Hepatic glucose uptake rapidly decreases after removal of the portal signal in conscious dogs

Hsien, Po-Shuan, Mary Courtney Moore, DoSs W. Neal, and Alan D. Cherrington. Hepatic glucose uptake rapidly decreases after removal of the portal signal in conscious dogs. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E987–E992, 1998.—The aim of this study was to assess the decay of the effect of the portal signal on net hepatic glucose uptake (NHGU). Experiments were performed on five 42-h-fasted conscious dogs. After the 40-min basal period, somatostatin was given peripherally along with insulin (1.8 pmol·kg⁻¹·min⁻¹) and glucagon (0.65 ng·kg⁻¹·min⁻¹) intraportally. In the first experimental period (Pe-GLU-1; 90 min), glucose was infused into a peripheral vein to double the glucose load to the liver (HGL). In the second experimental period (Po-GLU; 90 min), glucose (20.1 µmol·kg⁻¹·min⁻¹) was infused intraportally and the peripheral glucose infusion was reduced to maintain the same HGL. In the third period (Pe-GLU-2; 120 min), the portal glucose infusion was stopped and the peripheral glucose infusion was increased to again sustain HGL. Arterial insulin levels (42 ± 3, 47 ± 3, 43 ± 3 pmol/l) were basal and similar in the Pe-GLU-1, Po-GLU, and Pe-GLU-2 periods, respectively. Arterial glucagon levels were basal and similar (51 ± 3, 49 ± 2, 46 ± 2 ng/l) in the three experimental periods. The glucose loads to the liver were 251 ± 11, 274 ± 14, and 276 ± 12 µmol·kg⁻¹·min⁻¹, respectively. NHGU was 6.3 ± 2.4, 19.1 ± 2.8, and 9.2 ± 1.2 µmol·kg⁻¹·min⁻¹, and nonhepatic glucose uptake (non-HGU) was 23.6 ± 3.0, 53 ± 3.8, and 25.5 ± 3.7 µmol·kg⁻¹·min⁻¹ in the three periods, respectively. Cessation of the portal signal for only 10 min shifted NHGU and non-HGU to 9.4 ± 2.2 and 25.0 ± 2.8 µmol·kg⁻¹·min⁻¹, respectively; thus the effect of the portal signal was rapidly reversed both at the liver and peripheral tissues.

THE LIVER is important in the maintenance of glucose homeostasis. Studies concerned with glucose feeding (1, 4, 12, 15) have shown that splanchnic removal of glucose is greater after oral glucose ingestion than after peripheral intravenous glucose administration. The mechanism for this difference is still not clearly understood. Our previous data (14) and reports from others (15) have demonstrated that under physiological conditions a “portal signal” induced by the negative arterial-portal glucose gradient seen with feeding may allow the liver to differentiate between endogenous and exogenous glucose. Several studies have clearly shown that the portal signal can increase net glucose uptake by the liver (NHGU) and reduce net glucose uptake by muscle (2, 5, 13).

The characteristics of the effect of the portal signal on hepatic glucose uptake are not fully defined. Pagliassotti et al. (13) compared the time course of the effects of insulin and the portal signal on hepatic glucose uptake and glycogen metabolism. NHGU reached a maximum after only 15 min of exposure to the portal signal. In contrast, a fourfold rise in insulin required 75 min to produce a maximal increase in NHGU, even though the eventual response was of the same magnitude as that caused by the portal signal. Another report (3) has suggested that the response of the liver to the portal signal is neurally mediated, perhaps explaining why it is so rapid. It is still not clear, however, whether the effect of the portal signal subsides promptly once it is removed. This is a particularly important question because it is vital to know whether the liver, once exposed to the portal signal, remains in a receptive state for glucose for a prolonged period. This would be the case if the portal signal activates the liver by producing a change in enzyme activity that is not rapidly reversible. The aim of present study, therefore, was to determine the time course of the decay of the effect of the portal signal on net glucose uptake by the liver once the signal itself is removed.

