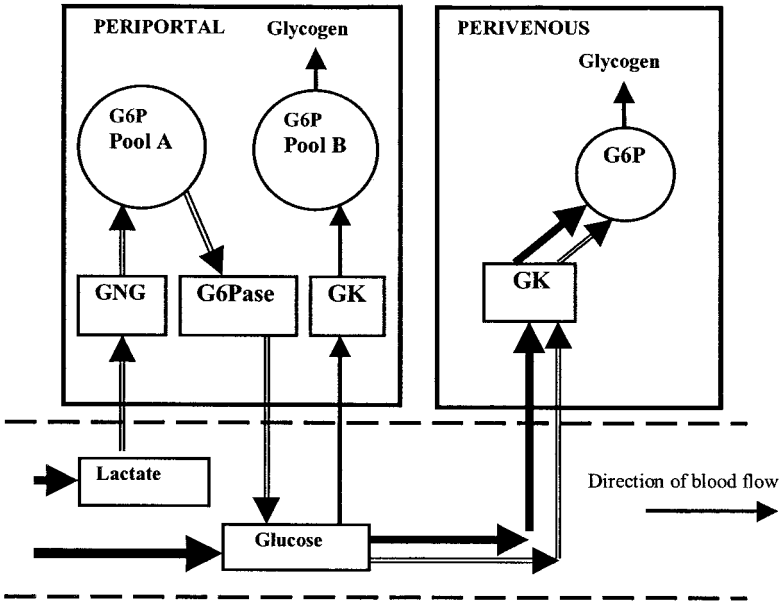

FIG. 6. Proposed scheme of hepatic glycogen synthesis. G6Pase, glucose 6-phosphatase; GNG, gluconeogenic pathway. Periportal G6P pools A and B do not communicate directly. Glycogen is formed from glucose via GK (solid arrows, direct pathway). Glycogen can be formed via GNG through pool A, through conversion to glucose and reuptake perivenously via GK (the TSP [open arrows]). Both pathways depend on GK activity.

tant function of nuclear sequestration of GK during starvation might be to delay degradation of GK protein, which is relatively easily inactivated by oxidation in vitro (19). This would facilitate rapid nuclear to cytoplasmic transport of "stored" GK when portal glucose concentration rises postprandially, providing increased GK activity before the appearance of newly synthesized GK. In the current studies, although GK-protein concentration was not measured directly, because the GK assay is independent of GKRP and as GK kinetics in MLP liver are unchanged (Patel et al., unpublished data), the activity data are likely to reflect total protein concentration. Additional studies of GK translocation in MLP liver, including the GKRP-mediated posttranscriptional regulatory mechanism, should be worthwhile.

In conclusion, we suggest that hepatic glucose output during refeeding is controlled by perivenous glucose uptake, which is responsive to portal blood concentration because of the abundance of high $S_{0.5}$ GK in those cells. In the early fasting → fed transition, the current view of the indirect pathway of glycogen synthesis needs to be modified taking into account hepatic intralobular heterogeneity of function. Previous evidence pointing to an indirect pathway via gluconeogenesis may be the result of intrasinusoidal transfer of glucose and communication with the perivenous cells via a TSP. A dominant direct pathway would have several clear advantages: *1*) it is more efficient energetically, *2*) it uncouples potentially conflicting demands of glucoregulation and the major hepatic role in acid-base homeostasis effected by lactate disposal (36), and *3*) it prevents inappropriate switching of gluconeogenic flux to glycogen. It is not presently possible to analyze directly the specific contribution of PVGU to regulation of hepatic glucose output in humans, although studies of glucose effectiveness provide indirect evidence of such a mechanism. However, it should be pointed out that current measures of hepatic glucose output should be regarded as the sum of gluconeogenesis plus glycogenolysis minus PVGU, rather than consisting of the first two components alone (37).

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