Characterization of the portal signal during 24-h glucose delivery in unrestrained, conscious rats

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Oghihara, N., W. Kawamura, K. Kasuga, Y. Hayashi, H. Arakawa, and M. Kikuchi. Characterization of the portal signal during 24-h glucose delivery in unrestrained, conscious rats. Am J Physiol Endocrinol Metab 286: E932–E940, 2004. First published January 6, 2004; 10.1152/ajpendo.00511.2002.—To characterize the “portal signal” during physiological glucose delivery, liver glycogen was measured in unrestrained rats during portal (Po) and peripheral (Pe) constant-rate infusion, with minimal differences in hepatic glucose load (HGL) and portal insulin between the delivery routes. Hepatic blood flows were measured by Doppler flowmetry during open surgery. Changes in hepatic glucose, portal insulin, glucagon, lactate, and free fatty acid concentrations were generally similar in either delivery except for glucagon at 4 h. Hepatic glycogen, however, increased continuously in Po and was higher than Pe at 8 and 24 h, although it decreased to the level of Pe upon the removal of Po at 8 h. There was a near-linear relationship between hepatic glycogen and HGL in either delivery, with the slope being twice as high in Po and the intercepts converging to basal HGL. The hepatic response to Po did not alter upon 80% replacement by Pe. These results suggest that negative arterial-portal glucose gradients increase the rate of hepatic glycogen synthesis in the incremental HGL in an all-or-nothing mode.

METHODS

Animal care. Male Sprague-Dawley rats (Charles River Breeding Laboratories Japan, Tokyo, Japan), aged 7–8 wk and weighing 220–250 g, were used. Immediately after surgery, they were housed individually in transparent cages under a controlled temperature (20°C) and a standard 12:12-h light (6:00 AM to 6:00 PM)-dark (6:00 PM to 6:00 AM) cycle. They were maintained on rat Purina chow and water ad libitum. Twenty-four hours before the study, the animals were deprived of food to deplete liver glycogen, but they were allowed free access to water. Only rats that had recovered their presurgery weight and exhibited normal activity were used. The protocol was approved by the institutional animal care committee.

Surgical procedures. Three days before the experiment, rats underwent carotid artery, jugular vein, and hepatic portal vein cannulation under pentobarbital sodium anesthesia (50 mg/kg ip; Abbott Laboratories, Tokyo, Japan). Two polyethylene catheters (PE-10, 0.28 mm ID, 0.61 mm OD; Intramedic, Clay Adams, NJ) for glucose infusion were introduced into the right jugular vein and extended to the level of the right atrium after blunt dissection. The left carotid artery was visualized after separation of the sternomastoides and sternohyoides muscles, whereupon another polyethylene catheter for blood sampling was advanced and implanted at the level of the aortic arch. A hepatic portal catheter for glucose infusion was inserted after making a 2- to 3-cm laparotomy incision. Briefly, after the cecum had been lifted out of the peritoneum, two mesenteric vessels draining to the ileocolic vein were located, and the area at the junction of these two vessels was cleared of fat. After a small incision was made, the ileocolic vein's distal part was ligated. The liver was exposed, and the left portal vein was ligated at the level of the hilus, while the right portal vein was ligated near the hepatic hilus. The portal vein was exposed at the level of the inferior edge of the right lobe, and a 1-cm incision was made in the anterior abdominal wall 0.5 cm away from the portal vein. The portal vein was then exposed by blunt dissection, and a 1.5-cm long incision was made to expose the portal vein. Two polyethylene catheters (PE-10, 0.28 mm ID, 0.61 mm OD; Intramedic, Clay Adams, NJ) for glucose infusion were introduced into the right jugular vein and extended to the level of the right atrium after blunt dissection. The left carotid artery was visualized after separation of the sternomastoides and sternohyoides muscles, whereupon another polyethylene catheter for blood sampling was advanced and implanted at the level of the aortic arch. A hepatic portal catheter for glucose infusion was inserted after making a 2- to 3-cm laparotomy incision. Briefly, after the cecum had been lifted out of the peritoneum, two mesenteric vessels draining to the ileocolic vein were located, and the area at the junction of these two vessels was cleared of fat. After a small incision was made, the ileocolic vein's distal part was ligated. The liver was exposed, and the left portal vein was ligated at the level of the hilus, while the right portal vein was ligated near the hepatic hilus. The portal vein was exposed at the level of the inferior edge of the right lobe, and a 1-cm long incision was made to expose the portal vein. Two polyethylene catheters (PE-10, 0.28 mm ID, 0.61 mm OD; Intramedic, Clay Adams, NJ) for glucose infusion were introduced into the right jugular vein and extended to the level of the right atrium after blunt dissection. The left carotid artery was visualized after separation of the sternomastoides and sternohyoides muscles, whereupon another polyethylene catheter for blood sampling was advanced and implanted at the level of the aortic arch. A hepatic portal catheter for glucose infusion was inserted after making a 2- to 3-cm laparotomy incision. Briefly, after the cecum had been lifted out of the peritoneum, two mesenteric vessels draining to the ileocolic vein were located, and the area at the junction of these two vessels was cleared of fat. After a small incision was made, the ileocolic vein's distal part was ligated. The liver was exposed, and the left portal vein was ligated at the level of the hilus, while the right portal vein was ligated near the hepatic hilus. The portal vein was exposed at the level of the inferior edge of the right lobe, and a 1-cm long incision was made to ex...
another polyethylene catheter was threaded toward the portal vein and positioned ~2 cm upstream of the bifurcation of the portal vein. The localization of the tip of the catheter was verified at autopsy. All of the catheters were tunneled under the skin, fixed with a ligature between the shoulders, and connected via tubing to an infusion pump (Harvard Apparatus, South Natick, MA) so that the animals could be studied in the awake, unrestrained state. To avoid clot formation, a heparinized (200 U/ml) saline solution was introduced into the catheters just before they were sealed.

