

Rat Small Intestine Is an Insulin-Sensitive Gluconeogenic Organ

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At variance with the current view that only liver and kidney are gluconeogenic organs, because both are the only tissues to express glucose-6-phosphatase (Glc6Pase), we have recently demonstrated that the Glc6Pase gene is expressed in the small intestine in rats and humans and that it is induced in insulinopenic states such as fasting and diabetes. We used a combination of arteriovenous balance and isotopic techniques, reverse transcription-polymerase chain reaction, Northern blot analysis, and enzymatic activity assays. We report that rat small intestine can release neosynthesized glucose in mesenteric blood in insulinopenia, contributing 20–25% of total endogenous glucose production. Like liver glucose production, small intestine glucose production is acutely suppressed by insulin infusion. In the small intestine, glutamine and, to a much lesser extent, glycerol are the precursors of glucose, whereas alanine and lactate are the main precursors in liver. Accounting for these metabolic fluxes: 1) the phosphoenolpyruvate carboxykinase gene (required for the utilization of glutamine) is strongly induced at the mRNA and enzyme levels in insulinopenia; 2) the glycerokinase gene is expressed, but not induced; 3) the pyruvate carboxylase gene (required for the utilization of alanine and lactate) is repressed by 80% at the enzyme level in insulinopenia. These studies identify small intestine as a new insulin-sensitive tissue and a third gluconeogenic organ, possibly involved in the pathophysiology of diabetes. *Diabetes* 50:740–746, 2001

Up to now, liver and kidney have been considered the only organs capable of releasing endogenous glucose because both are the only tissues to express glucose-6-phosphatase (Glc6Pase), the enzyme catalyzing the last enzymatic step common to glycogenolysis and gluconeogenesis, e.g., the hydrolysis of glucose-6-phosphate into glucose and inorganic phosphate (1–3). It had been accepted that Glc6Pase activity detected in most tissues, excluding liver and kidney, was due to unspecific phosphatase activities (3). However, using re-

verse transcription-polymerase chain reaction (RT-PCR), Northern blot analysis, and a highly specific Glc6Pase assay, we have recently shown that the Glc6Pase gene is also expressed (mRNA and activity) in human and rat small intestines (4). In addition, the Glc6Pase mRNA abundance and protein activity are markedly increased in 48-h-fasted and diabetic rats (4). This has strongly suggested that small intestine might possess the capacity to produce glucose in portal blood and that this production might be augmented in insulinopenic states. In keeping with the latter proposal, we recently reported that another major regulatory gene of gluconeogenesis, i.e., phosphoenolpyruvate carboxykinase (PEPCK), is also expressed in human and rat small intestine and is induced in insulinopenia (5). These data prompted us to address the question of the production of glucose by rat small intestine in vivo and of its possible enhancement in insulinopenic states.

Because the arteriovenous glucose balance technique measures net glucose exchanges, i.e., the sum of both uptake and release of glucose, it would not correctly evaluate the actual contribution of small intestine to glucose homeostasis. Therefore, we have simultaneously partitioned the release and uptake of glucose by small intestine using the combination of arteriovenous glucose balance and isotopic dilution techniques previously used in similar studies in kidney (6–8). The disappearance of the tracer assesses the removal of unlabeled glucose from the circulation. In addition, any decrease in tracer specific activity reveals the release of unlabeled glucose by small intestine. Part of this work has been published in abstract form (9).

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats (220–240 g) were obtained from Iffa-Credo (L'Arbresle, France) and were kept on a 12/12-h light-dark cycle. They were fed a standard laboratory diet (U.A.R., Epinay-sur-Orge, France). Fasted rats were deprived of food for 48 h, with access to water. Diabetic rats were used 3 days after a single intraperitoneal injection of streptozotocin (60 mg/kg body wt) (Sigma, La Verpillière, France). Postabsorptive normal and diabetic rats were deprived of food for 6 h before experiments and were allowed free access to water. All protocols described in this paper were performed according to the rules of our local ethics committee for animal experimentation.

Determination of intestinal blood flow. Hemodynamic studies were performed under the conditions of infusion experiments (see below), according to a radiolabeled microsphere technique (10). Rats were anesthetized with a single injection of pentobarbital (6 mg/100 g body wt). The right carotid artery was cannulated, and the catheter was advanced into the left heart ventricle. Its position was ascertained using a pressure probe. After laparotomy, the inferior mesenteric vein was ligated as described below; 500 μ l of microspheres labeled with ^{141}Ce in suspension in citrated-NaCl ($15 \pm 3 \mu\text{m}$ diameter, 2.7 GBq/g; DuPont-NEN, Boston, MA) was injected into the artery. The microsphere suspension (33 kBq) was injected over 10 s, followed by a double rinse with 100 μ l citrated-NaCl. The reference blood sample was drawn

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EGP, endogenous glucose production; FX, fractional extraction; Glc6Pase, glucose-6-phosphatase; IBF, intestinal blood flow; IGR, intestinal glucose release; IGU, intestinal glucose uptake; PEPCK, phosphoenolpyruvate carboxykinase; PC, pyruvate carboxylase; RT-PCR, reverse transcription-polymerase chain reaction.