MATERIALS AND METHODS

Animals and surgical procedures. Studies were carried out on five 42-h-fasted, conscious mongrel dogs of either sex, weighing between 20 and 32 kg. All animals were maintained on a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet no. 5006, Purina Mills, St. Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight. The protocol was approved by the Vanderbilt University Medical Center Animal Care Committee, and animals were housed according to American Association for the Accreditation of Laboratory Animal Care International guidelines. Approximately 16 days before study, each dog underwent a laparotomy under general anesthesia during which sampling catheters were inserted into a hepatic vein, the hepatic portal vein, and a femoral artery, as described in detail elsewhere (9). Catheters were also placed in a splenic and a jejunal vein for intraportal infusion of insulin and glucagon (Eli Lilly, Indianapolis, IN), glucose, and para-aminobenzoic acid (PAH; Sigma, St Louis, MO). Ultrasound flow probes (Transonic Systems, Ithaca, NY) were positioned around the portal vein and hepatic artery, and their proximal ends were placed in subcutaneous pockets.

Approximately 2 days before study, blood was drawn to determine the leukocyte count and the hematocrit of each animal. The dog was studied only if it had a leukocyte count of <18,000/mm³, a hematocrit of >35%, and normal stools, and it consumed all of its daily food ration. On the morning of the study...
study, the proximal ends of the flow probes and surgically implanted catheters were exteriorized, the catheters were cleared, the dog was placed in a Pavlov harness, and intravenous access was established in three peripheral veins.

Experimental design. At $-120 \text{ min}$, a primed ($36 \mu\text{Ci}$), continuous ($0.3 \mu\text{Ci/min}$) peripheral infusion of $[3-\text{H}]$glucose and a continuous peripheral infusion of indocyanine green dye (Becton-Dickinson, Cockeysville, MD; $4 \mu\text{g-kg}^{-1}\cdot\text{min}^{-1}$) were begun. The latter provided confirmation of hepatic vein catheter placement and a second measurement of hepatic blood flow. After $80 \text{ min}$ ($-120 \text{ to } -40 \text{ min}$) of dye equilibration, there was a $40\text{-min}$ ($-40 \text{ to } 0 \text{ min}$) basal period, followed by two $90\text{-min}$ and one $120\text{-min}$ experimental periods. At time $0$, constant infusions of several solutions were begun, and these infusions continued for three experimental periods. Somatostatin ($0.8 \mu\text{g-kg}^{-1}\cdot\text{min}^{-1}$; Bachem, Torrance, CA) was infused to suppress endogenous insulin and glucagon secretion. Insulin ($1.8 \text{ pmol-kg}^{-1}\cdot\text{min}^{-1}$) and glucagon ($0.65 \text{ ng-kg}^{-1}\cdot\text{min}^{-1}$) were infused intraportally at basal rates. In addition, a primed, continuous peripheral infusion of $50\%$ dextrose was begun at time $0$ so that the blood glucose could be quickly clamped at a desired value. In the first experimental period (Pe-GLU-1), glucose was infused into a peripheral vein to double the glucose load to the liver (HGL). In the second experimental period (Po-GLU), glucose was infused intraportally ($20.1 \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$) and the peripheral glucose infusion rate was reduced to maintain the HGL. In the third experimental period (Pe-GLU-2), the portal glucose infusion was stopped and the peripheral glucose infusion was increased to sustain the HGL. Dextrose ($20\%$) was used for the portal glucose infusion, and PAH (delivered at $1.7 \mu\text{mol-kg}^{-1}\cdot\text{min}^{-1}$) was added to the infusate to assess mixing of the infused glucose with blood in the portal and hepatic veins, as described previously (3, 13). Blood samples of $0.2 \text{ ml}$ were obtained from the artery every $5 \text{ min}$ to permit measurement of the plasma glucose concentrations to allow maintenance of the HGL. Larger blood samples ($5 \text{ to } 10 \text{ ml}$) for data acquisition were obtained from the artery, portal vein, and hepatic vein every $15 \text{ min}$ during the last $30 \text{ min}$ of Pe-GLU-1, every $15 \text{ to } 30 \text{ min}$ during Po-GLU, and every $10 \text{ to } 60 \text{ min}$ during Pe-GLU-2.

