Original Articles

Direct and Indirect Effects of Insulin on Glucose Uptake and Storage by the Liver

Shosuke Satake, Mary Courtney Moore, Kayano Igawa, Margaret Converse, Benjamin Farmer, Doss W. Neal, and Alan D. Cherrington

Studies were conducted in conscious 42-h–fasted dogs to determine how much of insulin's effect on hepatic glucose uptake arises from its direct hepatic action versus its indirect (extrahepatic) action. Each experiment consisted of equilibration, basal, and experimental periods. During the latter, somatostatin, basal intraportal glucagon, portal glucose (21.3 μmol · kg^{-1} · min^{-1}), and peripheral glucose (to double the hepatic glucose load) were infused. During the experimental period, insulin was infused intraportally at a basal rate (BI, $n = 6$), at a fourfold basal rate (PoI, $n = 6$), or via a peripheral vein to create a selective increase in the arterial insulin level similar to that in PoI (PeI, $n = 6$). Arterial and hepatic sinusoidal insulin levels (in picomoles per liter) during the experimental period were 31 ± 5 and 113 ± 15 in BI, 97 ± 11 and 394 ± 66 in PoI, and 111 ± 13 and 96 ± 9 in PeI, respectively. Net hepatic glucose uptake (NHGU) averaged 7.0 ± 1.1 μmol · kg^{-1} · min^{-1}, 15.7 ± 2.7 μmol · kg^{-1} · min^{-1} ($P < 0.05$ vs. BI), and 12.0 ± 2.4 μmol · kg^{-1} · min^{-1} ($P < 0.05$ vs. BI) in BI, PoI, and PeI, respectively. Net hepatic carbon retention was 4.4 ± 1.2 μmol glucose equivalents · kg^{-1} · min^{-1}, 12.3 ± 2.5 μmol glucose equivalents · kg^{-1} · min^{-1} ($P < 0.05$ vs. BI, $P < 0.05$ vs. PeI), and 7.1 ± 1.0 μmol glucose equivalents · kg^{-1} · min^{-1} ($P < 0.05$ vs. BI) in BI, PoI, and PeI, respectively. Both direct and indirect insulin actions increase NHGU, but the rise in hepatic sinusoidal insulin appears critical for efficient storage of glucose as hepatic glycogen. *Diabetes* 51:1663–1671, 2002

It is well known that there is an insulin concentration gradient between the hepatic portal and systemic circulations because insulin is secreted directly into the portal system and the liver degrades about half of the hormone that reaches it. However, this gradient is reversed in diabetic patients being treated with insulin because insulin is injected subcutaneously. In other words, systemic (arterial) insulin concentrations are always higher than portal or liver sinusoidal insulin concentrations in these patients. Recently, there has been great interest regarding the effect of this reversed insulin distribution on hepatic glucose metabolism, especially its effect on hepatic glucose production. Prager et al. (1) suggested that peripheral insulin infusion could suppress hepatic glucose production without any change in the portal insulin concentration. Sindelar et al. (2) observed that a selective rise in arterial insulin (portal insulin kept basal) could inhibit hepatic glucose production to the same extent as a selective rise in portal insulin (arterial insulin kept basal), and this effect was partly mediated by suppression of lipolysis (3). Other investigators have also noted the importance of the indirect (extrahepatic) effect of insulin on hepatic glucose production (4,5). It is thus apparent that insulin inhibits glucose production by the liver through both direct and indirect actions.

