Rapid reversal of the effects of the portal signal under hyperinsulinemic conditions in the conscious dog

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Hsieh, Po-Shiuan, Mary Courtney Moore, Doss W. Neal, Maya Emswiller, and Alan D. Cherrington. Rapid reversal of the effects of the portal signal under hyperinsulinemic conditions in the conscious dog. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E930–E937, 1999.—Experiments were performed on two groups of 42-h-fasted conscious dogs (n = 6/group). Somatostatin was given peripherally with insulin (4-fold basal) and glucagon (basal) intraportally. In the first experimental period, glucose was infused peripherally to double the hepatic glucose load (HGL) in both groups. In the second experimental period, glucose (21.8 µmol·kg\(^{-1}\)·min\(^{-1}\)) was infused intraportally and the peripheral glucose infusion rate (PeGIR) was reduced to maintain the precreating HGL in the portal signal (PO) group, whereas saline was given intraportally in the control (CON) group and PeGIR was not changed. In the third period, the portal glucose infusion was stopped in the PO group and PeGIR was increased to sustain HGL. PeGIR was continued in the CON group. The glucose loads to the liver did not differ in the CON and PO groups. Net hepatic glucose uptake was 9.6 ± 2.5, 11.6 ± 2.6, and 15.5 ± 3.2 vs. 10.8 ± 1.8, 23.7 ± 3.0, and 15.5 ± 1.1 µmol·kg\(^{-1}\)·min\(^{-1}\), and nonhepatic glucose uptake (non-HGU) was 29.8 ± 1.1, 40.1 ± 4.5, and 49.5 ± 4.0 vs. 26.6 ± 4.3, 23.2 ± 4.0, and 40.4 ± 3.1 µmol·kg\(^{-1}\)·min\(^{-1}\) in the CON and PO groups during the three periods, respectively. Cessation of the portal signal shifted NHGU and non-HGU to rates similar to those evident in the CON group within 10 min. These results indicate that even under hyperinsulinemic conditions the effects of the portal signal on hepatic and peripheral glucose uptake are rapidly reversible.

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min (−120 to −40) of dye equilibration, there was a 40-min (−40 to 0) basal period, followed by two 90-min and one 120-min experimental periods. At time 0, constant infusions of several solutions were begun, and these infusions were continued for the three experimental periods. Somatostatin (0.8 μg·kg⁻¹·min⁻¹; Bachem, Torrance, CA) was infused to suppress endogenous insulin and glucagon secretion. Insulin (7.2 pmol·kg⁻¹·min⁻¹) and glucagon (0.65 ng·kg⁻¹·min⁻¹) were replaced intraportally. 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 HGL in both groups. In the second experimental period (Po-GLU), glucose was infused intraportally (21.8 μmol·kg⁻¹·min⁻¹) and the peripheral glucose infusion rate was reduced to maintain the HGL in the portal signal (PO) group, whereas saline was given intraportally and the peripheral glucose infusion was continued unchanged in the control (CON) group. In the third experimental period (Pe-GLU-2), the portal glucose infusion was stopped and the peripheral glucose infusion rate was increased to sustain the HGL in the PO group. Peripheral glucose infusion was continued in the CON group. Dextrose (20%) was used for the portal glucose infusion, and p-aminohippuric acid (PAH; Sigma, St. Louis, MO; delivered at 1.7 μmol·kg⁻¹·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 (2, 23). Blood samples of 0.2 ml were obtained from the artery every 5 min to permit measurement of the plasma glucose concentrations to allow maintenance of the HGL. Larger blood samples (5–10 ml) for data acquisition were obtained from the artery, portal vein, and hepatic vein every 15 min during the last 30 min of Pe-GLU-1, every 15–30 min of Po-GLU, and every 10–60 min of Pe-GLU-2.

Processing and analysis of samples. Plasma glucose was assayed with the glucose oxidase method with a Beckman glucose analyzer (Fullerton, CA). Plasma insulin and glucagon concentrations were determined with radioimmunoassays (27). Blood glucose and blood lactate levels were determined from perchloric acid-treated samples according to the method of Lloyd et al. (16). PAH was also measured in perchloric acid-deproteinized blood as previously described (2, 20, 23).

