# Glucose Production and Gluconeogenesis in Postabsorptive and Starved Normal and Streptozotocin-Diabetic Rats

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**Using a 3-hour primed-continuous infusion of [3-$^{3}$H]glucose and [2-$^{13}$C]glycerol, we measured glucose production, gluconeogenesis from glycerol, and total gluconeogenesis (using mass isotopomer distribution analysis [MIDA] of glucose) in postabsorptive and starved normal and streptozotocin-diabetic rats. In normal rats, 48 hours of starvation increased ($P < .01$) the percent contribution of both gluconeogenesis from glycerol (from 14.4% ± 1.8% to 25.5% ± 4.0%) and total gluconeogenesis (from 52.2% ± 3.9% to 89.8% ± 1.3%) to glucose production, but the absolute gluconeogenic fluxes were not modified, since glucose production decreased. Diabetic rats showed increased glucose production in the postabsorptive state; this decreased with starvation and was comparable to that of controls after 48 hours of starvation. Gluconeogenesis was increased in postabsorptive diabetic rats (69.0% ± 1.3%, $P < .05$ *v* controls). Surprisingly, this contribution of gluconeogenesis to glucose production was not found to be increased in 24-hour starved diabetic rats (64.4% ± 2.4%). These rats had significant liver glycogen stores, but gluconeogenesis was also low (42.8% ± 2.1%) in 48-hour starved diabetic rats deprived of glycogen stores. Moreover, in 24-hour starved diabetic rats infused with [3-$^{13}$C]lactate, gluconeogenesis was 100% when determined by comparing circulating glucose and liver pyruvate enrichment, but only 47% ± 3% when calculated from the MIDA of glucose. Therefore, MIDA is not a valid method to measure gluconeogenesis in starved diabetic rats. This was not explained by differences in the labeling of liver and kidney triose phosphates: functional nephrectomy of starved diabetic rats decreased glucose production, but gluconeogenesis calculated by the MIDA method was only 48% ± 3.3%. We conclude that (1) diabetic rats have increased glucose production and gluconeogenesis in the postabsorptive state; (2) starvation decreases glucose production and increases the contribution of gluconeogenesis, but MIDA is not an appropriate method in this situation; and (3) the kidneys contribute to glucose production in starved diabetic rats.**


THE FASTING HYPERGLYCEMIA of diabetes is considered to result from both impaired glucose utilization and excessive glucose production.[1] The role of these two abnormalities is still debated, but a major role is usually attributed to glucose overproduction. This is based on the finding of large increases in endogenous glucose production (EGP) in diabetic patients[1] and experimental models of diabetes,[2-5] and of a positive correlation between fasting glucose production and hyperglycemia in diabetic subjects.[1]

The excessive glucose production was itself ascribed to an exaggerated gluconeogenesis.[6-7] However, these concepts have been recently challenged. First, there is convincing evidence that tracer-determined EGP was overestimated in diabetic patients[8-10] and in most studies of experimental models of diabetes.[11] Although there is indeed a positive relationship between EGP and fasting glucose level in diabetic patients,[12] significant increases in EGP are found only in patients with significant hyperglycemia.[8-9] Second, the method used to demonstrate increased gluconeogenesis in diabetic patients[6-7] was shown to be invalid for in vivo studies.[13-15] Recent studies using other approaches showed either no increase[16] or a 25% increase[17] in the percent contribution of gluconeogenesis to EGP in overnight-fasted type II diabetic humans; absolute gluconeogenesis was increased 40% to 60% in these two studies, since EGP was found to be elevated in diabetic subjects. Although the absolute gluconeogenic rate was not available in type I diabetics, the percent contribution was 45% of EGP after an overnight fast,[18] a value comparable to that of normal subjects.[16] This percent contribution increased to 90% after a 40- to 60-hour fast in normal volunteers.[18]

We anticipated that both starved normal and diabetic rats will demonstrate a similar 90% contribution of gluconeogenesis to EGP. In the present study, we measured EGP and gluconeogenesis in normal rats and in a model of insulinopenic (streptozotocin-induced) diabetes in rats. Both groups of rats were studied in the postabsorptive and starved states; gluconeogenesis was measured with [2-$^{13}$C]glycerol infusion to simultaneously determine gluconeogenesis from glycerol and, using mass isotopomer distribution analysis (MIDA) of glucose, total gluconeogenesis.[19-21]