**Experimental design.** Five to eight animals were used for each experimental condition. All animals were exposed to the same general protocol for stimulating hepatic glycogen deposition. On the day of the experiment, all catheters were opened and cleared of heparinized saline, and the first blood sample was taken; this time was identified as 0 min. The rats used for the following experiments were ones in which the plasma glucose levels in the first blood sample were <5 mmol/l.

Glucose (20% dextrose; Abbott Laboratories, North Chicago, IL) was infused through the portal vein catheter (Po) or the jugular vein catheter (Pe) over 24 h at the rate of 0, 12, 38, 48, 56, or 78 mmol/kg−1min−1 and coinfused intraportally at 16 mmol/kg−1min−1 and peripherally at 62 mmol/kg−1min−1, or Po was switched over to Pe at 8 h. Arterial blood samples were successively obtained at 0, 2, 4, 8, and 24 h or, in addition, at 5, 20, 40, and 60 min during glucose infusion to measure the plasma glucose levels. Separately, portal vein blood was sampled before and 2, 4, 8, or 24 h after the cessation of glucose infusion to measure plasma insulin, glucagon, lactate, and free fatty acid (FFA). Immediately after the last blood sampling, the abdomen was quickly opened under anesthesia with pentobarbital sodium (25 mg/kg) via an arterial catheter. After intestinal reflection, Doppler flow cuffs of a range-gated, ultrasonic transit time flowmeter (T106; Transonic System, Ithaca, NY) were placed around the hepatic artery and portal vein, and blood flows were measured, within 1 min, without any interruption of the glucose infusion. After blood flow monitoring, the hepatic venous blood was sampled, and small pieces from the three lobes of the liver were freeze-clamped with aluminum tongs precooled in liquid nitrogen to measure the hepatic glucose or the glycogen. The blood flow monitoring did not affect the hepatic glycogen content (see discussion). The control animals were treated in an identical fashion except that 0.9% saline was infused at the rate of 1 ml/h (1.3% of portal flow rate) instead of the glucose infusion (Table 1). The total blood volume sampled was 2.3 ml at the maximum. To avoid excessive bleeding, red blood cells of the rat were resuspended in saline and reinjected as soon as possible after each blood sampling (<20% of the rat’s total blood volume was removed). The glycogen concentrations were the means of the values from the three liver lobes in each animal. In a previous preliminary experiment, the hepatic glycogen concentration had been found to be similar in each lobe, and there was a high correlation in the mean values between the three lobes and the total liver (r = 0.82, n = 13).