Hyperinsulinemia, along with hyperglycemia and the route of glucose delivery (peripheral vein vs. portal vein), is one of the major determinants of the magnitude of hepatic glucose uptake in response to feeding (6). A comparison of the effects of portal versus peripheral insulin delivery on hepatic glucose uptake has not been carried out under controlled conditions similar to those encountered in the postprandial state (i.e., hyperglycemia, hyperinsulinemia, and a portal vein glucose concentration greater than the arterial concentration). Wilczek et al. (7) quantified net splanchnic glucose uptake in response to peripheral glucose infusion in pancreatic graft recipients whose insulin secretion was directed into the peripheral circulation and compared their response to normal adults and to individuals who had received renal transplants and were equivalently immunosuppressed. Net splanchnic glucose balance did not differ among the groups. The arterial insulin concentrations were higher in the pancreatic transplant patients than in the other groups, but portal insulin concentrations were not measured. Thus, the liver sinusoidal insulin level remains unknown. Likewise, the liver sinusoidal glucagon levels were neither controlled nor measured. In addition, because the glucose was given peripherally, it remains unclear what would have happened in the presence of oral glucose delivery. In only one study have the direct and indirect effects of insulin on net hepatic glucose uptake (NHGU) been measured in the presence of the portal signal. Kryshak et al. (8) studied

From the Department of Molecular Physiology and Biophysics, and Diabetes Research and Training Center, Vanderbilt University School of Medicine, Nashville, Tennessee.

Address correspondence and reprint requests to M.C. Moore, 702 Light Hall, Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232-0615. E-mail: genie.moore@mcmail.vanderbilt.edu.

Received for publication 28 September 2001 and accepted in revised form 21 February 2002.

AUC, area under the curve; BI, intraportal basal insulin infusion; dpm, disintegrations per minute; ICG, indocyanine green; NEFA, nonesterified fatty acid; NHGB, net hepatic glucose balance; NHGU, net hepatic glucose uptake; NHLO, net hepatic lactate output; PAH, *p*-aminohippuric acid; PeI, peripheral insulin infusion; PoI, intraportal high insulin infusion.

three groups of dogs. In the first group, the pancreatic drainage was transected and then re-anastomosed in its usual position (portal insulin delivery); in the second group, the pancreatic drainage was transected, and an anastomosis was created with the inferior vena cava (peripheral insulin delivery); and in the third group, a sham surgery was performed with no transection of the pancreatic drainage. After a radiolabeled mixed meal, the postprandial rates of glucose appearance and disappearance, as well as the suppression of hepatic glucose release, were virtually identical among the three groups. The systemic insulin concentrations were significantly greater in the peripheral insulin delivery group than in the other two groups, but neither the portal vein insulin and glucagon concentrations nor NHGU were assessed (8). Consequently, questions remain regarding the magnitude of the liver's role in disposition of the glucose derived from the meal, the insulin and glucagon concentrations existing within the hepatic sinusoids in the postprandial period, and the fate of the glucose taken up by the livers in the three groups. Therefore, the aim of the current study was to compare the direct and indirect effects of insulin in regulating glucose uptake and storage by the liver using the pancreatic-clamped conscious dog as a model.

RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were carried out on conscious 42-h–fasted mongrel dogs with a mean weight of 23.1 ± 0.4 kg. Each protocol included two female and four male dogs 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 animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care International guidelines, and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days before study, each dog underwent a laparotomy under general anesthesia, and silicone rubber catheters were inserted for sampling in a hepatic vein, the portal vein, and a femoral artery and for infusion into a splenic and a jejunal vein, as described in detail elsewhere (6,9). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and the hepatic artery. The free ends of the catheters and flow probe leads were placed in subcutaneous pockets. The dog was studied only if it had a leukocyte count $<18{,}000/mm^3$, a hematocrit >35%, a good appetite (as evidenced by consumption of at least three-quarters of the daily ration), and normal stools.

On the morning of the study, catheters and flow probe leads were exteriorized from their subcutaneous pockets using local anesthesia (6,9). The splenic and jejunal catheters were used for intraportal infusion of glucose, insulin (except the peripheral insulin infusion protocol), and glucagon (Eli Lilly, Indianapolis, IN). Angiocaths (Deseret Medical, Sandy, UT) were inserted into the left cephalic vein for [U-^{14}C]glucose and indocyanine green infusion (Sigma, St. Louis, MO), the right cephalic vein for peripheral glucose infusion, the left saphenous vein for somatostatin (Bachem, Torrance, CA) and *p*-aminohippuric acid (PAH) (Sigma) infusions, and the right saphenous vein (when necessary) for peripheral insulin infusion.