## MATERIALS AND METHODS

### *Materials*

Chemicals were acquired from Sigma Chemicals (St Louis, MO) or Merck (Darmstadt, Germany), and enzymes were from Boehringer (Mannheim, Germany). [2-$^{13}$C]glycerol (99% atoms $^{13}$C) was from Isotec (St Quentin, France), [6,6-$^{2}H_2$]glucose and [1-$^{13}$C]glucose from Eurositop (Gif sur Yvette, France), [6-$^{13}$C]glucose from Sigma Chemicals, and [3-$^{13}$C]lactate from Mass Trace (Woburn, MA). [3-$^{3}$H]glucose was from Isotopchim (Ganogobie-Peyrins, France).

### *Protocols*

Male Sprague-Dawley rats were obtained from IFFA-Creddo (L'Arbresle, France) and housed under controlled temperature (22°C) and lighting (lights on 8 AM to 8 PM). They were divided into control and diabetic groups. Diabetes was induced by intraperitoneal injection of streptozotocin in 24-hour fasted rats (70 mg/kg freshly prepared in 150 mmol/L citrate buffer, pH 4.5); only rats with glycemia above 15 mmol/L were studied. Normal and diabetic rats were studied in the postabsorptive state (6 hours after food removal, ie, food was removed at 8 AM and tracer infusion started at 2 PM: normal, n = 6, 250 to 300 g body weight; diabetic, n = 6, 200 to 230 g body weight) or after 48

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hours (normal, n = 7, 180 to 210 g body weight; diabetic, n = 3, 180 to 200 g body weight) or 24 hours (diabetic rats only) of starvation. Diabetic rats starved for 24 hours were divided into two groups, one infused after ligature of the kidney artery and vein (n = 8, 180 to 225 g body weight) and one without ligature (n = 7, 240 to 250 g body weight). After anesthesia with sodium pentobarbital (6 mg/100 g body weight intraperitoneally), catheters were inserted into the right atrium through the right jugular vein and into the aorta through the left carotid. [2-$^{13}$C]glycerol (10 μmol · kg$^{-1}$ · min$^{-1}$) and [3-$^{3}$H]glucose (0.12 and 0.24 μCi/min in starved and postabsorptive control rats and 0.48 μCi/min in diabetic rats) were infused through the jugular vein for 180 minutes after a priming dose (infusion rate × 10 for 1 minute). Arterial blood was sampled before tracer infusion and at 150, 165, and 180 minutes. After the last blood sampling, the abdomen was quickly opened, portal venous blood was collected, and the liver was freeze-clamped with tongs cooled in liquid nitrogen.

Additional groups of diabetic rats were infused in the postabsorptive state (n = 5, 220 to 250 g body weight) or after 24 hours of starvation (n = 7, 225 to 265 g body weight) with [3-$^{13}$C]lactate (10 μmol · kg$^{-1}$ · min$^{-1}$ in the postabsorptive group and 6 μmol · kg$^{-1}$ · min$^{-1}$ in the fasted group) for 4 hours after a priming dose (infusion rate × 20 for 1 minute). Blood was collected before tracer infusion and at 210, 255, and 240 minutes. The liver was freeze-clamped at the end of the experiments.

### *Analytical Procedures*

Plasma glucose, lactate, and glycerol levels were measured by enzymatic assays.[22] Glucose and glycerol were purified from neutralized perchloric extracts of plasma by sequential ion-exchange chromatography. Part of the neutralized extract was dried before chromatography to measure glucose radioactivity. The neutral eluate from the ion-exchange chromatography was split into two parts. One was used to measure glycerol enrichment by gas chromatography–mass spectrometry using the triacetate derivative as previously described.[23] The other part was used to prepare the aldonitrile pentaacetate derivative of glucose.[24] This derivative was used for gas chromatographic–mass spectrometric measurement of the total enrichment and the MIDA of glucose; these procedures have been described in detail previously.[20-21] Frozen liver samples were crushed in a mortar cooled in liquid nitrogen and homogenized in cold perchloric acid (6% wt/vol). After centrifugation, the supernatant was neutralized with 3 mol/L $K_2CO_3$, and the glycogen concentration was measured by enzymatic assay.[25] $^{13}$C enrichment of the glucosyl units of glycogen was measured using the aldonitrile pentaacetate derivative of glucose. For experiments with labeled lactate infusion, liver lactate and pyruvate enrichment were measured as previously described.[26] All isotopic enrichment (IE) determinations were performed on a gas chromatograph (HP581; Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (OV1701; Chrompack, Bridgewater, NJ) and interfaced with a mass spectrometer (HP5971A; Hewlett-Packard) operating in the electron-impact ionization mode. Standard curves prepared by mixing known amounts of natural and labeled compounds were analyzed with each series of biological samples. Special care was taken to obtain comparable ion peak areas between the standard and biological samples, adjusting the injection volume or the split ratio when necessary.[20]