**Analytic procedures.** Blood glucose was determined by means of a glucose oxidase method using a Beckman glucose analyzer (Fullerton, CA), whereas hepatic tissue glucose was determined spectrophotometrically by the method of Bergmeyer et al. (4), using hexokinase and glucose-6-phosphate dehydrogenase. The liver glucose concentration was measured directly and calculated using total liver water as the denominator on the basis of a report that the extracellular component is ~30% of the liver water, whereas the intracellular component is 70% (11, 26). Plasma insulin was measured using a species-specific double-antibody radioimmunoassay (Phadeseph Insulin Test; Pharmacia Diagnostics, Uppsala, Sweden) and the plasma glucagons with a commercially available kit (Daichii, Tokyo, Japan), with interassay coefficients of variability of 11 and 8%, respectively. Plasma samples for glucagon determination were preserved by the addition of aprotinin (Trasylol; Bayer, Kankakee, IL) at collection. Plasma lactate was analyzed by the method of Lloyd et al. (18). Plasma concentrations of the FFAs were determined using microfluorometric methods. The liver samples were ground to powder with mortars and pestles cooled with liquid nitrogen and were then stored at −70°C. Glycogen was extracted from the liver sample by use of the HSO4 extraction and ethanol precipitation method described by Good et al. (10). The glucose hydrolyzed from glycogen was assayed by a glucose oxidase method. The concentration of liver glycogen was expressed as micromoles of glucosyl units per gram wet weight.

**Calculation of HGL.** The load of glucose entering the liver (HGL) during Pe was calculated according to the equation: \( [G]_A \times (F_{HA} + F_{PV}) \), in which \([G]_A \) represents the blood glucose concentration in an artery, \( F_{HA} \) is the blood flow rate of the hepatic artery, and \( F_{PV} \) is that of the portal vein. During Po, the portal vein glucose concentration cannot be used to calculate HGL, since the portal blood sample is taken downstream from the site of entry of the glucose infusion and the mixing of the blood and glucose is problematic. Therefore, the HGL during intraportal glucose infusion was determined indirectly according to the equation: \( [G]_A \times (F_{HA} + F_{PV}) + GUG \times GUG \), where \( F_{HA} \) is the intraportal glucose infusion rate and \( GUG \) is the gut uptake of glucose and is assumed to be negligible since, in a preliminary experiment, the \( GUG \) values during Pe at 78 mmol/kg−1min−1 had been found to be less than or similar to the basal values (basal: 0.16 ± 0.19 mmol/kg−1min−1, n = 4) and were not dose dependent. The plasma glucose value was corrected to whole plasma glucose with the equation (the blood glucose/plasma glucose ratio had been verified independently): \( \text{[G]plasma} = 0.83 \times \text{[G]plasma} + 15.4, n = 60, r = 0.97 \).

**Statistical analysis.** Data are expressed as means ± SE. The time course data were analyzed with repeated-measures ANOVA within groups. Statistical comparisons between groups were made using independent t-tests. Where appropriate, the correlation coefficients were calculated, and when significant correlations were found, the method of least squares was used to obtain a linear regression equation. Differences in the slopes of the regression lines against the

<p>| Table 1. Arterial glucose level, A-P glucose gradient, hepatic blood flows, and hepatic glucose load during Po and Pe |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Infusion Rate, mmol/kg−1min−1</th>
<th>Delivery Group</th>
<th>Arterial Glucose, mmol/l</th>
<th>A-P Glucose Gradient, mmol/l</th>
<th>Hepatic Arterial Blood Flow, ml/min</th>
<th>Portal Venous Blood Flow, ml/min</th>
<th>Hepatic Glucose Load, μmol/kg−1min−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>Po</td>
<td>4.6 ± 0.1</td>
<td>0</td>
<td>3.9 ± 0.5</td>
<td>13.0 ± 0.9</td>
<td>9.1 ± 0.7</td>
</tr>
<tr>
<td>22</td>
<td>Po</td>
<td>4.5 ± 0.1</td>
<td>0.42 ± 0.06</td>
<td>3.8 ± 0.6</td>
<td>13.1 ± 0.8</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>22</td>
<td>Pe</td>
<td>6.9 ± 0.3</td>
<td>0</td>
<td>4.5 ± 1.1</td>
<td>13.3 ± 2.2</td>
<td>14.8 ± 2.3</td>
</tr>
<tr>
<td>47</td>
<td>Po</td>
<td>7.0 ± 0.1</td>
<td>0.69 ± 0.04</td>
<td>3.4 ± 0.4</td>
<td>13.6 ± 1.7</td>
<td>10.0 ± 2.6</td>
</tr>
<tr>
<td>47</td>
<td>Pe</td>
<td>7.5 ± 0.3</td>
<td>0.78 ± 0.2</td>
<td>3.6 ± 0.4</td>
<td>15.2 ± 1.7</td>
<td>17.0 ± 0.8</td>
</tr>
<tr>
<td>78</td>
<td>Po</td>
<td>8.3 ± 0.2</td>
<td>1.14 ± 0.10</td>
<td>3.9 ± 0.9</td>
<td>15.9 ± 1.5</td>
<td>21.2 ± 2.1</td>
</tr>
<tr>
<td>78</td>
<td>Pe</td>
<td>8.5 ± 0.2</td>
<td>0</td>
<td>4.4 ± 0.4</td>
<td>16.0 ± 1.7</td>
<td>20.2 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). A-P, arterial-portal; Po and Pe, portal and peripheral glucose delivery, respectively. *Significant difference within the groups (ANOVA) P < 0.05. †Significant difference between delivery routes (nonpaired t-test) P < 0.05. ‡Mean values expressed as cumulative areas under the curves (AUCs) divided by 24 h. §Measured at 24 h during glucose delivery at open surgery.