TABLE 1

Determination of intestinal glucose fluxes in normal postabsorptive, 48-h-fasted, and diabetic rats using [^3H]glucose tracer data and glucose balance measurements

Rat	[^3H]glucose-specific activity (dpm/ μmol)		Glucose concentration (mmol/l)		Fractional extraction	Glucose balance	IGU	IGR	EGP
	Artery	Vein	Artery	Vein					
Normal ($n = 6$)	20,715 \pm 1,357	20,966 \pm 1,045 (NS)	10.29 \pm 0.58	9.49 \pm 0.38 ($P < 0.02$)	0.06 \pm 0.01	17.7 \pm 6.1	14.8 \pm 4.1	-2.9 \pm 2.4	75.5 \pm 6.0
48-h-fasted ($n = 8$)	20,903 \pm 938	19,942 \pm 780 ($P < 0.001$)	7.79 \pm 0.33	7.43 \pm 0.28 (NS)	0.09 \pm 0.02	9.2 \pm 4.5	17.8 \pm 3.8	8.6 \pm 1.5 (21% EGP)	41.3 \pm 1.0
Diabetic ($n = 10$)	13,646 \pm 946	13,464 \pm 470 ($P < 0.01$)	30.76 \pm 1.41	31.03 \pm 1.39 (NS)	0.004 \pm 0.01	-13.18 \pm 7.7	9.19 \pm 15.4	22.4 \pm 9.7 (19% EGP)	119 \pm 5

Data are means \pm SE. Glucose balance, IGU, IGR, and EGP are given in micromoles per kilogram per minute. P values were determined by Student's t test for paired data.

through the left femoral artery, previously catheterized, by a syringe infusion withdrawal pump (PHD 2000, Harvard Instruments). The pump was started 5 s before blood was drawn, and the sample was collected for 150 s. Rats were killed by pentobarbital injection, and the tissues were quickly dissected, washed, dried, and weighed. Organ blood flows were calculated by multiplying the withdrawal rate of the blood reference sample by the ratio of the radioactivity in the organ to the radioactivity of the reference sample (10). The distribution of the cardiac output to the two kidneys was very similar and the distribution to the lungs was $<5\%$ in all cases, validating the accuracy of the determinations (11,12).

Determination of intestinal glucose fluxes by [^3H]glucose infusion. Rats were catheterized and infused with [^3H]glucose under the conditions described in detail in our previous reports (13,14), including some additional procedures. After infusion for 10 min, laparotomy was performed; the cecum was retracted on the left. Inferior mesenteric circulation was stopped by ligation to exclude the blood flow coming from the cecum and the colon. The guts were replaced in the abdominal cavity, and the incision was protected with a wet gauze. Preliminary monitorings of the glycemia and of the specific activity of blood glucose indicated that a steady-state equilibrium was obtained during the last 60 min of the total 90-min infusion experiment. At that final time, blood samples were gently taken simultaneously in the carotid artery and the superior mesenteric vein. Blood plasma was immediately separated by centrifugation at 4°C , deproteinized, and used for triplicate determinations of glucose concentration and ^3H -radioactivity after evaporation to dryness. The fractional extraction (FX) of glucose across small intestine was calculated as $[(^3\text{H})\text{glucose SA}_{\text{artery}} \times \text{glucose concentration}_{\text{artery}}] - (^3\text{H})\text{glucose SA}_{\text{vein}} \times \text{glucose concentration}_{\text{vein}} / (^3\text{H})\text{glucose SA}_{\text{artery}} \times \text{glucose concentration}_{\text{artery}}$, where SA is specific activity. The intestinal glucose uptake (IGU) was calculated as $\text{IBF} \times \text{glucose concentration}_{\text{artery}} \times \text{FX}$, and the intestinal glucose balance (IGB) was calculated as $\text{IBF} \times (\text{glucose concentration}_{\text{artery}} - \text{glucose concentration}_{\text{vein}})$. The intestinal glucose release (IGR) was calculated as IGU minus IGB. Total endogenous glucose production (EGP) was obtained from the [^3H]glucose infusion rate and the specific activity of arterial blood glucose (13,14).

Determination of small intestine gluconeogenic fluxes from ^{14}C -labeled precursor infusions. L-[U- ^{14}C]glutamine (8.69 GBq/mmol; DuPont-NEN), L-[U- ^{14}C]alanine (6.5 GBq/mmol; DuPont-NEN), or L-[U- ^{14}C]lactic acid (6.5 GBq/mmol; DuPont-NEN) diluted in NaCl 1% containing 1 g/l serum bovine albumin was infused into the right jugular vein in rats set up as above. Each ^{14}C -labeled metabolite (0.185 MBq/ml) was infused at 10.7 kBq/min for 1 min, followed by a continuous infusion at 1.07 kBq/min for 90 min. Preliminary experiments of peripheral infusion of the four gluconeogenic precursors studied (see below also) indicated that a steady-state equilibrium of incorporation of labeled carbons into glucose was reached from 60 to 120 min. The plasma [^{14}C]glucose specific activity was thus determined in both arterial and mesenteric blood sampled as above at 90 min. Glucose was purified from neutralized perchloric acid extracts by sequential anion-cation exchange chromatography (15). Glucose concentration was determined and ^{14}C -radioactivity was measured after drying. Glutamine specific activity was determined in mesenteric blood. Plasma was separated and deproteinized by addition of 2% (mass/volume, final concentration) sulfosalicylic acid containing D-glucosaminic acid as an internal standard and was frozen at -20°C . Glutamine was purified and quantified by ion-exchange liquid chromatography on a Beckman 6300 amino acid analyzer. The small intestine gluconeogenic flux from [^{14}C]glutamine was calculated according to the equation of Stumvoll et al. (16) as $\text{IBF} \times [(^{14}\text{C})\text{glucose SA}_{\text{vein}} \times \text{glucose concentration}_{\text{vein}}] - (1 - \text{FX}) \times [(^{14}\text{C})\text{glucose SA}_{\text{artery}} \times \text{glucose concentration}_{\text{artery}}] / (1.2 \times [^{14}\text{C}] \text{glutamine SA}_{\text{vein}})$, where SA is specific activity.