### *Calculations*

The glucose turnover rate (Rt) was calculated from the glucose specific activity and the tracer infusion rate using an equation for the steady state; in these conditions, glucose Rt is equal to EGP. The endogenous glycerol appearance rate (Ra) was calculated from the tracer infusion rate (I) and glycerol IE measured in arterial blood and expressed as moles percent excess (MPE) using the equation for steady-state Ra: (I/IE) − I. The glycerol clearance rate was calculated as the ratio of total glycerol (endogenous plus tracer infusion rate) Rt to glycerol concentration. Gluconeogenesis from glycerol was calculated from the $^{13}$C enrichment in glucose and glycerol with the equation, percent glucose formed from glycerol = (glucose IE/[2 × glycerol IE]) × 100, using the enrichment of glycerol measured in portal venous blood.[21] $^{13}$C glucose IE was calculated from the fractional abundance of the isotopomers of glucose, g0 (no excess $^{13}$C), g1 (one excess $^{13}$C), and g2 (two excess $^{13}$C; g0 + g1 + g2 = 1), as IE = g1 + (2 × g2). Calculation of total gluconeogenesis from the MIDA of glucose has been described in detail previously.[20] In short, from the ratio r = g1/g2, one can calculate the enrichment of triose phosphates as p = 2/(r + 2). This holds theoretically only if the labeling of glucose is symmetrical; however, we and others[19-20] have shown that significant errors occur only when large asymmetry is observed, a situation not encountered in this study. Comparison of the calculated IE of triose phosphates with half the IE of glucose yields the percent contribution of gluconeogenesis to EGP. For experiments with labeled lactate infusion, gluconeogenesis was calculated (1) from the IE of circulating glucose and of liver lactate or pyruvate as percent of glucose formed from gluconeogenesis = $^{13}$C glucose IE/(2 × lactate or pyruvate IE) and (2) from the MIDA of glucose.

Results are shown as the mean ± SEM. Comparisons were performed using Student's *t* test for paired data (within-group comparison) or one-way ANOVA (between-group comparison).

## RESULTS

All diabetic rats, including the 48-hour starved group, had glycemia above 15 mmol/L, versus 10.0 ± 0.3 and 7.1 ± 0.4 mmol/L in postabsorptive and 48-hour starved normal rats. Glucose concentration was stable during the study for all groups except 24-hour starved diabetic rats with ligature of the kidney vein and artery. In this group, glucose increased after the ligature from 19.1 ± 1.4 to 30.4 ± 1.5 mmol/L and remained stable thereafter. To obtain accurate measurements of the MIDA of glucose, we had to use a high infusion rate of labeled glycerol. This resulted in an increase of glycerol above the basal value. However, glycerol concentrations were stable during the 150 to 180 minutes of the sampling period (200 to 230 μmol/L). The increase was more important (to 380 to 410 μmol/L) in 24-hour starved diabetic rats with ligature of the kidney vein and artery, since glycerol increased after the ligature before starting the tracer infusions. Rats were infused in the venous-arterial mode, and glycerol enrichment was therefore higher in arterial blood than in portal venous blood (Table 1). Endogenous glycerol Ra was not modified by starvation in control rats (Table 2). Diabetic rats in the postabsorptive state had an endogenous glycerol Ra comparable to that of the controls; this Ra was not modified by starvation for 24 hours, but a 50% decrease was observed in the 48-hour starved diabetic group (Table 2). Kidney-vessel ligature also decreased ($P < .01$ *v* the group without ligature) the glycerol Ra and induced a large decrease of the glycerol clearance rate (from 131 ± 11 to 64 ± 6 mL · kg$^{-1}$ · min$^{-1}$, $P < .01$). As expected, starvation decreased the glucose Rt in both normal and diabetic rats. Compared with the control group, diabetic rats had increased glucose production in the postabsorptive state, but this difference disappeared in the 48-hour starved groups (Table 2). Lastly, the glucose Ra was moderately lower ($P < .05$) in diabetic rats with kidney ligature than in the corresponding group without ligature.