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horizontal axis were assessed with contrasts of ANOVA. Statistical significance was accepted at $P < 0.05$.

RESULTS

Arterial plasma glucose, A-P glucose gradients, blood flows, and HGL during glucose infusion at different rates. Figure 1 shows the changes in the arterial glucose concentration over 24 h during glucose infusion in Po and Pe at the rates of 0, 22, 47, and 78 $\mu$mol-kg$^{-1}$min$^{-1}$. The concentration of plasma glucose did not change from baseline in the control rats, whereas it increased rapidly and reached peaks of from 1.4 to $\sim$2.0-fold basal within 4 h in either delivery mode; thereafter, a plateau or a slight decline was observed. Table 1 shows the mean cumulative values over 24 h of arterial plasma glucose, the A-P glucose gradient, and the HGL, as well as the hepatic arterial and portal blood flows. These parameters increased dose dependently except for the hepatic arterial blood flow. They did not differ between Po and Pe at any rate except for the negative A-P gradient. The HGL was proportional to the glucose infusion rate and the negative A-P gradients in either route of delivery (Po: $r = 0.99$, Pe: $r = 0.98$ for the infusion rates; Po and Pe, $r = 0.995$ for the A-P gradients).

Portal plasma insulin, glucagon, lactate, and FFA levels during glucose delivery at 78 $\mu$mol-kg$^{-1}$min$^{-1}$. Figure 2 shows the time courses of plasma hormone and metabolite excursions in the portal vein over 24 h during glucose infusion at 78 $\mu$mol-kg$^{-1}$min$^{-1}$ and saline infusion as a control via the portal or the peripheral route. As is depicted in Fig. 2A, portal insulin tended to increase to an initial peak at 2 h in Po, with a second, slower rise thereafter, whereas it increased gradually in Pe without any changes in the control. Portal glucagon promptly increased and nearly reached the maximum of three times the basal by 2 h in the control, whereas it started to rise at 8 h in Pe or increased gradually in Po, reaching the control levels at 24 h in either method of delivery (Fig. 2B). The lactate concentration reached a near peak at 2 h and leveled off thereafter in either method of delivery (Fig. 2C). FFAs decreased to nadir at 4–8 h without any further changes (Fig. 2D). There was no significant difference in the concentrations and the areas under the curves (AUCs) of the hormones or metabolites over 24 h between Po and Pe, except for the glucagon concentration at 4 h.

HGL and net hepatic glycogen synthesis during glucose delivery at 78 $\mu$mol-kg$^{-1}$min$^{-1}$. Table 2 shows the time courses of the hepatic glucose concentration directly measured during glucose infusion at 78 $\mu$mol-kg$^{-1}$min$^{-1}$. The postload values of hepatic glucose increased by 11–17% compared with those of arterial glucose, nearly reached maximum by 2 h, and maintained a similar level thereafter. However, there was no difference in hepatic glycemia at any time between the delivery

![Fig. 1. Changes in arterial plasma glucose concentrations over 24 h during portal (Po) or peripheral glucose delivery (Pe) at the rates of 22, 47, and 78 $\mu$mol-kg$^{-1}$min$^{-1}$. Data represent the mean of each group of 5 rats. Mean values were expressed as cumulative areas under the curves (AUCs) up to the arbitrary time point divided by the minute number.](http://ajpendo.physiology.org/)