Determination of small intestine gluconeogenic flux from ^{13}C -labeled glycerol. A primed 16 $\mu\text{mol}/\text{min}$ infusion of [^{13}C]glycerol ([2- ^{13}C ,99 AP]glycerol; Mass Trace, Woburn, MA) diluted in NaCl/albumin as above, was performed for 1 min, followed by a continuous 90-min infusion at 1.6 $\mu\text{mol}/\text{min}$. Arterial and mesenteric plasma was sampled as above. Glucose and glycerol were purified and derived into aldonitrile penta-acetate glucose and glycerol triacetate as described previously (15,17). The [^{13}C]glucose and [^{13}C]glycerol isotopic enrichments were obtained by gas chromatography-mass spectrometry according to previously validated procedures (15,17,18). Glucose and glycerol isotopic enrichments were expressed as molar ratios (percentages) and further converted as mole percentage excess for the calculation of the glycerol flux into glucose.

Enzyme assays and other analytical procedures. At the end of the experiments, a liver lobe and the first 5 cm of the proximal jejunum were freeze-clamped in situ using tongues precooled in liquid nitrogen. Homogenates were prepared from frozen tissues as previously described (4,13,14) and were used to determine the total activity of enzymes (V_{max}). Phosphoenolpyruvate carboxykinase (PEPCK) was assayed according to the decarboxylation assay described by Jomain-Baum et al. (19). Pyruvate carboxylase (PC) V_{max} was determined using a [^{14}C]bicarbonate incorporation assay under saturating conditions of substrates and cofactors (20). Glycerokinase V_{max} was determined according to the method of Bergmeyer et al. (21). Total RNAs were extracted and analyzed by RT-PCR and Northern blotting as described in our previous reports (4,5,22). Plasma glucose concentration was determined in triplicate in the supernatants of $\text{Ba}(\text{OH})_2$ and ZnSO_4 precipitate by the enzymatic assay of Bergmeyer et al. (23), and plasma lactate was determined by the enzymatic assay of Gutmann and Wahlefeld (24). Glycogen content was determined as described by Keppler and Decker (25).

Statistical analyses. Statistical analyses were performed by analysis of variance. When statistical differences were established, the differences were tested for significance using a two-tailed Student's t test for paired or unpaired values as relevant.

RESULTS

Quantification of IGR in postabsorptive, fasted, and diabetic rats. In postabsorptive rats, total EGP determined in the peripheral arterial blood was $75.5 \pm 6.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, in keeping with previous results (13,14). The blood glucose concentration in the mesenteric vein was significantly lower than that in the artery (by $\sim 0.8 \text{ mmol/l}$), whereas arterial and venous [^3H]glucose specific activity were not different (Table 1). This was a first indication that no newly synthesized unlabeled glucose molecule had been released into the circulation by small intestine. The mean IGU, calculated from the individual FX (average value 0.06 ± 0.01 , mean \pm SE, $n = 6$) and the IBF ($5.8 \pm 1.1 \text{ ml}/\text{min}$), was $14.8 \pm 4.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Table 1). The mean IGR, calculated from the individual IGUs and glucose balances, was very close to zero ($-2.9 \pm 2.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; Table 1). These data strongly suggested that there was no detectable production of endogenous glucose by small intestine in the postabsorptive state.

TABLE 2

Estimation of intestinal glucose fluxes in diabetic rats using lactate and glucose balance measurements

Rat	Lactate concentration (mmol/l)		Glucose concentration (mmol/l)		Glucose balance	IGU (1 glucose = 2 lactate)	IGR
	Artery	Vein	Artery	Vein			
Diabetic ($n = 15$)	1.42 ± 0.09	1.93 ± 0.13 ($P < 0.001$)	29.92 ± 1.1	30.27 ± 1.0 (NS)	-17.1 ± 9.5	12.3 ± 1.9	29.4 ± 10.7 (25% EGP)
Diabetic ($n = 38$)	—	—	30.77 ± 0.69	31.06 ± 0.68 ($P < 0.05$)	-15.0 ± 7.6	0	15.0 ± 7.6 (13% EGP)

Data are means \pm SE. Glucose balance, IGU, and IGR are given in micromoles per kilogram per minute. P values were determined by Student's t test for paired data.

In 48-h-fasted rats, EGP was lower than in postabsorptive rats ($41.3 \pm 1.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; Table 1). This also agreed with previous data (14). As in postabsorptive rats, the blood glucose concentration in the vein tended to be lower than that in the artery (Table 1). However, the difference was less than in postabsorptive rats (~ 0.3 – 0.4 mmol/l) and was not statistically significant. In contrast with postabsorptive rats, the [3 - ^3H]glucose specific activity was lower (by $4.5 \pm 0.7\%$, $P < 0.001$) in the venous than in the arterial blood (Table 1). This indicated that unlabeled glucose had been released by small intestine. The mean IGU, calculated as above from the individual FX (0.09 ± 0.02 , mean \pm SE, $n = 8$) and the IBF (6.5 ± 1.0 ml/min), was $17.8 \pm 3.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Table 1). The IGR, calculated from the individual data of IGU and glucose balance, averaged to a positive value of $8.6 \pm 1.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (mean \pm SE, $n = 8$). This represented 21% of EGP (Table 1).