Processing and analysis of samples. Blood glucose, lactate, and hematocrit and plasma glucose, insulin, and glucagon concentrations were determined as described previously (9). PAH was measured in perchloric acid-proteinized blood as previously described (3, 10, 13).

Calculation. When substrates are infused intraportally, the possibility of poor mixing with the blood in the laminar flow of the portal circulation is of concern. Mixing of the infused glucose in the portal vein was assessed by comparing the recovery of PAH, which was mixed with the portal glucose infusate, in the portal and hepatic veins with the PAH infusion rate (2, 10, 13). Because of the magnitude of the coefficient of variation of the method for assessing PAH balance, samples were considered statistically unmixed (i.e., $95\%$ confidence that mixing did not occur) if hepatic or portal vein recovery of PAH was $40\%$ greater or less than the actual amount of PAH infused (3, 10, 13). An experiment was defined as having poor mixing (and was excluded from the database) if a PAH recovery-to-infusion ratio of $>1.4$ or $<0.6$ was observed at more than one of the four time points in the portal glucose infusion phase. Six dogs were studied; one is not included because of poor mixing. The ratio of PAH recovery in the portal vein to the intraportal PAH infusion rate was $0.9 \pm 0.1$, and the ratio of PAH recovery in the hepatic vein to the PAH infusion rate was $0.8 \pm 0.1$ (a ratio of $1.0$ would represent perfect mixing). When a dog was retained in the database, all of the points were used whether they were mixed or not, because mixing errors occur randomly.

Hepatic blood flow (HBF) was calculated by two methods, ultrasonic flow probes and dye extraction (9), and was normalized by body weight. The results obtained with ultrasonic flow probes and indocyanine green were not significantly different, but the data shown in Figs. 1-6 are those obtained with the flow probes, because their measurement did not require an assumption regarding the distribution of the arterial and portal vein contribution to hepatic blood flow.

The rate of substrate delivery to the liver, or hepatic substrate load, was calculated by a direct (D) method as

$$\text{load}_D = (\text{load}_A \times \text{ABF}) + (\text{load}_P \times \text{PBF})$$

where $\text{load}_A$ is the substrate concentration, subscripts A and P refer to artery and portal vein, respectively, and ABF and PBF refer to blood flow through the hepatic artery and portal vein, respectively. A similar method was used to calculate the hepatic sinusoidal insulin and glucagon concentrations.

$$\text{H}\text{IG}_{\text{HS}} = [(\text{H}\text{IG}_A \times \text{ABF}) + (\text{H}\text{IG}_P \times \text{PBF})/(\text{ABF} + \text{PBF})]$$

where $\text{H}$ is the hormone concentration, and subscript HS refers to hepatic sinusoid. To avoid any potential errors arising from either incomplete mixing of glucose during intraportal glucose infusion or a lack of precise measurement of the distribution of hepatic blood flow, hepatic glucose load was also calculated by an indirect (I) method

$$\text{load}_I = (\text{G}_{\text{A}} \times \text{HBF}) + \text{GI}_P - \text{GUG}$$

where $G$ is the blood glucose concentration, GI$_P$ is the intraportal infusion rate, and GUG is the uptake of glucose by the gastrointestinal tract, calculated on the basis of the previously described relationship between the arterial blood glucose concentration and GUG (3, 10, 13). The load of a substrate exiting the liver was calculated as

$$\text{load}_{\text{out}} = \text{S}_i \times \text{HBF}$$

where subscript $i$ represents the hepatic vein.