![Fig. 2. Changes in portal plasma insulin (A), glucagon (B), lactate (C), and free fatty acid (FFA; D) concentrations over 24 h during Po or Pe at the rate of 78 $\mu$mol-kg$^{-1}$min$^{-1}$. Data represent means ± SE of each group of 8 rats. Mean values were expressed as cumulative AUCs up to the arbitrary time point divided by the minute number. #Significant differences between portal and peripheral delivery ($P < 0.05$).](http://ajpendo.physiology.org/)
routes. On the other hand, the net hepatic glycogen synthesis continued to rise in Po, whereas it remained equilibrated after 8 h in Pe, although it increased similarly over 4 h in either mode of delivery (Fig. 3). As a result, the amount of hepatic glycogen was significantly greater at 8 h and was even more so at 24 h in Po compared with that of Pe. There was also a significant difference between the 8-h and 24-h values in Po (189.5 ± 21.9 for 8 h vs. 234.8 ± 12.1 μmol·kg⁻¹·min⁻¹ for 24 h, P < 0.05). Therefore, in the following experiments, the hepatic glycogen was measured after a 24-h glucose infusion.

Portal insulin and net hepatic glycogen synthesis 24 h after glucose delivery at different rates. When glucose was infused over 24 h intraportally or peripherally at the rates of 0, 11, 22, 38, 47, 58, and 78 μmol·kg⁻¹·min⁻¹, the portal insulin at 24 h increased dose dependently with no significant difference between the delivery routes (Table 3). In contrast, the amounts of hepatic glycogen were significantly higher in Po than in Pe at a glucose dose of >47 μmol·kg⁻¹·min⁻¹ (Table 4).

Relation between hepatic glycogen and HGL or A-P gradients. Figure 4 illustrates the relationships between hepatic glycogen deposition at 24 h and the mean values of the cumulative amount of HGL over 24 h. Hepatic glycogen increased approximately linearly with the increasing HGL in either method of delivery. The slope of the regression line was about twice as high in Po as in Pe (23.2 ± 3.8 for Po vs. 12.2 ± 4.7 for Pe, n = 8; contrast of ANOVA, P < 0.05), and the intercepts on the abscissa converged to near-basal HGL values [Po: 10.4 ± 3.2 vs. Pe: 8.6 ± 2.7 μmol·kg⁻¹·min⁻¹, n = 8, P = not significant (NS)]. Similarly, there was a linear relationship between hepatic glycogen and A-P gradients in Po (r = 0.997).

A-P gradients and hepatic glycogen after partial replacement of Po by Pe. When glucose was simultaneously infused intraportally at 16 μmol·kg⁻¹·min⁻¹ and peripherally at 62 μmol·kg⁻¹·min⁻¹ (20%Po/80%Pe), the A-P gradient was lowered by 77%, from −1.14 to −0.26 mmol/l, but arterial glycogen, hepatic blood flow, and HGL did not change (Table 4).
5). Portal insulin, glucagon, and hepatic glycogen deposition did not differ at 8 and 24 h compared with those in Po (Fig. 5). As a result, the net hepatic glycogen synthesis was dissociated from its linear relation to the negative A-P glucose gradient (Fig. 6).

**Hepatic glycogen synthesis after switchover from Po to Pe.** When Po or the coinfusion via the two routes (20%Po/80%Pe) was discontinued at 8 h and followed by Pe, arterial glucose, A-P gradients, hepatic blood flows, and HGL (Table 6), like the portal insulin and glucagon levels, did not differ compared with those of Po (Fig. 7). However, the net hepatic glycogen synthesis was reduced to that of Pe.

**DISCUSSION**

The induction of a negative A-P glucose gradient is very closely associated with increases in both the HGL and portal insulin levels during physiological glucose delivery. Therefore, we undertook to minimize any potentially confounding effects of different HGL and plasma insulin concentrations between Po and Pe by prolonging the glucose infusion periods in conscious, unrestrained rats. As is shown in Figs. 1 and 2 and Table 2, the time courses of the portal glucose, insulin, glucagon, lactate, and FFA excursions, as well as the hepatic glucose concentration, were generally similar over 24 h in either method of glucose delivery. In contrast, as is depicted in Fig. 3, the cumulative amount of hepatic glycogen in Po increased significantly at 24 h over that of 8 h, and it was larger at 8 and 24 h than that of Pe. The initial temporal change in plasma glucose, insulin, or glucagon over 8 h is unlikely to affect the hepatic glycogen at 24 h because the hepatic glycogen decreased to the level of Pe when Po was discontinued and Pe was started at 8 h (Fig. 7). On this occasion, there was no difference in HGL over 24 h, or in portal insulin and glucagon at 24 h, compared with those in Po throughout (Table 6). Evidence also showed that the on-and-off time constants for the effect of the portal signal on the liver are rapid (12, 13).