In postabsorptive diabetic rats, EGP was higher than in normal postabsorptive rats ($119 \pm 5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; Table 1). In contrast with postabsorptive and fasted rats, the venous glucose concentrations tended to be higher (by ~ 0.3 mmol/l) than the arterial concentrations, without being statistically different, however (Table 1). As in fasted rats, there was a significant ($P < 0.01$) decrease in the [3 - ^3H]glucose specific activity in the vein as compared with the artery (Table 1). This decrease was much lower than in fasted rats ($-1.3 \pm 0.4\%$, mean \pm SE, $n = 10$). A reason for the latter was that the IBF was dramatically enhanced in diabetic rats as compared with normal rats: 12.3 ± 1.6 vs. 5.8 ± 1.1 ml/min ($P < 0.01$, Student's t test, unpaired data). This high IBF associated with high glucose concentrations (in the range of 30 mmol/l) strongly affected the accuracy of the determination of glucose specific activity. Consequently, there was a high dispersion in the FX values (averaging close to nil; 0.004 ± 0.01) and thus in the IGU values ($9.19 \pm 15.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (see Table 1), revealing that the mean FX value was obtained from a set of positive and negative values. The negative values were considered the consequence of experimental error and were included in all calculations. Noteworthy, in spite of this imprecision in the FX determinations, the mean calculated IGR ($22.4 \pm 9.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, mean \pm SE, $n = 10$) represented 19% of total EGP, a value very close to that determined in 48-h-fasted rats (Table 1).

To bypass the difficulty encountered in the FX determinations in diabetic rats, we specified the IGR using two other approaches. First, using a group of 15 diabetic rats, we calculated the IGU from the lactate release by small intestine, based on the assumption that two lactate mole-

cules are representative of one glucose molecule taken up. As shown in Table 2, there was substantial lactate release by small intestine; the lactate concentration in the mesenteric vein was 0.5 mmol/l higher than that in the artery. This allowed us to estimate an average IGU of $12.3 \pm 1.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (mean \pm SE, $n = 15$), which was comparable to that calculated from the FX data (compare with Table 1). The venous glucose concentration also tended (not significantly) to be higher than the arterial concentration in this group (Table 2). The mean IGR, calculated from the individual IGU and glucose balance data, was $29.4 \pm 10.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, representing 25% of the EGP. Second, we carried out statistical analysis of glucose balances from 38 diabetic rats used in this study. This revealed a plasma glucose concentration higher in the mesenteric vein than in the artery ($P < 0.05$), indicating a significant net release of glucose by small intestine (Table 2). The IGR (estimated from the individual glucose balances only, thus neglecting the IGU) was $15.0 \pm 7.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. This represented 13% of total EGP.

We studied the effect of insulin infusion (480 pmol/h) under conditions of euglycemia (13,14) in 48-h-fasted rats, in which accurate determinations of glucose specific activity could be performed (see above). EGP was significantly suppressed by insulin infusion: 25.0 ± 3.6 vs. $41.3 \pm 1.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in saline-infused rats ($P < 0.01$, Student's t test, unpaired data). In contrast with saline-infused fasted rats, there was no decrease in the glucose specific activity in the mesenteric vein as compared with the artery: $6,957 \pm 595$ vs. $6,959 \pm 461$ dpm/ μmol (means \pm SE, $n = 4$, NS). This strongly suggested that the release of glucose neosynthesized in small intestine was totally suppressed upon insulin infusion. In keeping with the latter, the IGR calculated from these tracer data were close to zero ($-1.0 \pm 4.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). As a consequence, there occurred a marked decrease (by 1 mmol/l) in the glucose concentration in the vein as compared with the artery: 6.62 ± 0.39 vs. 7.63 ± 0.51 mmol/l, respectively ($P < 0.05$, Student's t test, paired data). This contrasted with the nonsignificant lowering observed in the same rats infused with saline (see above).

Identification of small intestine gluconeogenic precursors. We studied the incorporation of carbon-labeled precursors in glucose in 48-h-fasted rats. All four gluconeogenic precursors infused were incorporated into glucose (Table 3). The specific activity of glucose was 3.4% higher ($P < 0.01$) in the mesenteric vein than in the artery when [U - ^{14}C]glutamine was infused, indicating that glucose molecules neosynthesized from [U - ^{14}C]glutamine were released by the small intestine. In a similar manner, the

TABLE 3
Quantification of intestinal gluconeogenic fluxes from carbon-labeled precursors in 48-h-fasted and diabetic rats

Precursor infused	48-h-fasted rats			Diabetic rats		
	[¹⁴ C]glucose specific activity (dpm/μmol)		Precursor→glucose (μmol · kg ⁻¹ · min ⁻¹)	[¹⁴ C]glucose specific activity (dpm/μmol)		Precursor→glucose (μmol · kg ⁻¹ · min ⁻¹)
	Artery	Vein		Artery	Vein	
[U- ¹⁴ C]glutamine (n = 6)	669 ± 36 (Δ _{v-a} = 3.4%, P < 0.01)	692 ± 35	4.8 ± 5.0 (57% IGR)	317 ± 31 (Δ _{v-a} = 1.9%, P < 0.02)	323 ± 30	10.1 ± 3.3 (45% IGR)
[U- ¹⁴ C]alanine (n = 6)	2,152 ± 96 (Δ _{v-a} = 0.4%, NS)	2,162 ± 91	—	—	ND	—
[U- ¹⁴ C]lactate (n = 6)	2,044 ± 133 (Δ _{v-a} = 0.3%, NS)	2,037 ± 121	—	—	ND	—
	Molar ratio (%)			Molar ratio (%)		
[2- ¹³ C]glycerol (n = 5, 48 h-fast) (n = 7, diabetic)	21.26 ± 4.32 (Δ _{v-a} = 3.1%, P < 0.05)	21.96 ± 4.28	1.6 ± 0.7 (19% IGR)	6.35 ± 0.62 (Δ _{v-a} = 0.3%, NS)	6.37 ± 0.66	—

Data are means ± SE. P values were determined by Student's *t* test for paired data. ND, not determined.