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. In the PO group, 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, 20, 23). 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 than or less than the actual amount of PAH infused (2, 20, 23). 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. In the PO group, 11 dogs were studied; five dogs were not included because of poor mixing. In the six animals that were retained, the ratio of PAH recovery in the portal vein to the PAH infusion rate was 0.9 ± 0.1, whereas the ratio of PAH recovery in the hepatic vein to the PAH infusion rate was 0.8 ± 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.

HBF was calculated by two methods, ultrasonic flow probes and dye extraction (19). 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 use did not require an assumption regarding the distribution of arterial and portal contribution to HBF.

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

\[
\text{load}_D = ([S]_A \times ABF) + ([S]_P \times PBF)
\]

where [S] 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. 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 HBF, the HGL in the portal glucose infusion period was also calculated with an indirect (I) method

\[
\text{load}_I = \frac{G \times HBF}{GR + GUG}
\]

where G is the blood glucose concentration, GR 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 (2, 20, 23).

The load of a substrate exiting the liver was calculated as

\[
\text{load}_\text{out} = [S]_H \times HBF
\]

where subscript H represents the hepatic vein.

Direct and indirect methods were used in calculation of net hepatic balance (NHB). The direct calculation was NHB_D = load_out - load_in. The indirect calculation was NHB_I = load_out - load_in. Both equations were used in calculation of net hepatic glucose balance (NHGB). The data depicted in Figs. 1–6 were obtained with the indirect calculation. We chose this approach because it is the one we used in our earlier publications and because the indirect method is less subject to mixing noise. NHGU was 18% less during portal glucose delivery when the direct as opposed to the indirect calculation was used to assess liver glucose uptake, but this difference had no impact on our conclusion. The direct method was employed only to calculate net hepatic lactate balance. Net fractional substrate extraction by the liver (direct or indirect) was calculated as the ratio of NHB to load_in. Nonhepatic glucose uptake (non-HGU) was calculated by subtracting the rate of NHGU from the total glucose infusion rate. The NHB of glucose equivalents was calculated as the sum of the balances of NHGB (I) and lactate, once the latter was converted to glucose equivalents.

To calculate glucose balance, plasma glucose values were converted to whole blood glucose values with 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 results similar 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.
A similar method was used to calculate the hepatic sinusoidal insulin and glucagon concentrations: 
$$[H]_{HS} = \frac{([H]_A \times ABF) + ([H]_P \times PBF))}{(ABF + PBF)}$$, where $[H]$ is the hormone concentration and subscript $HS$ refers to the hepatic sinusoid.

Rates of glucose production ($R_g$) and utilization ($R_d$) were assessed by tracer dilution method (5) during the three experimental periods, respectively.

Data are presented as means ± SE. SYSTAT (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. Arterial and liver sinusoidal insulin concentrations rose three times the basal level in both groups (Table 1 and Fig. 1A) during the experimental periods, thus mimicking insulin concentrations seen in the postprandial state. Arterial plasma glucagon levels remained basal and did not differ between groups (Table 1 and Fig. 1B).

Blood glucose levels and HBF. In the PO group, peripheral glucose infusion doubled the blood glucose level. Intraportal glucose infusion in the second experimental period produced an arterial-portal blood glucose gradient of $-0.7 ± 0.1$ mM and thereby presented the liver with a portal signal. Cessation of the portal glucose infusion rapidly reversed the arterial-portal glucose gradient (Fig. 2A). The glucose level was also doubled in the CON group, but a positive arterial-portal glucose gradient was maintained throughout (Fig. 2B).