Table 5. *Arterial glucose level, A-P glucose gradient, hepatic blood flows, and hepatic glucose load over 8 and 24 h during Po in 80% replacement by Pe*

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Delivery Group</th>
<th>Arterial Glucose, mmol/l</th>
<th>A-P Glucose Gradient, mmol/l</th>
<th>Hepatic Arterial Blood Flow, ml/min</th>
<th>Portal Venous Blood Flow, ml/min</th>
<th>Hepatic Glucose Load, μmol/kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Po</td>
<td>8.2±0.3</td>
<td>1.11±0.12</td>
<td>4.1±0.7</td>
<td>15.6±1.3</td>
<td>21.5±0.6</td>
</tr>
<tr>
<td></td>
<td>20%Po/80%Pe</td>
<td>8.4±0.4</td>
<td>0.22±0.01</td>
<td>4.2±0.6</td>
<td>15.2±1.8</td>
<td>20.4±1.8</td>
</tr>
<tr>
<td>24</td>
<td>Po</td>
<td>8.3±0.2</td>
<td>1.14±0.10</td>
<td>3.9±0.9</td>
<td>15.9±1.5</td>
<td>21.2±2.1</td>
</tr>
<tr>
<td></td>
<td>20%Po/80%Pe</td>
<td>9.3±0.5</td>
<td>0.26±0.02</td>
<td>4.4±0.9</td>
<td>13.7±1.5</td>
<td>19.5±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). *Significant difference between delivery routes (nonpaired t-test), P < 0.05. ¹Mean values expressed as cumulative AUCs divided by 24 h. ²Measured at 24 h during glucose delivery at open surgery.
24 h; I
Pe, switchover from Po to Pe at 8 h.
3
glucose via Po at 16 mol/kg \(\cdot \) min
over 24 h. A similar hepatic glycogen synthesis over
h in Po than in Pe (Fig. 2B) and by the lower glycemic levels in Po at 78 mol/kg \(\cdot \) min
than those in Pe for much of the first 4 h (Fig. 1).
The linear relationship between net hepatic glycogen
synthesis and HGL in rats (Fig. 4) was similar to that
achieved between NHGU and HGL in dogs (28). The slope of the
regression lines of the net hepatic glycogen against the HGL
axis was two times higher in Po than in Pe in rats, whereas that
of NHGU was four times higher than Pe in dogs, with the
intercepts on the HGL axis converging near the basal values in
both animals. These results are in concert with those in the
work by Pagliassotti et al. (29), who demonstrated that most of
the glucose equivalents were retained by the liver and were
diverted into glycogen, not oxidized or stored as fat. Our
findings extend the earlier observations of Myers and col-
leagues (7, 24), who demonstrated that the portal signal re-
duced the HGL threshold for NHGU, whereas the incremental NHGU is largely the same
regardless of the HGL. In contrast, the different slopes in our
studies favor the interpretation that the portal signal increases
the rate of NHGU or hepatic glycogen synthesis relative to the
increase in HGL above the basal level.
The 80% replacement of Po by Pe elucidates the relationship
between the negative A-P gradients and the net hepatic glyco-

Table 6. Arterial glucose level, A-P glucose gradient, hepatic blood flows, and hepatic glucose load on partial replacement and switchover at 8 h of Po by Pe