[¹³C]glucose molar ratio was significantly higher (3.1%) in the vein than in the artery (Table 3), indicating that glucose molecules that had incorporated [2-¹³C]glycerol were released by small intestine. These data allowed us to rule out definitively the possibility that the decrease in glucose specific activity in the mesenteric blood evidenced above could only be due to recycling of [3-³H]glucose after detritiation at the level of 3-carbon compounds. In contrast, after infusion of [U-¹⁴C]lactate or [U-¹⁴C]alanine, there was no significant difference between the glucose specific activity in the vein and that in the artery (Table 3). This strongly suggested that the incorporation of [U-¹⁴C]lactate and [U-¹⁴C]alanine into glucose did not take place in small intestine. The small intestine gluconeogenic fluxes from glutamine and glycerol to glucose, calculated from the specific activity of glucose and its precursors, represented 57 and 19%, respectively, of the total IGR determined as above (Table 3).

In diabetic rats infused with [U-¹⁴C]glutamine, there was a significant increase in the mesenteric vein glucose specific activity as compared with arterial glucose (Table 3). The calculated gluconeogenesis from glutamine (10.1 ± 3.3 μmol · kg⁻¹ · min⁻¹) accounted for ~45% of the total IGR (Table 3). In contrast, we did not measure any increase in the [¹³C]glucose specific activity in the mesenteric vein with regards to the artery after infusion of [2-¹³C]glycerol.

Molecular studies. We specified the gene expressions (mRNA and activity) of the enzymes required for the utilization of the four substrates in the liver gluconeogenesis, i.e., PEPCK, PC, and glycerokinase. All three genes were expressed at the mRNA and enzyme levels in small intestine in the three groups of rats (Fig. 1). Note that, for the three enzymes, the V_{\max} determined in small intestine in postabsorptive rats was ~10% of the V_{\max} determined in the liver of the same rats (not shown). The same feature was also observed for Glc6Pase expression in rat small intestine (4). It must be mentioned that another key enzyme in gluconeogenesis, fructose-1,6-bisphosphatase, was not studied here because it is well known that it is expressed in substantial amount in the small intestine of different species (26–28). PEPCK gene expression was dramatically enhanced in 48-h-fasted and diabetic rats, as

compared with postabsorptive rats, at both the mRNA (by about 12 times) and the enzyme activity (by 2–2.5 times) level (Fig. 1). In contrast, PC gene expression was not altered at the mRNA level. However, a marked decrease (by 80%) in the PC V_{\max} was observed in both 48-h-fasted and diabetic rats, as compared with postabsorptive rats (Fig. 1). On the other hand, there was no modification of the glycerokinase gene expression (mRNA and V_{\max} levels) induced by fasting or diabetes (Fig. 1). We also determined the small intestine glycogen contents. The latter was not altered in 48-h-fasted rats, as compared with postabsorptive rats, but was markedly increased (by ~400%) in diabetic rats (Fig. 1).

DISCUSSION

In this work, we have used a combination of arteriovenous glucose balance and tracer dilution techniques to partition the uptake and release of glucose by the small intestine in rats under different nutritional and hormonal conditions. In postabsorptive rats, [3-³H]glucose specific activity measurements were accurate enough to determine a significant FX of glucose. Thus, an IGU could also be accurately calculated. From the positive glucose balance and IGU, the IGR was found to be close to nil. A comparable accuracy was obtained in 48-h-fasted rats, and a significant FX could be determined. This allowed us to calculate a significant glucose uptake. Despite the fact that the arteriovenous glucose balance was positive, i.e., the mean uptake of glucose surpassed the mean release, we could determine with confidence a significant IGR of 8.6 ± 1.5 μmol · kg⁻¹ · min⁻¹, accounting for 21% of total EGP.

In contrast, in diabetic rats, the mean FX was not different from zero because of the occurrence of positive and negative values. Since negative FX values are theoretically impossible, they might reflect experimental errors in the determination of small differences in [3-³H]glucose specific activity, complicated in diabetic rats by the markedly increased blood flow and plasma glucose concentration. A comparable impediment has been encountered in FX determinations for kidney (8). As previously pointed out, setting the negative values to zero or repeating the measures until a “theoretically possible” positive value is