**Table 1. IE of Circulating Glycerol and Glucose**

| Group | Glycerol IE | | Glucose IE | |
|---|---|---|---|---|
| | Carotid Artery | Portal Vein | g1 | g2 |
| Normal rats | | | | |
| PA | 45.2 ± 1.7 | 31.3 ± 3.0* | 9.61 ± 1.21 | 0.54 ± 0.08 |
| 48 h S | 40.7 ± 1.3 | 37.6 ± 1.6* | 13.51 ± 0.95 | 0.62 ± 0.09 |
| Diabetic rats | | | | |
| PA | 47.1 ± 3.5 | 43.4 ± 2.9* | 11.15 ± 0.45 | 0.57 ± 0.07 |
| 24 h S | 49.3 ± 1.1 | 44.5 ± 2.1* | 10.72 ± 0.33 | 0.56 ± 0.04 |
| 24 h S LK | 62.1 ± 4.6 | 55.8 ± 3.5* | 12.65 ± 1.14 | 1.17 ± 0.24 |
| 48 h S | 60.0 ± 4.6 | 58.9 ± 0.6 | 11.81 ± 1.00 | 1.03 ± 0.16 |

NOTE. IE is presented as the MPE (mean ± SEM).

Abbreviations: PA, postabsorptive; S, starved; LK, with kidney ligature. (These abbreviations are also used in Tables 2 to 4.)

*$P < .05$ *v* the value in arterial blood.

The contribution of glycerol to glucose production expressed as a percentage or absolute value is shown in Table 3 In normal rats, the percent contribution was increased ($P < .05$) by starvation but the absolute value was not significantly modified, since glucose production was lower in the starved group. Postabsorptive diabetic rats had a percent contribution of glycerol to glucose production similar to that of postabsorptive controls and a higher absolute flux ($P < .01$) due to an enhanced production of glucose. Contrary to what was observed in normal rats, this percent contribution of glycerol was not increased but instead was decreased by starvation, and the absolute contribution, already decreased in the 24-hour starved group, was markedly decreased ($P < .01$) in the 48-hour starved group compared with both postabsorptive diabetic and 48-hour starved normal rats. Lastly, in addition to decreasing the glycerol Ra and glucose Ra, kidney ligature decreased the absolute gluconeogenesis from glycerol ($P < .01$).

The total contribution of gluconeogenesis to glucose production as calculated from the MIDA of glucose was increased by starvation, as expected, in normal rats when expressed as a percent of total glucose production (Table 4),. but was unchanged when calculated as the absolute flux. Compared with the corresponding control group, postabsorptive diabetic rats had an increased gluconeogenic rate when expressed as a percent contribution ($P < .05$) or an absolute value ($P < .05$). Surprisingly, the percent contribution of gluconeogenesis did not increase when diabetic rats were starved for 24 hours and was lower than in the starved normal group ($P < .01$). These

**Table 2. Endogenous Production of Glucose and Glycerol ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)**

| Group | Glucose Ra | Glycerol Ra |
|---|---|---|
| Normal rats | | |
| PA | 68.1 ± 3.1 | 12.2 ± 0.8 |
| 48 h S | 50.8 ± 2.4* | 11.7 ± 0.7 |
| Diabetic rats | | |
| PA | 110.9 ± 7.2‡ | 12.6 ± 1.8 |
| 24 h S | 83.9 ± 3.9* | 13.5 ± 0.6 |
| 24 h S LK | 69.1 ± 2.5*† | 10.2 ± 0.7† |
| 48 h S | 58.1 ± 8.7* | 6.3 ± 1.1*†‡ |

*$P < .01$ *v* corresponding PA group.

†$P < .01$ *v* 24 h S group.

‡$P < .01$ *v* corresponding group of normal rats.