Direct and indirect methods were used in calculation of net hepatic balance (NHB). The direct calculation was $\text{NHB}_D = \text{load}_{\text{out}} - \text{load}_I$ (D). The indirect calculation was $\text{NHB}_I = \text{load}_{\text{out}} - \text{load}_I$ (I). Both equations were used in calculation of net hepatic glucose balance (NHGB), but only the direct calculation was employed for calculation of lactate balance. Net fractional substrate extraction by the liver (direct or indirect) was calculated as the ratio of NHB to load$_I$. Net nonhepatic glucose uptake was calculated by subtracting the rate of net hepatic glucose uptake from the total glucose infusion rate. The net hepatic balance of glucose equivalents was calculated as the sum of the balances of NHGB (I) and lactate, once the latter was converted to glucose equivalents. This parameter was calculated to provide an index of glycogen deposition. The approach assumes that glucose distribution did not increase during glucose uptake by the liver (8). To calculate glucose balance, plasma glucose values were converted to whole blood glucose values by using a correction factor obtained by averaging the ratio of the whole blood glucose value to the plasma glucose value in each period and each vessel throughout the study. Calculations performed with plasma glucose values converted to blood glucose gave similar results to those performed with blood glucose values per se, but the variance was reduced because of the accuracy of plasma arteriovenous glucose differences, which can be obtained without a deproteinization step. Use of whole blood
Glucose ensures accurate NHGB measurements regardless of the characteristics of glucose entry into the erythrocyte.

Data are presented as means ± SE. SYSTAT (Evanston, IL) was used for statistical analysis. The time course data were analyzed with repeated-measures ANOVA with post hoc analysis by univariate F tests. Results were considered statistically significant at P < 0.05.

**RESULTS**

Plasma insulin and glucagon concentrations. The arterial (48 ± 9 vs. 44 ± 4 pmol/l; Table 1) and hepatic sinusoidal (140 ± 8 vs. 126 ± 12 pmol/l; Fig. 1, top) plasma insulin concentrations were slightly lower in the experimental periods than in the control period, but they remained basal and equal throughout all three experimental periods. Arterial (49 ± 3 vs. 50 ± 3 ng/l; Table 1) and hepatic sinusoidal (56 ± 2 vs. 59 ± 4 ng/l; Fig. 1, bottom) plasma glucagon levels were basal and equal in the control and experimental periods.

Blood glucose and lactate levels and hepatic blood flow. During the portal glucose infusion period, arterial glucose levels were slightly lower (9.6 ± 0.2 vs. 9.4 ± 0.2 mM) and portal glucose levels were significantly greater (9.4 ± 0.2 vs. 10.0 ± 0.2 mM, P < 0.05) than during the peripheral glucose infusion periods. The intraportal glucose infusion produced an arterial-portal blood glucose gradient of [minus]0.6 mM and thereby presented the liver with a portal signal (Fig. 2, top). After cessation of portal glucose infusion, the arterial-portal glucose gradient was immediately reversed (Fig. 2, top).

Blood lactate levels in the femoral artery, portal vein, and hepatic vein increased simultaneously two- to threefold in response to peripheral glucose infusion. The hepatic vein lactate level switched from being lower than the portal vein lactate level during the control period to being higher than it was during the experimental periods (Fig. 2, bottom). This indicates that the liver switched from net lactate uptake to net lactate output.

HBF in the four periods averaged 33.2 ± 1.7, 28.5 ± 1.6, 29.7 ± 1.7, and 27.8 ± 1.9 ml·kg⁻¹·min⁻¹, respectively. The ratios of hepatic artery to total liver blood flow were 0.20 ± 0.02, 0.32 ± 0.02, 0.34 ± 0.02, and 0.32 ± 0.02. The HBF and the ratios of hepatic artery to total liver blood flow were significantly different among periods (P < 0.05).

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**Table 1. Arterial plasma insulin and glucagon concentrations and total glucose infusion rates during basal and glucose infusion periods in 5 42-h-fasted conscious dogs**

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Pe-Glu-1</th>
<th>Po-Glu</th>
<th>Pe-Glu-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial plasma insulin, pmol/l</td>
<td>48 ± 9</td>
<td>42 ± 3</td>
<td>47 ± 2</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Arterial plasma glucagon, ng/l</td>
<td>49 ± 3</td>
<td>51 ± 3</td>
<td>49 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Total glucose infusion rate, µmol·kg⁻¹·min⁻¹</td>
<td>32 ± 4</td>
<td>26 ± 2</td>
<td>31 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Basal, average of 2 values taken at 0 and 30 min; Pe-glu-1, average of 3 values taken between 0 and 90 min; Po-Glu, average of 4 values taken between 90 and 180 min; Pe-Glu-2, average of 6 values taken between 180 and 300 min. No significant differences were observed among periods in these parameters.