Experimental design. Each experiment consisted of a 90-min equilibration period (−120 to −30 min), a 30-min basal period (−30 to 0 min), and a 240-min experimental period (0 to 240 min). At −120 min, priming doses of $NaH^{14}CO_3$ (0.22 μCi/kg) and [U-^{14}C]glucose (11 μCi/kg) were given, and constant infusions of [U-^{14}C]glucose (0.4 μCi/min) and indocyanine green (ICG) dye (0.14 mg/min; Sigma) were initiated. In addition, a constant peripheral infusion of PAH (1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was started and continued until the intraportal infusion of glucose mixed with PAH was started at 0 min. At 0 min, a constant peripheral infusion of somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was begun to suppress endogenous insulin and glucagon secretion, and glucagon (0.6 $ng \cdot kg^{-1} \cdot min^{-1}$) was replaced intraportally to maintain basal levels. The dogs were divided into three groups at 0 min. In the first group, insulin was infused into the portal vein at 2.4 $pmol \cdot kg^{-1} \cdot min^{-1}$ to maintain basal levels (intraportal basal insulin infusion [BI], $n = 6$). In the second group, insulin was infused into the portal vein at 7.2 $pmol \cdot kg^{-1} \cdot min^{-1}$ to mimic insulin levels seen after a meal (intraportal high insulin infusion [PoI], $n = 6$). In the third group, insulin was infused into a peripheral vein at 3.6 $pmol \cdot kg^{-1} \cdot min^{-1}$ to create a reversed insulin distribution (peripheral insulin infusion [PeI], $n = 6$). The latter infusion rate was chosen so that the peripheral insulin level in PeI could be kept the same as that in PoI (increased approximately fourfold), whereas the sinusoidal insulin level would be the same as that in BI (basal). A constant intraportal glucose (21.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; 20% dextrose; Abbott Laboratories)/PAH infusion (PAH mixed with dextrose in such a way that the PAH infusion rate was 1.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was also started at 0 min. In addition to the constant infusions, a primed-continuous variable rate peripheral infusion of 50% dextrose was begun at 0 min in each group, so that the blood glucose could be quickly clamped at its desired value. Both of the glucose infusates were mixed with [3-^{3}H]glucose to permit assessment of the amount of hepatic glycogen synthesized via the direct pathway at the end of the experiment.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 15 min during the basal period (−30 to 0 min), at 15 and 30 min, and every 30 min thereafter as previously described (6,9). Arterial blood samples were also taken every 5 min throughout the experimental period to monitor glucose level (6,9). After completion of each experiment, the animal was killed with an overdose of pentobarbital, and a tissue sample from each of the seven liver lobes was rapidly frozen with clamps prechilled in liquid nitrogen. The tissue was stored at −70°C for later analysis.

Processing and analysis of samples. Hematocrit; blood glucose, lactate, alanine, glycerol, and $^{14}CO_2$; plasma glucose, insulin, and glucagon concentrations; and [^{3}H]glucose and [^{14}C]glucose were determined as described previously (6,9,10). Plasma nonesterified fatty acid (NEFA) concentrations were determined enzymatically (NEFA C kit; Wako Chemicals, Richmond, VA). Four to eight 10-μl aliquots of plasma from each sample were immediately analyzed for glucose using the glucose oxidase technique in a glucose analyzer (Beckman Instruments, Fullerton, CA).

Glycogen was extracted by using boiling 30% KOH and 96% ethanol, and its concentration was determined by enzyme degradation (Sigma) (11). Net incorporation of ^{3}H into glycogen was measured after liquid scintillation counting of the processed samples. Hepatic glucose-6-phosphate concentrations were determined as described by Michal (12).

Calculations and data analysis. Hepatic blood flow was measured by ultrasonic flow probes and by use of ICG dye extraction. The two methods yielded similar results, but the data reported here were calculated with the ultrasonic-determined flows because their measurement did not require an assumption regarding the relative contribution of arterial and portal flow to total hepatic blood flow.