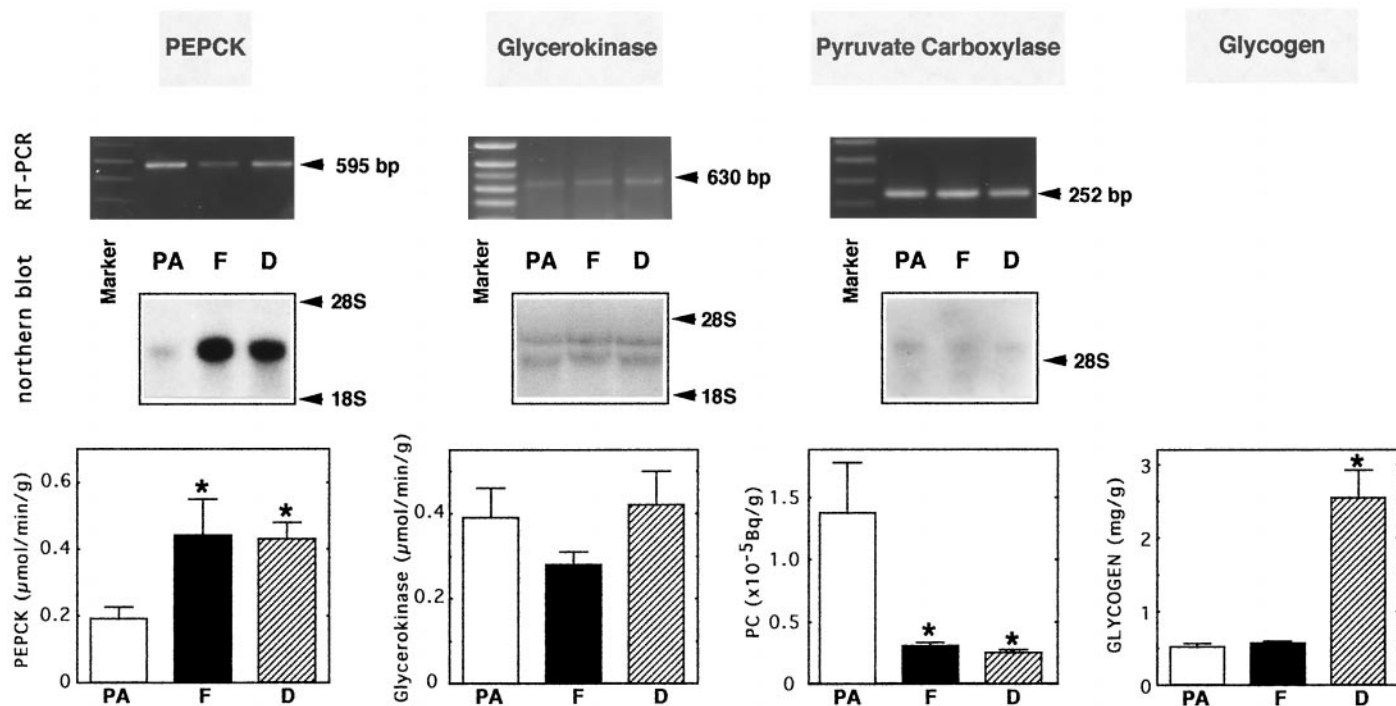


FIG. 1. Effect of fasting and diabetes on PEPCK, PC, glycerokinase, and glycogen in rat small intestine. RT-PCR (top panels), Northern blots (middle panels), and glycogen contents and enzymatic activity determinations under conditions of maximal velocity (bottom panels) were performed from total RNA and homogenates prepared from a 5-cm freeze-clamped jejunum sample. The expected length of c-DNAs (RT-PCR) is indicated on the right of the top panels, and the position of 28S and 18S RNAs on Northern blot panels. In bottom panels, the results are expressed as means \pm SE of at least four animals per group. *Value significantly different from postabsorptive (PA) value ($P < 0.05$, *t* test for unpaired data). PA, normal postabsorptive rats; F, 48-h-fasted rats; D, streptozotocin-induced diabetic rats.

obtained may introduce bias, resulting in the determination of artificially high glucose uptake and consequently the overestimation of the glucose release (8). It is interesting to note that incorporating these negative values in all calculations, a mean IGR of $22.4 \pm 9.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, representing 19% of the EGP (a percentage very close to that which could be accurately determined in fasted rats), was obtained in diabetic rats. We could not quantify small intestine glucose uptake by means of the 2-deoxy[^3H]glucose approach, since the latter is not suitable in tissues expressing Glc6Pase (29). Therefore, we estimated small intestine glucose uptake by means of small intestine lactate release. This allowed us to calculate an average IGR of $29.4 \pm 10.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, representing 25% of the total EGP, close to that determined from tracer data (see above). Finally, IGR was estimated from the sole significant negative glucose balance in 38 diabetic rats used in this study. This is a minimal value because it neglects the amount of glucose taken up by the small intestine. From the latter rationale, it is strongly suggested that a minimal IGR in diabetic rats could not be $<13\%$ of EGP (see Table 2).

Our results also strongly suggest that from the four main gluconeogenic precursors, only glutamine and, to a lesser extent, glycerol could be incorporated into neosynthesized glucose released by small intestine. In contrast, alanine and lactate, which were more actively incorporated into systemic glucose than was glutamine (compare the arterial [^{14}C]glucose specific activity from the three [^{14}C] precursors in Table 3), were not incorporated at the level of small intestine. Because both are well known to constitute the best liver gluconeogenic substrates, one may hypoth-

esize that incorporation of these substrates into glucose mainly took place in this tissue. Our results are consistent with known liver and intestinal metabolic fluxes. It is indeed well established that glutamine is actively taken up and metabolized by small intestine (30,31). In contrast, alanine and lactate are released by small intestine in the portal vein and are then actively taken up by the liver (32,33).

In fasted rats, we calculated that gluconeogenesis from glutamine and glycerol could account for 57 and 19% of total IGR, respectively. Glutamine enters gluconeogenesis through the Krebs cycle at the level of α -ketoglutarate. Because of carbon exchanges through cycling, the glutamine flux to glucose, determined herein, might be underestimated by up to 40% (16,34). In contrast, glycerol enters gluconeogenesis at the level of 3-carbon compounds and does not undergo similar carbon exchanges within the Krebs cycle. Because the small intestine glycogen storage capacity ($\sim 0.5 \text{ mg/g}$ wet tissue) is not altered in fasting (Fig. 1), the amount of glucose released from small intestine glycogenolysis in fasted rats should be very low. Taking into account that the actual small intestine gluconeogenesis from glutamine might be up to 40% higher than that measured, $\sim 80\%$ (instead of 57%) of the total IGR, it may be postulated that total IGR in 48-h-fasted rats might be accounted for by gluconeogenesis from glutamine for a bulk part and from glycerol for a remaining marginal part ($\sim 20\%$). In diabetic rats, the small intestine gluconeogenic flux from glutamine accounted for about half of the IGR, close to that calculated in fasted rats. We could not, however, obtain evidence for the incorporation of glycerol carbons into venous glucose. This might be

because of the reasons of imprecision outlined above. Alternatively, the hypothesis that a substantial proportion of the IGR in diabetic rats might be derived from glycogen stores is plausible, since the latter were substantial in diabetic rats (Fig. 1).