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**Fig. 1. Estimated plasma insulin (top) and glucagon (bottom) levels in hepatic sinusoid in 42-h-fasted, conscious dogs during basal and 3 experimental periods. Data are means ± SE; n = 5.**

**Fig. 2. Blood glucose (top) and lactate (bottom) levels in femoral artery, portal vein, and hepatic vein in 42-h-fasted, conscious dogs during basal and 3 experimental periods. Data are means ± SE; n = 5.**
and portal flow were similar throughout all three experimental periods.

NHGB, fractional glucose extraction, and hepatic glucose load. In the control period, net hepatic glucose output averaged 7.6 ± 1.0 µmol·kg⁻¹·min⁻¹. Peripheral glucose infusion resulted in net hepatic uptake of 6.3 ± 2.4 µmol·kg⁻¹·min⁻¹. Intraportal glucose infusion caused NHGU to increase to 19.1 ± 2.8 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. Pe-GLU-1). Cessation of the portal signal caused NHGU to promptly (10 min) decrease to 9.2 ± 2.3 µmol·kg⁻¹·min⁻¹ (Fig. 3, top). The hepatic fractional extraction of glucose showed a similar trend. The means were 1.8, 7.2, and 2.7% in the three periods, respectively (Fig. 3, middle).

The hepatic glucose loads were 251 ± 11, 274 ± 14, and 276 ± 12 µmol·kg⁻¹·min⁻¹ in three periods (Fig. 3, bottom), respectively. Thus a change in the glucose load could not account for the reduction in NHGU seen on removal of the portal signal.

Nonhepatic glucose uptake. Activation of the portal signal caused nonhepatic glucose uptake to fall from 23.6 ± 3.0 to 5.3 ± 1.8 µmol·kg⁻¹·min⁻¹. Furthermore, elimination of the portal signal for only 10 min shifted net nonhepatic glucose uptake from 5.3 ± 1.8 to 25.5 ± 3.7 µmol·kg⁻¹·min⁻¹ (Fig. 4). The total glucose infusion rates were not significantly different in the three experimental periods (Table 1).

Net hepatic lactate balance. In response to peripheral glucose infusion, net hepatic lactate balance switched rapidly from uptake (−6.3 ± 0.9 µmol·kg⁻¹·min⁻¹) to output (6.7 ± 1.5 µmol·kg⁻¹·min⁻¹). Net hepatic lactate output was not significantly different in the three periods (Fig. 5), indicating that the changes in NHGB for the most part reflect changes in glycogen deposition.

Net hepatic balance of glucose equivalents. The net balance of glucose equivalents across the liver switched from output of 4.4 ± 1.2 to uptake of 2.9 ± 2.9 µmol·kg⁻¹·min⁻¹ in response to peripheral glucose infusion. Intraportal glucose infusion further increased uptake to 14.6 ± 3.3 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. Pe-GLU-1). Cessation of the portal signal rapidly (10 min) returned the uptake of glucose equivalents to 5.4 ± 2.4 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. Po-GLU; Fig. 6).
Previous studies have shown that the portal signal induced by a negative arterial-portal glucose gradient triggers an important response that allows the liver to differentiate between endogenous and exogenous glucose. The characteristics of the effects of the portal signal on hepatic glucose metabolism, however, are still not fully defined. In the present study, we sought to determine the time required for the effects of the portal signal on the liver to subside. The results demonstrate that in the presence of basal insulin the effect of the portal signal on NHGU is eliminated within 10 min. This means that the “on” and “off” times of the effect are similar and both very rapid.