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Delivery Group</th>
<th>Arterial Glucose, mmol/l</th>
<th>A-P Glucose Gradient,1 mmol/l</th>
<th>Hepatic Arterial Blood Flow,2 ml/min</th>
<th>Portal Venous Blood Flow,2 ml/min</th>
<th>Hepatic Glucose Load,3 (\mu\text{mol/kg} \cdot \text{min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Po</td>
<td>8.2±0.3</td>
<td>1.11±0.12</td>
<td>4.1±0.7</td>
<td>15.6±1.3</td>
<td>21.5±0.6</td>
</tr>
<tr>
<td></td>
<td>Pe</td>
<td>8.5±0.4</td>
<td>0</td>
<td>4.0±0.6</td>
<td>15.0±1.4</td>
<td>21.4±1.2</td>
</tr>
<tr>
<td></td>
<td>Po→Pe</td>
<td>8.2±0.3</td>
<td>1.11±0.12</td>
<td>4.2±0.5</td>
<td>15.6±1.6</td>
<td>21.3±1.1</td>
</tr>
<tr>
<td></td>
<td>20%Po/80%Pe→Pe</td>
<td>8.4±0.4</td>
<td>0.22±0.01</td>
<td>4.1±0.8</td>
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<td>Po</td>
<td>8.3±0.2</td>
<td>1.14±0.10</td>
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<td>15.9±1.5</td>
<td>21.2±2.1</td>
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<tr>
<td></td>
<td>Pe</td>
<td>8.5±0.2</td>
<td>0</td>
<td>4.4±0.4</td>
<td>16.0±1.7</td>
<td>20.2±1.5</td>
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<tr>
<td></td>
<td>Po→Pe</td>
<td>8.4±0.4</td>
<td>0</td>
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<td></td>
<td>20%Po/80%Pe→Pe</td>
<td>8.6±0.5</td>
<td>0</td>
<td>4.1±0.8</td>
<td>15.5±1.9</td>
<td>19.8±1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). *Significant difference between delivery routes (nonpaired t-test), \(P < 0.05\). 1Mean values expressed as cumulative AUCs divided by 24 h. 2Measured at 24 h during glucose delivery at open surgery.
gen synthesis. The resultant 77% reduction in the A-P gradients did not decrease the amount of hepatic glycogen at 24 h compared with that in Po (NS) at the level of equalized portal insulin and glucagons or at the HGL (Figs. 5 and 6 and Table 5). As a consequence, the hepatic response to the A-P gradients was differentiated from the response to the HGL (Fig. 7), because the HGL was proportional to the A-P gradients (see methods). It is conceivable, therefore, that the A-P gradient increases the slope in an all-or-nothing manner. This finding also extends a previous observation in the canine study by Pagliassotti et al. (31). They demonstrated that a 56% reduction in the plasma A-P glucose gradient evoked a 53% reduction in NHGU when HGL and plasma insulin were held constant. It seems, therefore, that NHGU is dependent on the magnitude of the A-P gradients but not on the HGL. This difference may be attributed, at least in part, to the difference in the experimental conditions. The A-P gradients were linearly related not only to hepatic glycogen synthesis in this study but also to NHGU in the previous study in dogs (28), whereas NHGU was saturated with increasing A-P gradients at greater than ~1 mmol in their study. The 80% replacement of Po by Pe achieved a comparable reduction in the A-P gradients in our study, whereas it evoked a lesser reduction in the A-P gradients in their study (31). Generally speaking, then, the liver appears to be more sensitive to the A-P gradients in the nonsteady hyperglycemic state than in the hyperglycemic and pancreatic clamp states. Therefore, the far smaller A-P gradients than in the clamp study could not reduce hepatic glycogen synthesis at all.

Portal glucose delivery at 16 μmol·kg⁻¹·min⁻¹ alone is unlikely to enhance the hepatic glycogen deposition, since the amount of HGL is estimated to be <17.6 μmol·kg⁻¹·min⁻¹, less than the threshold values for hepatic glycogen synthesis (Fig. 4 and Table 4). However, when the tiny portal glucose delivery was replaced by the peripheral delivery at 8 h, the cumulative hepatic glycogen decreased to the level of Pe at 24 h at the equalized levels of portal insulin, glucagon, and HGL (Figs. 6 and Fig. 8 and Table 6). This finding supports the postulate that hepatic glycogen synthesis is enhanced as long as the negative glucose gradient is present. Likewise, portal plasma insulin increases with an increase in the negative glucose gradients. However, portal insulin is less likely to play a major role in the enhancement of net hepatic glycogen synthesis in Po; there was no difference in the amount of portal insulin between the delivery routes (Table 3). Judging from these results, it is conceivable that the liver handles endogenous and exogenous glucose indiscriminately; in other words, HGL is a determinant of the amount of hepatic glycogen synthesis. The increase in the slope may be interpreted in terms of the activation of a rate-limiting enzyme or enzymes in the glycogenic pathway. The linear HGL dependency of hepatic glucose uptake and the glycogen synthesis suggests that glucokinase is one of the most plausible candidate enzymes. Cherrington (7) has hypothesized that the portal signal causes a translocation of glucokinase from the nucleus into the cytoplasm to phosphorlyse glucose.