**Table 3. Contribution of Glycerol to EGP Expressed as a Percent (%) or as an Absolute Value ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)**

| Group | Gluconeogenesis From Glycerol | |
|---|---|---|
| | % | $\mu mol \cdot kg^{-1} \cdot min^{-1}$ |
| Normal rats | | |
| PA | 14.4 ± 1.8 | 9.9 ± 1.3 |
| 48 h S | 25.2 ± 4.0* | 12.8 ± 2.0 |
| Diabetic rats | | |
| PA | 14.6 ± 1.2 | 16.0 ± 1.0‡ |
| 24 h S | 13.5 ± 0.8 | 11.3 ± 0.6* |
| 24 h S LK | 11.5 ± 0.8 | 7.9 ± 0.5*† |
| 48 h S | 11.8 ± 1.0‡ | 6.4 ± 0.3*†‡ |

*$P < .01$ *v* corresponding PA group.

†$P < .01$ *v* 24 h S group.

‡$P < .01$ *v* corresponding group of normal rats.

24-hour starved diabetic rats had a significant concentration of liver glycogen when measured either at the end of the experiments (16.6 ± 1.6 mg/g liver $v < 0.1$ in 48-hour starved control rats) or, in an additional group, 3 hours earlier, ie, corresponding to time 0 of the tests (21.0 ± 2.3 mg/g liver). However, when gluconeogenesis was calculated in 48-hour starved diabetic rats, which had no measurable liver glycogen, we found a further decrease in both the percent contribution and the absolute gluconeogenic flux (Table 4). There was no significant incorporation of $^{13}C$ in the liver glycogen of 24-hour starved diabetic rats. The calculated gluconeogenic fluxes were also decreased when the kidneys were ligatured in 24-hour starved diabetic rats. Given these results, we infused two additional groups of diabetic rats (one in the postabsorptive state and one 24-hour fasted) with [3-$^{13}C$]lactate and estimated gluconeogenesis by comparing the enrichment of plasma glucose versus those of liver lactate and pyruvate. In postabsorptive diabetic rats, the percent contribution of gluconeogenesis estimated from enrichment of plasma glucose and liver lactate and pyruvate was, respectively, 44% ± 4% and 73% ± 6%. In the starved group, the corresponding estimates were 63% ± 5% and 100% ± 9%. However, the contribution of gluconeogenesis to total glucose production calculated from the MIDA of glucose was 64% ± 4% in the postabsorptive group (total glucose enrichment, 7.13% ± 0.35%) but only 47% ± 3% in the starved rats (total glucose enrichment, 7.41% ± 0.38%).

**Table 4. Calculated Contribution of Gluconeogenesis to EGP Expressed as a Percent (%) or as an Absolute Value ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)**

| Group | Gluconeogenesis | |
|---|---|---|
| | % of EGP | $\mu mol \cdot kg^{-1} \cdot min^{-1}$ |
| Normal rats | | |
| PA | 52.2 ± 3.9 | 37.2 ± 3.3 |
| 48 h S | 89.8 ± 1.3* | 45.2 ± 3.4 |
| Diabetic rats | | |
| PA | 69.0 ± 4.1‡ | 75.3 ± 5.7§ |
| 24 h S | 64.4 ± 2.4 | 54.1 ± 3.1* |
| 24 h S LK | 48.0 ± 3.3*†§ | 33.2 ± 2.7*†‡ |
| 48 h S | 42.8 ± 2.1*†§ | 23.4 ± 0.2*†§ |

*$P < .01$ *v* corresponding PA group.

†$P < .05$ *v* 24 h S group.

‡$P < .05$, §$P < .01$: *v* corresponding group of normal rats.