The recovery of PAH across the liver was measured as reported previously (6,9) and allowed assessment of mixing of the infusate with the portal blood. A total of 29 dogs were studied. One was not included in the database because of malfunction of a sampling catheter, one was excluded because of an unsuccessful pancreatic clamp, and nine were omitted because of poor mixing. In the 18 animals included in the database, the ratio of recovered to infused PAH in the portal and hepatic veins was 0.8 ± 0.1 and 0.9 ± 0.1, respectively (with a ratio of 1.0 representing ideal mixing).

The rate of substrate delivery to the liver, or hepatic substrate load, was calculated by a direct (D) method as follows: $\text{Load}_{in}(D) = ([S]_A \times \text{ABF}) + ([S]_P \times \text{PBF})$, where [$S$] is the substrate concentration, A and P refer to artery and portal vein, respectively, and ABF and PBF refer to blood or plasma flow (as appropriate) through the hepatic artery and portal vein, respectively. To avoid any potential errors arising from either incomplete mixing of glucose during intraportal infusion or lack of precise measurements of the distribution of hepatic blood flow, hepatic glucose load was also calculated by an indirect (I) method: $\text{Load}_{in}(I) = (G_A \times \text{HBF}) + \text{GIR}_{Po} - \text{GUG}$, where G_A is the arterial blood glucose concentration, HBF refers to blood flow through the liver, GIR_{Po} is the intraportal glucose infusion rate, and GUG is the uptake of glucose by the gastrointestinal tract, calculated based on the previously described relationship between the arterial blood concentration and GUG (6,9).

The load of a substrate exiting the liver was calculated as follows: $\text{Load}_{out} = ([S]_H \times \text{HBF})$, where H represents the hepatic vein. Direct and indirect methods were used in the calculation of net hepatic balance (NHB). The direct calculation was $\text{NHB}(D) = \text{Load}_{out} - \text{Load}_{in}(D)$, and the indirect calculation was $\text{NHB}(I) = \text{Load}_{out} - \text{Load}_{in}(I)$. A negative value indicates net uptake by the liver. Both equations were used in calculation of net hepatic glucose balance (NHGB), and the results did not differ between the two methods. The results shown in this report were based on the direct calculation. Only the direct calculation was used for substrates other than glucose because they were not infused intraportally. Nonhepatic glucose uptake was calculated as the glucose infusion rate minus NHGB. Net fractional substrate extraction by the liver was calculated as $\text{NHB} \div \text{Load}_{in}$.

TABLE 1
Arterial and calculated hepatic sinusoidal plasma insulin and glucagon concentrations in 42-h–fasted conscious dogs receiving insulin intraportally at 1.8 or 7.2 pmol · kg^{-1} · min^{-1} (BI and PoI groups, respectively) or peripherally at 3.6 pmol · kg^{-1} · min^{-1} (PeI group)