Thorens’ group [Burcelin et al. (5)] has shown that portal glucose delivery at the rate of basal endogenous glucose production caused hypoglycemia in mice. However, hypoglycemia did not occur during intraportal glucose infusion at the rates of 38 and 47 μmol·kg⁻¹·min⁻¹, the rate comparable to the basal endogenous glucose production rate in rats (15, 19).

The result is in agreement with a dog study (20) or a human study (35).

It is worth noting that portal glucagon rapidly reached a near-maximal level of three times basal in the control and in Po, whereas it did not change over 8 h in Pe (Fig. 2B). The surge of glucagon disagrees with the previous canine studies in which portal glucagon did not increase in either method of delivery over 3 h (14, 22). Presumably, the instantaneous glucagon response to isotonic saline via the portal route is due to the stress or the load of the isotonic saline at 1.0 ml/h in rats, since putative baroreceptors have been reported to exist in the hepatoponortal region (23). Because glucose infusion with insulin release could have suppressed the glucagon release, the difference in portal glucagon between Po and Pe may also be partly attributed to the animals being stressed. The increase in the amount of portal glucagon from 8 h in Pe is unlikely to have lowered the quantity of hepatic glycogen compared with that of Po, because there was no significant difference between the different delivery routes either in portal glucagon at 8 and 24 h (Fig. 2B) or in the increments of portal glucagon from 8 to 24 h [Po, 76.8 ± 44.9 pg/ml vs. Pe, 116.6 ± 52.1 pg/ml (NS), n = 5]. If anything, the greater glucagon release in Po over 8 h would minimize the increment in glycogen deposition and cause the effect of the portal signal to be underestimated. The postoperative days are unlikely to have affected the results, as the portal glucagon and the hepatic glycogen deposition at 24 h
in Po did not differ at the 4th and 7th postoperative days [7 days: 60.2 ± 12.8 vs. 4 days: 52.0 ± 10.3 μg/ml for glucagon, n = 5 (NS); 7 days: 56.2 ± 15 vs. 4 days: 61.2 ± 8.4 μmol·g liver⁻¹·min⁻¹ for glycogen, n = 4 (NS)]. Thus portal glucagon excursion would not disturb our conclusions.

The accuracy of the HGL measurement needs some comment. HGL is determined by multiplying the arterial and portal glucose concentrations by the hepatic blood flows. In the present study, HGL was calculated indirectly using the arterial glucose concentration to minimize any errors introduced by inadequacies in the mixing of blood and glucose in the portal vein. The indirect method was found to be more precise than the direct measurement in dogs (28). Furthermore, we measured the liver tissue glucose concentration directly to verify the indirect measurement of the HGL (Table 2). Actually, the hepatic glucose concentrations were slightly (11–17%), but insignificantly, higher than those of arterial glucose at 2, 8, and 24 h but did not differ between the delivery routes. On the other hand, the continuous monitoring of hepatic blood flows is not possible at present in unrestrained rats. In the current study, hepatic blood flows measured under anesthesia were applied to conscious animals, so the HGL is qualitative in this study. However, the results were in good agreement with those obtained using radioactive-microsphere techniques in fasting, conscious rats (16, 17, 27, 34), although Sasaki and Wagner (32) have reported that splanchic blood flows increase slightly in anesthetized rats. The hepatic blood flow rates (F_ha + F_po) did not differ at 24 h between glucose and saline infusion under anesthesia in either method of delivery (NS, ANOVA), and there was no difference between the delivery routes (Table 1). Hepatic blood flows are known not to alter during glucose delivery in conscious dogs (21, 31). If we take all of these results together, it is conceivable that the hepatic blood flow does not change over 24 h before and during glucose infusion. If anything, such changes should have affected the rats equally during either peripheral or portal glucose infusion.

In summary, we found a near-linear relationship between the cumulative amount of hepatic glycogen and HGL in either Po or Pe over 24 h, with the slope being twice as high in Po and the intercepts converging near the basal HGL at comparable portal plasma insulin levels. The hepatic glycogen response to Po was unaltered by a 73% reduction in the negative A-P glucose gradients. These results suggest that 1) the liver handles endogenous and exogenous glucose as indiscriminately as the HGL, 2) a negative A-P glucose gradient increases, in an all-or-nothing mode, the slope for hepatic glycogen deposition against the amount of glucose newly entering the liver (incremental HGL), and 3) plasma insulin appears unlikely to play a positive role in the increase in the slope by the portal signal in a nonsteady, physiological, hyperglycemic state.

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