During the basal and three experimental periods (basal period, −40–0 min; Pe-GLU-1 period, 60–90 min; Po-GLU, 120–180 min; Pe-GLU-2 period, 190–300 min), HBF in the two groups was 33 ± 6, 33 ± 2, and 33 ± 2 vs. 31 ± 2, 26 ± 2, 26 ± 1, and 28 ± 2 ml·kg$^{-1}$·min$^{-1}$ in the PO and CON groups, respectively. In the basal period, net hepatic extraction of glucose, and HGL. The mean HGL was not significantly different between the two groups or between the different test periods (Fig. 3C; 125 ± 7, 244 ± 20, 277 ± 19, and 277 ± 23 vs. 137 ± 11, 249 ± 25, 244 ± 12, and 264 ± 20 µmol·kg$^{-1}$·min$^{-1}$ in the PO and CON groups, respectively). In the basal period, net hepatic glucose output did not differ between PO and CON groups (9.9 ± 1.3 vs. 9.1 ± 2.0 µmol·kg$^{-1}$·min$^{-1}$, respectively). In the first experimental period, peripheral glucose infusion resulted in NHGU of 10.8 ± 1.8 and 9.6 ± 2.5 µmol·kg$^{-1}$·min$^{-1}$ in the PO and CON groups, respectively. During the second experimental period, NHGU increased to an average of 23.7 ± 3.0 µmol·kg$^{-1}$·min$^{-1}$ in the PO group ($P < 0.05$) but changed minimally (11.6 ± 2.6 µmol·kg$^{-1}$·min$^{-1}$) in the CON group. During the second experimental period, when the direct method of calculation was used, NHGU was slightly but not significantly less (19.6 ± 2.6 µmol·kg$^{-1}$·min$^{-1}$) in the PO group and still differed significantly from that in the control group ($P < 0.05$). Cessation of the portal signal caused NHGU to promptly (10 min) decrease to 14.9 ± 1.5 µmol·kg$^{-1}$·min$^{-1}$ ($P < 0.05$ vs. Po-GLU), so that in the last period NHGU was again similar in the two groups (15.5 ± 1.1 vs. 15.5 ± 3.2 µmol·kg$^{-1}$·min$^{-1}$).

**Table 1.** Arterial plasma insulin and glucagon concentrations during the basal period and 3 experimental periods in portal and control groups of 42-h-fasted conscious dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal</th>
<th>Pe-GLU-1</th>
<th>Po-GLU</th>
<th>Pe-GLU-2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Arterial plasma insulin, pmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>55 ± 3</td>
<td>129 ± 15</td>
<td>136 ± 10</td>
<td>148 ± 13</td>
</tr>
<tr>
<td>Control</td>
<td>58 ± 10</td>
<td>134 ± 24</td>
<td>148 ± 21</td>
<td>156 ± 21</td>
</tr>
<tr>
<td></td>
<td>Arterial plasma glucagon, ng/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal</td>
<td>40 ± 3</td>
<td>44 ± 3</td>
<td>42 ± 3</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>35 ± 2</td>
<td>39 ± 2</td>
<td>37 ± 2</td>
<td>36 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Basal period, average of 2 values taken at −40 and 0 min. Pe-GLU-1 period, average of 3 values taken between 0 and 90 min; Po-GLU period, average of 4 values taken between 90 and 180 min; Pe-GLU-2 period, average of 7 values taken between 180 and 300 min. No significant differences were observed among experimental periods or between 2 groups in above variables.
Hepatic glucose uptake and the portal signal

**Discussion**

Previous studies have demonstrated that the portal signal is an important component of the metabolic response to feeding. However, the characteristics of the effects of the portal signal on whole body glucose metabolism are still not fully defined. Our previous data showed that hepatic glucose uptake decreased rapidly after elimination of the portal signal in the presence of basal insulin (12). The present study sought to define whether, in the presence of hyperinsulinemia, the reversibility of the effect of the portal signal on NHB would still be rapid. The present results demonstrate clearly that hyperinsulinemia did not alter the “off” time of the effect of the portal signal on hepatic or peripheral glucose uptake.

The portal signal and insulin are both important regulators of NHB. However, their interaction in the control of hepatic glucose metabolism is still not fully defined. Several studies (8, 13, 15, 23) have shown that the ability of insulin to stimulate glucose uptake by the liver reaches its peak slowly. Nevertheless, under postprandial conditions, the liver responds rapidly to the incoming glucose load, suggesting that an additional signal must be involved. Our previous work established...
that portal glucose delivery creates such a signal (12, 23). The present study demonstrates that the decay of the effect of the portal signal on net glucose uptake by the liver was rapid (within 10 min) even in the presence of hyperinsulinemia. Because that was also the case in the presence of basal insulin levels (12), it appears that the off time of the effect of the portal signal is not influenced by insulin. The rapid “on” (23) and off time constants of the effect of the portal signal on the liver not only provide a way to tightly match glucose absorption to hepatic glucose uptake but also to link hepatic glucose uptake to liver glycogen deposition. The portal signal thus appears to be critical for the normal distribution of dietary glucose among the various tissues of the body.