Parameter and group	Basal period	Experimental period (min)								
		15	30	60	90	120	150	180	210	240
Arterial plasma insulin (pmol/l)										
BI	37 ± 5	26 ± 6	24 ± 6	30 ± 6	29 ± 4	31 ± 6	32 ± 4	35 ± 4	35 ± 4	34 ± 6
PoI	35 ± 4	74 ± 18*	79 ± 13*	100 ± 16*	94 ± 20*	98 ± 16*	100 ± 13*	99 ± 15*	120 ± 18*	108 ± 13*
PeI	41 ± 8	98 ± 19*	106 ± 19*	109 ± 20*	114 ± 16*	116 ± 20*	111 ± 14*	107 ± 15*	110 ± 7*	128 ± 13*
Hepatic sinusoidal plasma insulin (pmol/l)										
BI	101 ± 18	108 ± 27	103 ± 20	106 ± 16	112 ± 19	122 ± 14	109 ± 12	115 ± 16	121 ± 10	105 ± 10
PoI	109 ± 19	277 ± 46*	348 ± 51*	424 ± 67*	397 ± 64*	378 ± 83*	396 ± 69*	413 ± 60*	402 ± 56*	443 ± 75*
PeI	129 ± 33	93 ± 9	87 ± 12	98 ± 11	100 ± 12	97 ± 15	99 ± 10	94 ± 8	91 ± 8	105 ± 10
Arterial plasma glucagon (ng/l)										
BI	34 ± 4	32 ± 3	33 ± 0	32 ± 2	30 ± 2	29 ± 1	30 ± 2	28 ± 1	28 ± 1	26 ± 1
PoI	34 ± 6	33 ± 6	33 ± 5	36 ± 5	33 ± 5	33 ± 5	32 ± 5	33 ± 5	32 ± 5	33 ± 5
PeI	40 ± 4	40 ± 4	41 ± 3	38 ± 1	34 ± 3	35 ± 2	37 ± 2	33 ± 2	38 ± 3	33 ± 3
Hepatic sinusoidal plasma glucagon (ng/l)										
BI	40 ± 5	41 ± 4	43 ± 3	41 ± 4	43 ± 4	41 ± 3	38 ± 2	39 ± 3	40 ± 2	34 ± 2
PoI	39 ± 8	42 ± 7	47 ± 9	45 ± 6	43 ± 8	43 ± 9	43 ± 9	42 ± 8	39 ± 7	41 ± 7
PeI	45 ± 4	47 ± 2	48 ± 3	48 ± 3	45 ± 3	42 ± 3	46 ± 2	40 ± 2	47 ± 4	42 ± 4

Data are means ± SE. n = 6/group. Basal values are the mean of the values at −30 and 0 min. *$P < 0.05$ vs. BI.

For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using correction factors (ratio of the blood to the plasma concentration) as previously described (9). Blood glucose concentrations were used for the calculation of NHGB because the use of whole blood glucose ensures accurate hepatic balance measurements regardless of the characteristics of glucose entry into the erythrocyte.

Hepatic sinusoidal insulin and glucagon concentrations were calculated as $Load_{in}$ (D) ÷ hepatic flow, using plasma concentrations and plasma flow data. This calculation provides an estimate of the hormone concentrations at the beginning of the sinusoid, where the hepatic artery and portal vein blood become confluent.

Net hepatic glycogen synthesis was calculated as the difference between the final glycogen concentration and the mean concentration in the livers of 11 dogs that were killed at the end of a 42-h fast. Net carbon retention by the liver (representing glucose storage as glycogen in the liver) was NHGU − (net hepatic lactate output + net hepatic $^{14}CO_2$ output), with all factors in glucose equivalents. Glycogen synthesis via the direct pathway was derived by dividing the hepatic [^{3}H]-labeled glycogen (disintegrations per minute [dpm]/g liver) by the average inflowing plasma [^{3}H]glucose specific activity (dpm/mg glucose). Indirect hepatic glycogen synthesis was calculated as the difference between net synthesis and synthesis via the direct pathway. Area under the curve (AUC) was calculated by the trapezoidal rule.

Statistical analysis. All data are presented as means ± SE. Time course data were analyzed with repeated-measures ANOVA, and univariate F tests were used for post hoc comparisons. One-way ANOVA was used for comparisons of mean data and AUC. Statistical significance was accepted at $P < 0.05$.

RESULTS

Plasma insulin and glucagon concentrations. The plasma insulin levels during the basal period were almost identical among the three groups (Table 1). The arterial and hepatic sinusoidal insulin concentrations were kept basal (31 ± 5 and 113 ± 15 pmol/l, respectively) throughout the control study (BI). When the portal insulin infusion rate was increased, the arterial and hepatic sinusoidal plasma insulin levels were increased to 97 ± 11 and 394 ± 66 pmol/l, respectively. Peripheral insulin infusion at 3.6 pmol · kg^{-1} · min^{-1} increased the arterial insulin level to 111 ± 13 pmol/l without altering the hepatic sinusoidal level (96 ± 9 pmol/l) appreciably. This step allowed us to examine the effects of a selective rise in arterial insulin on NHGU as well as the combined effect of simultaneous increases in both arterial and sinusoidal insulin. Arterial and hepatic sinusoidal plasma glucagon concentrations remained basal and indistinguishable in the three groups (Table 1).