It is noteworthy that the gene expression studies provided a strong molecular basis accounting for these metabolic fluxes. In the liver, PC is considered to be the first committed enzyme in gluconeogenesis, accounting for the utilization of alanine and lactate after conversion in pyruvate by alanine transaminase and lactate dehydrogenase, respectively. A dramatic lowering in PC V_{max} in small intestine occurred in both fasted and diabetic rats. The effect likely took place at a posttranslational level because there was no alteration in the abundance of PC mRNA. This is in keeping with the fact that alanine and lactate were not incorporated into glucose produced by small intestine. On the other hand, we measured a twofold increase in PC V_{max} in the liver of the same rats (not shown), in agreement with previous data (35) and with the utilization of both alanine and lactate as major substrates of liver gluconeogenesis. In contrast with PC, a strong induction of PEPCK gene expression, at both the mRNA and enzymatic activity levels, occurred in small intestine in insulinopenic states. The latter result is in agreement with our previous data (5), in spite of very different protocols of sampling. In the liver, PEPCK is considered a major regulatory enzyme of gluconeogenesis from oxaloacetate, accounting for the utilization of both pyruvate (after conversion in oxaloacetate by PC) and substrates cycling through the Krebs cycle. This is the fate of glutamine after conversion in glutamate and α -ketoglutarate. In small intestine, the coordinate inverse regulation of PC and PEPCK may thus account for the utilization of glutamine as a major substrate of gluconeogenesis, as well as for the nonutilization of alanine and lactate. With regard to glycerol utilization, this substrate enters gluconeogenesis at the level of 3-carbon compounds after phosphorylation by glycerokinase. The presence (without induction) of this enzyme in small intestine is in keeping with the lesser role of glycerol as a substrate for gluconeogenesis in fasted rats. In diabetic rats, our data suggest that the IGR not arising from glutamine may derive from glycogenolysis rather than from glycerol gluconeogenesis. In keeping with an active glycogen metabolism occurring in small intestine, the presence of glycogen stores and of glycogen synthase and phosphorylase has been previously reported in both rodent and human small intestine and intestinal cell lines (36–38). Of note, the abnormal accumulation of glycogen is also known to occur in the diabetic kidney (39), and it has recently been suggested that kidney glycogenolysis might contribute EGP in type 1 diabetic patients (40).

In conclusion, following our recent report that Glc6Pase and PEPCK genes are expressed in rat small intestine and are strongly induced in fasted and diabetic states (4,5), we show here that, at variance with the current view that only the liver and the kidney are glucose-producing organs, small intestine contributes ~20–25% of systemic EGP in insulinopenia in rats. In addition, small intestine glucose production may be suppressed by insulin. This identifies small intestine as a new insulin-sensitive tissue. Finally,

we have specified the metabolic and molecular mechanisms underlying small intestine glucose production, pointing out the crucial role of glutamine as a precursor. Because the major regulatory genes of gluconeogenesis (i.e., Glc6Pase, PEPCK, and fructose-1,6-bisphosphatase) are expressed in human small intestine (4,5,28) and because glutamine is a crucial substrate for this tissue too, we believe that these results will certainly enable a better understanding of the mechanisms involved in the pathophysiology of human diabetes.

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REFERENCES

- Mittelman S, Bergman RN: Liver glucose production in health and disease. *Curr Opin Endocrinol Diabetes* 5:126–135, 1999
- Adroge H: Glucose homeostasis and the kidney. *Kidney Int* 42:1266–1282, 1992
- Mithieux G: New knowledge regarding glucose-6 phosphatase gene and protein and their roles in the regulation of glucose metabolism. *Eur J Endocrinol* 136:137–145, 1997
- Rajas F, Bruni N, Montano S, Zitoun C, Mithieux G: The glucose-6 phosphatase gene is expressed in human and rat small intestine: regulation of expression in fasted and diabetic rats. *Gastroenterology* 117:132–139, 1999
- Rajas F, Croset M, Zitoun C, Montano S, Mithieux G: Induction of PEPCK gene expression in insulinopenia in rat small intestine. *Diabetes* 49:1165–1168, 2000
- Cersosimo E, Judd RL, Mile JM: Insulin regulation of renal glucose metabolism in conscious dogs. *J Clin Invest* 93:2584–2589, 1994
- Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J: Uptake and release of glucose by the human kidney. *J Clin Invest* 96:2528–2533, 1995
- Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J: Contribution by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes* 48:292–298, 1999
- Mithieux G, Croset M, Zitoun C, Hurot JM: Contribution of small intestine to endogenous glucose production in insulinopenia states in rats (Abstract). *Diabetes* 48 (Suppl. 1):279A, 1999
- Lebrech D, Blanchet L: Effect of two models of portal hypertension on splanchnic organ blood flow in the rat. *Clin Sci* 68:23–28, 1985
- McDewitt DG, Nies AS: Simultaneous measurements of cardiac output and its distribution with microspheres in the rat. *Cardiovascular Res* 10:494–498, 1976
- Barbee RW, Perry BD, Ré RN, Murgu JP: Microsphere and dilution techniques for the determination of blood flows and volumes in conscious mice. *Am J Physiol* 263:R728–R733, 1992
- Minassian C, Tarpin S, Mithieux G: Role of glucose-6 phosphatase, glucokinase, and glucose-6 phosphate in liver insulin resistance and its correction by metformin. *Biochem Pharmacol* 55:1213–1219, 1998
- Guignot L, Mithieux G: Mechanisms by which insulin, associated or not with glucose, may inhibit hepatic glucose production in the rat. *Am J Physiol* 277:E984–E989, 1999
- Beylot M, Previs SF, David F, Brunengraber H: Determination of the ^{13}C -labeling pattern of glucose by gas-chromatography-mass spectrometry. *Anal Biochem* 212:526–531, 1993
- Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J: Human kidney and liver gluconeogenesis: evidence for organ substrate selectivity. *Am J Physiol* 274:E817–E826, 1998
- Peroni O, Large V, Beylot M: Measuring gluconeogenesis with $2[^{13}\text{C}]$ -glycerol and mass isotopomer distribution analysis of glucose. *Am J Physiol* 269:E516–E523, 1995
- Croset M, Brossard N, Pachiaudi C, Normand S, Lecerf J, Chirouze V, Riou