## DISCUSSION

### *EGP and Endogenous Glycerol Production*

EGP has usually been reported to be elevated in insulin-dependent diabetic animals,[2-5] suggesting that excessive glucose production plays an important role in the hyperglycemia of these experimental models of diabetes. This view was recently challenged, at least in the postabsorptive state, by Wi et al.[11] They infused (without a priming dose) normal and diabetic rats in the postabsorptive state with [6-$^3$H]glucose for 6 hours. In these conditions, stable glucose specific activity was obtained within 3 hours in normal rats, but not before 5 hours in the diabetic group. EGP measured after plateau specific activity was achieved was 22% higher in the diabetic group, but this difference failed to reach statistical significance. Comparable data have been reported in non–insulin-dependent diabetic subjects.[8-10] We infused labeled glucose for 3 hours; therefore, we considered the possibility that we overestimated EGP in diabetic rats in the present study. The maximal error can be estimated from the data of Wi et al[11] to be 20% (which would yield in postabsorptive diabetic rats an EGP of 88 ± 5 instead of 111 ± 7 μmol/kg/min, still higher than in control postabsorptive rats, $P < .05$). However, this overestimation is unlikely since we used a priming dose, contrary to Wi et al, and except for rats with kidney ligature, glucose specific activity was the same at 150 and 180 minutes (eg, EGP in postabsorptive diabetic rats was calculated to be 113 ± 5 and 109 ± 6 μmol/kg/min at 150 and 180 minutes, respectively, $P > .60$; the corresponding values were 86 ± 4 and 81 ± 2 in 24-hour starved diabetic rats without kidney ligature, $P > .40$). Endogenous glycerol Ra was not modified by diabetes or starvation, except in 48-hour starved diabetic rats and 24-hour starved diabetic rats with kidney ligature, which had a lower Ra. Diabetic rats starved 48 hours had a large decrease in body weight, with a near-total exhaustion of subcutaneous adipose tissue explaining the decreased lipolytic rate. The moderate decrease of endogenous glycerol Ra in the group with kidney ligature agrees with the demonstration that the kidney contributes 4% to 7% to glycerol Ra in dogs.[27] The decrease in glycerol clearance rate also agrees with the well-known role of the kidney in glycerol utilization.[27-28] EGP was also decreased in diabetic rats with kidney ligature. This could be the consequence of the increased glucose level induced by the suppression of glycosuria. However, Wi et al[11] showed that EGP was not modified when the glycemia of diabetic rats was normalized by phlorizin, suggesting that the ability of glucose to regulate EGP is impaired in streptozotocin-induced diabetes. Therefore, we believe our data confirm that the kidneys significantly contribute to glucose production in diabetic rats, as shown in dogs[29] and in rats with functional hepatectomy.[30] This interpretation is also supported by the increase in kidney glucose-6-phosphatase activity induced by starvation and streptozotocin-diabetes in rats.[31]

### *Gluconeogenesis From Glycerol and Total Gluconeogenesis*

In normal rats, the percent contribution of both glycerol and total gluconeogenesis to EGP was increased by starvation but the absolute gluconeogenic fluxes were not significantly enhanced, since total EGP decreased. This suggests that the increase in the percent contribution was merely the result of the decrease in the glycogenolytic rate with the progressive exhaustion of liver glycogen stores. Postabsorptive diabetic rats had an elevated percent contribution of gluconeogenesis, as measured by glucose MIDA, but not of the percent contribution of glycerol, suggesting that gluconeogenesis from other substrates was enhanced. Surprisingly, the percent contribution of total gluconeogenesis to EGP did not increase in 24-hour starved diabetic rats. This could signify either that there was actually always a significant glycogenolytic rate in these rats or that the estimate of gluconeogenesis obtained by MIDA is not valid in this situation. The first possibility is supported by the parallel lack of an increase in the contribution of glycerol to EGP and the presence of significant glycogen stores in the liver. However, glycogenolysis estimated from comparison of liver glycogen content measured at 0 and 180 minutes would represent only 5 to 8 μmol/kg/min glucose production. Moreover, gluconeogenesis estimated by the MIDA of glucose was only 43% of EGP in 48-hour starved diabetic rats deprived of liver glycogen stores. Lastly, gluconeogenesis in 24-hour starved diabetic rats infused with [3-$^{13}$C]lactate was 100% of EGP when estimated by comparison of plasma glucose and liver pyruvate enrichment, but only 47% when calculated from the MIDA of glucose. This last low estimate of gluconeogenesis with MIDA contrasts with the plausible value (97%) found in 2-day starved normal rats infused with [U-$^{13}$C]lactate.[32] Therefore, we believe that the MIDA of glucose is not a valid method to estimate total gluconeogenesis in diabetic rats (at least during starvation, since estimates of gluconeogenesis obtained by the MIDA method during [2-$^{13}$C]glycerol or [3-$^{13}$C]lactate infusion and by the comparison of plasma glucose and liver pyruvate enrichment during [3-$^{13}$C]lactate infusion are consistent in postabsorptive diabetic rats). Limitations in the use of MIDA to measure gluconeogenesis have been pointed out by Previs et al[32] and Landau et al,[33] who found underestimates of gluconeogenesis in 2-day fasted rats and 60-hour fasted humans, respectively. They ascribed these underestimates to heterogeneity of the labeling of the precursor triose phosphate pool, which itself is due to a periportal to pericentral decrease of glycerol concentration[33] and enrichment.[32] In contrast, we[20-21] and Neese et al[19] found that gluconeogenesis measured by the MIDA method accounted for 90% to 100% of EGP in fasted rats. We had no clear explanation for these discordant results. We suggested that in our experiments and in those of Neese et al the increase in glycerol concentration and hepatic glycerol load induced by a high infusion rate of labeled glycerol allowed obtainment of homogenous labeling of the triose phosphate pool. This explanation does not hold for starved diabetic rats, since we used the same tracer infusion rate and observed comparable increases of glycerol. We hypothesized that the gluconeogenesis underestimation could be due to a difference in triose phosphate labeling between the liver and kidney, but the results obtained in rats with kidney ligature rule out this possibility. We do not presently have a clear explanation for this discrepancy in the measurement of gluconeogenesis by the MIDA method between normal and diabetic rats.