Blood glucose concentrations, hepatic blood flow, and hepatic glucose load. The arterial and portal vein blood glucose levels were similar among the three groups and increased twofold during the experimental periods (Fig. 1). The arterial-portal glucose gradient was ~1 mmol/l in all groups. Likewise, the hepatic blood flow was similar among the three groups during both the basal period (31 ± 2, 28 ± 2, and 31 ± 3 ml · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively) and the experimental period (27 ± 1, 27 ± 1, and 28 ± 2 ml · kg^{-1} · min^{-1}, respectively). The hepatic glucose load doubled during the glucose infusion period and did not differ significantly among the three groups (Fig. 1).

NHGB, net hepatic fractional glucose extraction, glucose infusion rates, and nonhepatic glucose uptake. The groups exhibited a similar rate of net hepatic glucose production during the basal period. Coincident with the start of the experimental period, they switched from net production to net uptake, with the rate reaching a pseudoplateau at ~60 min (Fig. 2). The mean rates of NHGU during the 4-h infusion period were 7.0 ± 1.1, 15.7 ± 2.7, and 12.0 ± 2.4 μmol · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively, using the direct method of calculation ($P <$ 0.05 for BI vs. both PoI and PeI; $P = 0.15$ for PoI vs. PeI). The rates obtained using the indirect calculation were similar (8.6 ± 1.5, 15.5 ± 3.3, and 11.1 ± 3.2 μmol · kg^{-1} · min^{-1}, respectively). The net hepatic fractional extraction