The mechanisms by which the portal signal and insulin affect hepatic glucose uptake are not fully understood. Hepatic glucose-6-phosphatase and hepatic glucokinase are key determinants of hepatic glucose flux (7, 10, 18) and are therefore both potential sites of action of the portal signal and insulin. An earlier study showed that insulin can exert a dominant negative effect on the mRNA levels of glucose-6-phosphatase in different nutritional and hormonal states (6). Regulation of glucokinase activity in the hepatocyte is controlled by the transcription of the glucokinase gene and translocation of glucokinase protein within the cell (25). Several in vitro studies have shown that insulin can induce glucokinase gene expression (14). In addition, one in vitro study suggested that high insulin levels (10 nM) can potentiate glucose (or fructose)-induced translocation of glucokinase (3). The action of insulin on intracellular glucokinase distribution in vivo is still unknown. Whereas translocation is known to occur rapidly (26) and could quickly alter NHGU, the effects of insulin on gene transcription occur somewhat slowly and would result in a delayed increase of the NHGU. The slowly developing effects of insulin on hepatic glucose uptake (8, 13, 15, 23) suggest that its primary action is to induce transcriptional alterations.

The effects of the portal signal on glucokinase and glucose-6-phosphatase, on the other hand, are still unexplored. As noted above, recent reports have sug-

Fig. 3. Net hepatic glucose balance (NHGB; A), net hepatic fractional extraction of glucose (B), and hepatic glucose load (C) in 42-h-fasted conscious dogs during basal and 3 experimental periods in portal and control groups (n = 6 for each). *Post hoc comparison revealed differences (P < 0.05) between 2 groups at this time point.
gested that the translocation of glucokinase within the hepatocyte from the nucleus to the cytosol is a major determinant of hepatic glucose uptake (26) and glycogen synthesis (4). An earlier in vitro study showed that the movement of glucokinase induced by glucose or fructose is rapid and reversible, occurring within 30 min (9). We have previously suggested that glucokinase translocation within the hepatocyte is a key determinant of hepatic glucose uptake by the conscious dog (26). In that regard, we have shown that the on and off time constants for the complete translocation of glucokinase induced by fructose in the conscious dog are short (30 min; M. Shiota, unpublished observations). Given the time course of glucokinase movement and the impact the enzyme has on liver glucose uptake, it is

![Graph](image)

**Fig. 4.** Nonhepatic glucose uptake in 42-h-fasted conscious dogs during basal and 3 experimental periods in portal and control groups (n = 6 for each). *Post hoc comparison revealed differences (P < 0.05) between 2 groups at this time point.

**Fig. 5.** Arterial blood lactate levels (A) and net hepatic lactate balance (B) in 42-h-fasted conscious dogs during basal and 3 experimental periods in portal and control groups (n = 6 for each). There are no differences between 2 groups during 3 experimental periods.

**Table 2.** Average values for total glucose infusion rate, total Rₐ, and Rₖ during the 3 experimental periods in portal and control groups of 42-h-fasted conscious dogs