- JP, Tayot JL, Lagarde M: In vivo compartmental metabolism of ^{13}C -docosahexaenoic acid, studied by gas chromatography-combustion isotope ratio mass spectrometry. *Lipids* 31: S109–S115, 1996
19. Jomain-Baum M, Schramm VL, Hanson RW: Mechanism of 3-mercaptopicolinic acid inhibition of hepatic phosphoenolpyruvate carboxykinase (GTP). *J Biol Chem* 251:37–44, 1976
 20. Ballard FJ, Hanson RW: Phosphoenolpyruvate carboxykinase and pyruvate carboxylase in developing rat liver. *Biochem J* 104:866–871, 1967
 21. Bergmeyer HU, Gawehn K, Grassl M: Enzymes as biochemical reagents. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Deerfield Beach, FL, Verlag-Chemie, 1974, p. 425–522
 22. 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
 23. Bergmeyer HU, Bernt E, Schmidt F, Stork M: D-glucose. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Deerfield Beach, FL, Verlag-Chemie, 1974, p. 1196–1201
 24. Guthmann I, Wahlefeld AW: L-(+)-lactate-determination with lactate dehydrogenase and NAD. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Deerfield Beach, FL, Verlag-Chemie, 1974, p. 1464–1468
 25. Keppler D, Decker K: Glycogen: determination with amyloglucosidase. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Deerfield Beach, FL, Verlag-Chemie, 1974, p. 1127–1131
 26. Anderson JW: Glucose metabolism in jejunal mucosa of fed, fasted and streptozotocin-diabetic rats. *Am J Physiol* 226:226–229, 1974
 27. Bismut H, Hers HG, Van Schaftingen E: Conversion of fructose to glucose in the rabbit small intestine. *Eur J Biochem* 213:721–726, 1993
 28. Froesh RE: Essential fructosuria and hereditary fructose intolerance. In *The Metabolic Basis of Inherited Disease*. Stanbury JB, Wyngaarden JB, Fredrickson DS, Eds. New York, McGraw Hill, 1972, p. 131–138
 29. Ferré P, Leturque A, Burnol AF, Penicaud L, Girard J: A method to quantify glucose utilization in vivo in skeletal muscle and white adipose tissue of the anaesthetized rat. *Biochem J* 228:103–110, 1985
 30. Windmueller HG, Spaeth AE: Identification of ketone bodies and glutamine as the main respiratory fuels in vivo for postabsorptive rat small intestine. *J Biol Chem* 253:69–76, 1978
 31. Windmueller HG, Spaeth AE: Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats: quantitative importance of glutamine, glutamate and aspartate. *J Biol Chem* 255:107–112, 1980
 32. Owen OE, Reichle FA, Mozzoli MA, Kreulen T, Patel MS, Effenbein IB, Golsorkhi M, Chang KHY, Rao NS, Sue HS, Boden G: Hepatic, gut, and renal substrate flux rates in patients with hepatic cirrhosis. *J Clin Invest* 68:240–252, 1981
 33. Remesy C, Demigne C, Aufrère J: Interorgan relationships between glucose, lactate and aminoacids in rats fed on high-carbohydrate or high-protein diets. *Biochem J* 170:321–329, 1997
 34. Consoli A, Kennedy F, Miles J, Gerich J: Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the overall contributions of gluconeogenesis and glycogenolysis to overall hepatic glucose output in man. *J Clin Invest* 80:1303–1310, 1987
 35. Jitrapakdee S, Wallace J: Structure, function and regulation of pyruvate carboxylase. *Biochem J* 340:1–16, 1999
 36. Anderson JW, Jones A: Biochemical and ultrastructural study of glycogen in jejunal mucosa of diabetic rats. *Proc Soc Exp Biol Med* 145:268–272, 1974
 37. Field JB, Epstein S, Egan T: Studies in glycogen storage diseases. I. Intestinal glucose-6 phosphatase activity in patients with von Gierke's disease and their parents. *J Clin Invest* 44:1240–1247, 1965
 38. Rousset M, Paris H, Chevalier G, Terrain B, Murat JC, Zweibaum A: Growth-related enzymatic control of glycogen metabolism in cultured human tumor cells. *Cancer Res* 44:154–160, 1984
 39. Holck P, Rasch R: Structure and segmental localization of glycogen in the diabetic rat kidney. *Diabetes* 42:891–900, 1993
 40. Mitrakou A, Meyer C, Tolia A, Platanisiotis D, Vlachos L, Gerich J, Raptis SA: Evidence for increased glycogenolysis and gluconeogenesis in type 1 diabetes mellitus (Abstract). *Diabetologia* 42 (Suppl. 1):165A, 1999