### *Liver Glycogen in Diabetic Rats*

Liver glycogen stores are depleted in 24-hour starved normal rats.[34] The persistence of liver glycogen in 24-hour starved but

not 48-hour starved insulinopenic diabetic rats was reported more than 30 years ago by Friedmann et al,[34] confirming previous observations (see Friedmann et al[34] for earlier references). There is also evidence for the presence of large glycogen stores in the liver of fasted diabetic subjects.[35] In addition, Friedmann et al[34] showed that when glycogen stores of 24-hour starved diabetic rats were depleted by glucagon, there was a rapid return of glycogen to initial levels even in the absence of an exogenous glucose load. However, this ability to accumulate glycogen appeared to be limited, since starved diabetic rats stored less glycogen after refeeding than starved-refed normal rats.[34,36] The mechanisms leading to this persistence of glycogen stores in 24-hour starved diabetic rats are not clear. There is evidence for a decreased glycogenolytic rate in non–insulin-dependent diabetic humans.[18] The small decrease in liver glycogen in a 3-hour period in the present report in insulinopenic rats would also be indicative of a low glycogenolytic rate. However, this result should be interpreted cautiously, since glycogen levels were measured in two different groups of diabetic rats. Moreover, there could be a simultaneous synthesis and breakdown of glycogen. Significant glycogen synthesis in these conditions seems unlikely, as neither Friedmann et al[34] nor Wi et al[11] found incorporation of labeled glucose into liver glycogen (incorporation was only found after depletion of glycogen stores when net glycogen deposit occurred). Incorporation through the indirect, neoglucogenic pathway is also unlikely, since we found no incorporation of $^{13}C$ in glycogen despite the high infusion rate of labeled glycerol. Therefore, it is probable that liver glycogen turnover is low in 24-hour fasted diabetic rats. The return of liver glycogen to initial values after glucagon-induced depletion involved glycogen synthesis through both direct and indirect pathways, as shown by the redistribution of the labeled carbon of [1-$^{14}C$]glucose in position 6 of the glucosyl unit of glycogen.[34] The direct pathway requires phosphorylation of glucose. The phosphorylation rate should be decreased in diabetic rats since glucokinase activity is low in insulinopenic diabetes,[37] and this should limit glycogen synthesis, as there is evidence that glucokinase is limiting for liver glucose utilization, including glycogen synthesis.[38] However, glucose is also phosphorylated by liver hexokinase I. The high glucose level of diabetic rats could maintain a significant flux through this enzyme, contributing to the slightly elevated glucose-6-phosphate level found in alloxan-diabetic rats[36] and to the glycogen deposition observed upon refeeding or after glucagon-induced glycogen depletion in starved diabetic rats. Moreover, it has been shown that glucose-6-phosphate produced by hexokinase I, contrary to that produced by glucokinase, does not promote glycogen synthase activation.[39] This could explain why the capacity of the liver to accumulate glycogen is reduced in diabetic rats.

In conclusion, postabsorptive EGP is moderately increased in streptozotocin-induced diabetes in rats. Gluconeogenesis (despite limitations in the use of MIDA to measure it in starved diabetic rats) contributes to this increased EGP. The kidney participates in both gluconeogenesis and glycerol metabolism. Moreover, there are also abnormalities of glycogen metabolism in this model of insulinopenic diabetes.

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