Pagliassotti et al. (13) demonstrated that the portal signal produced a rapid stimulation of NHGU and a rapid activation of glycogen synthase, which resulted in glycogen accumulation. Our results indicate that the off time of the portal signal on NHGU is as quick as its on time. Taken together, these data support the concept that the portal signal brings about its effects via the nervous system. Furthermore, it appears that the signal probably causes an activation of glucokinase or an inhibition of glucose-6-phosphatase, thereby quickly altering the entry rate of glucose into the liver. The most likely candidate enzyme would appear to be glucokinase. Glucokinase is viewed as the glucose sensor and a key metabolic control point in pancreatic β-cells and hepatocytes (8). Glucokinase translocation from the nucleus to the cytosol can be induced by a high concentration of glucose in vivo (16). Our preliminary data showed that the portal signal enhances glucokinase translocation in dog liver and that the time course of translocation from the nucleus to the cytosol is rapid and tightly linked to the augmentation of NHGU. It is known that the activity of glycogen synthase is increased in the presence of the portal signal (13). It seems likely, however, that the latter effect is secondary to activation of glucokinase and the resulting increase in glucose 6-phosphate levels (13). The latter metabolite has recently been shown to be the primary regulator of glycogen synthase activity in the liver (17).

After the portal signal was removed, nonhepatic glucose uptake also rapidly returned to the rate seen before portal glucose infusion. These data support the hypothesis put forward by Adkins et al. (2) and Pagliassotti et al. (13) that the portal signal not only enhances hepatic glucose uptake but also induces peripheral insulin resistance. More recently Galassetti et al. (5) have shown by directly measuring net glucose uptake across the hindlimb that the portal signal can reduce glucose uptake in skeletal muscle. In accord with this, Xie and Lautt (18) have suggested that hepatic parasympathetic nerves regulate the release of a liver-generated factor that selectively controls insulin effectiveness in skeletal muscle. The present study showed that, in the presence of basal insulin, the portal signal almost completely suppressed the augmentation of nonhepatic glucose uptake induced by doubling the peripheral glucose load. It suggests that the suppressive effect of the portal signal on peripheral (muscle) glucose uptake is powerful and insulin independent. Regardless of its etiology, the effect of the portal signal on peripheral glucose uptake is rapid in onset and rapid in elimination.

The current data indicate somewhat higher rates of NHGU during both the peripheral and portal glucose infusion periods compared with the rates observed under similar conditions in the studies of Pagliassotti et al. (13) and Adkins et al. (2). The differences most likely result from the fact that in the present study the rise in the plasma glucose level was significantly higher (20–40 mg/dl) than in the other two studies. Moreover, in the study of Adkins et al., arterial glucagon levels were significantly higher than in the current study. Because small elevations in glucagon have been shown to impair NHGU (6), this would reduce glucose uptake by the liver in the study of Adkins et al.

In the present study, the net balance of glucose equivalents across the liver represents the combination of glucose and lactate balances (after the latter were converted to glucose equivalents). Previous studies (5) have demonstrated that, under similar conditions, the majority of glucose equivalents retained by the liver were diverted into glycogen and not oxidized or stored as fat. This calculation, therefore, allows one to estimate the glucose available for glycogen synthesis and serves as an indicator of glycogen deposition during the study. This result suggests that the liver rapidly reduced glycogen deposition once the portal signal was removed.

In the present study, we clamped insulin at a basal level throughout the three experimental periods to isolate the effect of the portal signal. However, a rise in...
insulin is normally an important component to the feeding response, because it plays an important role in stimulating the activity of liver glycogen synthase (13). Moreover, insulin controls glucokinase transcription in liver (7, 11). It is important, therefore, to note that in the presence of high levels of insulin, the reversibility of the effect of the portal signal on NHGU may not be as rapid as in the presence of basal insulin levels.

In summary, the portal signal induces a rapid shift of the liver into net glucose uptake. Once the signal is removed, the liver rapidly reduces net glucose uptake. Moreover, the portal signal brings about an equally fast suppression of nonhepatic glucose uptake, which is likewise quickly reversed once the signal is removed. These results indicate that the on or off time constants for the effects of the portal signal on liver and muscle glucose uptake are short, and this is consistent with their being mediated by a neural mechanism.

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