<table>
<thead>
<tr>
<th></th>
<th>Pe-GLU-1</th>
<th>Po-GLU</th>
<th>Pe-GLU-2</th>
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<tbody>
<tr>
<td><strong>Portal group</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TGIR</td>
<td>37.4 ± 4.1</td>
<td>46.9 ± 3.4</td>
<td>56.0 ± 3.6</td>
</tr>
<tr>
<td>Total Rₐ</td>
<td>42.7 ± 2.6</td>
<td>49.8 ± 3.2</td>
<td>59.5 ± 3.1</td>
</tr>
<tr>
<td>Rₖ</td>
<td>39.0 ± 2.4</td>
<td>48.6 ± 3.2</td>
<td>59.0 ± 3.2</td>
</tr>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGIR</td>
<td>39.4 ± 2.6</td>
<td>51.7 ± 3.1</td>
<td>65.0 ± 4.9</td>
</tr>
<tr>
<td>Total Rₐ</td>
<td>44.7 ± 2.7</td>
<td>51.9 ± 2.8</td>
<td>61.4 ± 3.5</td>
</tr>
<tr>
<td>Rₖ</td>
<td>39.4 ± 2.2</td>
<td>51.7 ± 3.1</td>
<td>65.0 ± 4.9</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in units of µmol·kg⁻¹·min⁻¹. TGIR, total glucose infusion rate; Rₐ and Rₖ, glucose appearance and disappearance rate, respectively, with calculated tracer dilution method; Pe-GLU-1 period, average of 3 values taken between 0 and 90 min; Po-GLU period, average of 4 values taken between 90 and 180 min; Pe-GLU-2 period, average of 7 values taken between 180 and 300 min. No significant differences were observed throughout 3 test periods or between 2 groups in above variables.

![Graph](image)

**Fig. 6.** Net hepatic balance of glucose equivalents in 42-h-fasted conscious dogs during basal and 3 experimental periods in portal and control groups (n = 6 for each). Net balance of glucose equivalents is calculated as sum of net balance of glucose and lactate, the latter converted to glucose equivalents. *Post hoc comparison revealed differences (P < 0.05) between 2 groups at this time point.
possible that the portal signal is bringing about its effect through translocation of glucokinase. The activity of glucose-6-phosphatase decreases slowly (~1 h) in response to feeding (17). The current data indicate that the off time constant for the effects of the portal signal on liver is rapid (~10 min), and therefore this enzyme is unlikely to play a role in the action of the portal signal.

Another possible site of action for insulin and the portal signal is liver glycogen synthase. It has been shown in the dog (23) and human (24) that insulin has a stimulatory effect on liver glycogen synthase and might thereby “pull” glucose into glycogen. The portal signal has also been shown to activate glycogen synthase (23), although this may be secondary to a rise in glucose-6-phosphatase, which results from the increased entry of glucose into the hepatocyte. In the present study, cessation of the portal signal, even under hyperinsulinemic conditions, rapidly reversed its effects on NHGU and liver glycogen deposition simultaneously. This suggests that the translocation of glucokinase may be a key to the control of hepatic glycogen synthase.

Several studies also have reported that the portal signal has a suppressive effect on peripheral glucose uptake (mostly in muscle) (1, 11, 12, 23). The present results show that the off time for the effect of the portal signal is equally rapid at muscle and liver. Although non-HGU increased significantly after removal of the portal glucose infusion, it was slightly lower during Pe-GLU-2 in the PO group than in the CON group. It is hard to know whether the slightly decreased non-HGU during Pe-GLU-2 in the PO group was due to the prior presence of the portal signal. It might also have been caused by the random difference in the sensitivity of glucose utilization to insulin in the two groups. In support of this, no obvious difference was observed in the percent rise in nonhepatic glucose utilization between Pe-GLU-1 and Pe-GLU-2 in the two groups (the increment in non-HGU was 64 ± 17 vs. 68 ± 17% in PO and CON groups, respectively).

It would appear from the coordinated and rapid responses of non-HGU that a neurally mediated mechanism is involved in bringing about the effects of the portal signal on muscle. However, Xie and Lautt (28–30) have suggested that a circulating factor may be released by the liver that regulates the responsiveness of skeletal muscle to insulin. Furthermore, these authors have shown that its effect is dependent on intact hepatic parasympathetic nerves. The question as to what is the link between the effect of the portal signal on the liver and peripheral tissues remains unresolved.

In summary, the effects of the portal signal on liver and peripheral tissues rapidly reverse once the portal signal is removed, even under hyperinsulinemic conditions. The results suggest that the on and off time constants for the effects of the portal signal on liver and peripheral tissues are rapid and independent of physiological changes in insulin. The portal signal and insulin appear to play opposing roles in the regulation of peripheral glucose uptake but synergistic roles in regulating glucose uptake by the liver.

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