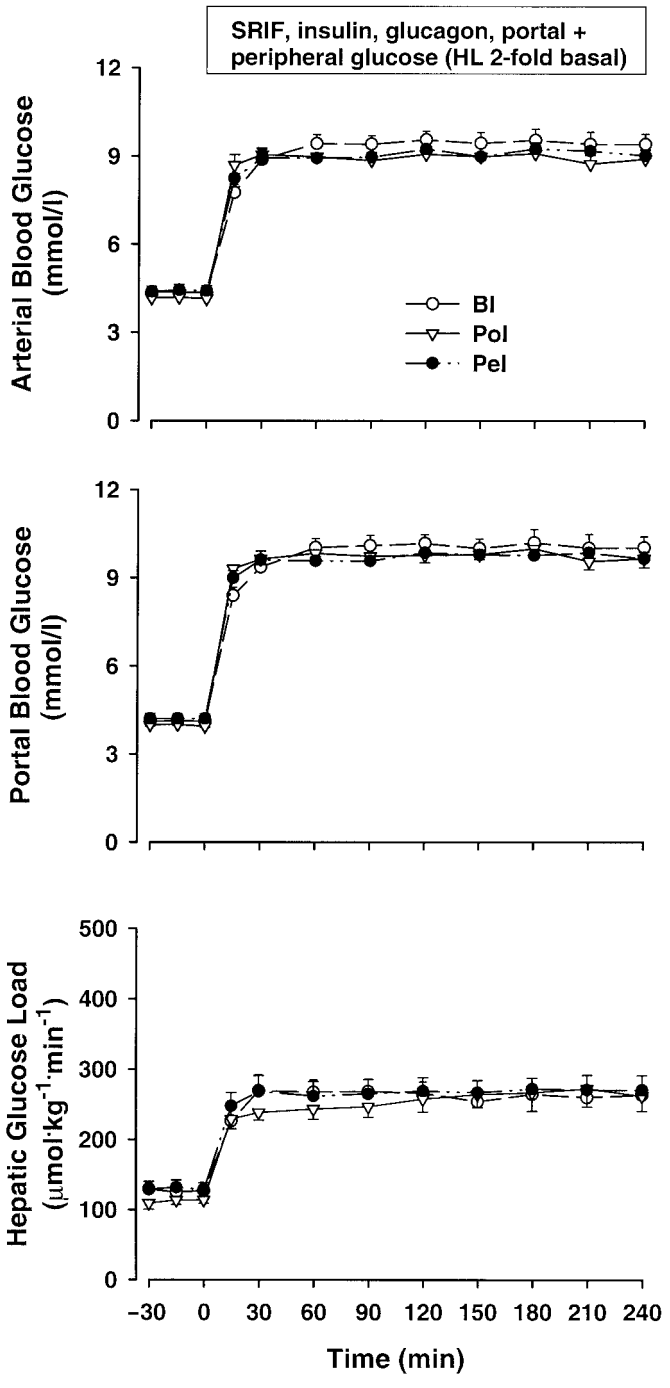

FIG. 1. Arterial and portal blood glucose concentrations and hepatic glucose load in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), and elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. There were no differences among the groups. HL, hepatic glucose load; SRIF, somatostatin.

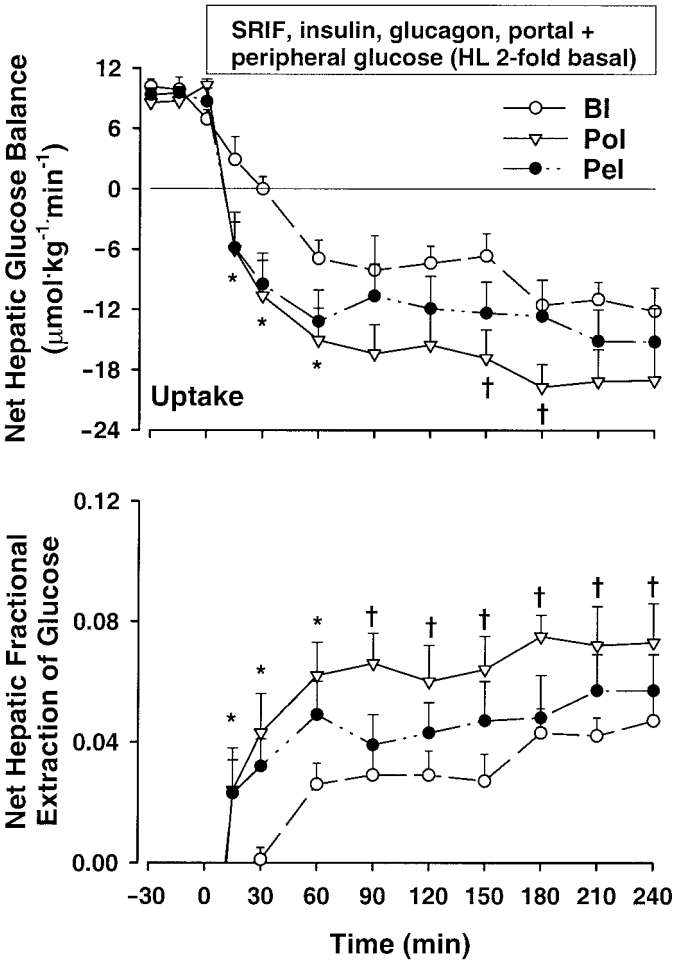

FIG. 2. NHGB and net hepatic fractional glucose extraction in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), and elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. *P < 0.05 for both PoI and PeI vs. BI; †P < 0.05 for PoI vs. BI. There were no significant differences between PoI and PeI. HL, hepatic glucose load; SRIF, somatostatin.

(direct calculation) averaged 0.026 ± 0.004, 0.061 ± 0.010, and 0.045 ± 0.008 in BI, PoI, and PeI, respectively (P < 0.05 for both PoI and PeI vs. BI; P = 0.09 for PoI vs. PeI).

The mean rate of glucose infusion (portal plus peripheral) was significantly (P < 0.005) lower in BI than in either PoI or PeI (28.3 ± 2.1, 62.3 ± 8.6, and 54.6 ± 3.0 μmol · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively) (Fig. 3). Nonhepatic glucose uptake averaged 17.9 ± 1.7, 43.6 ± 8.6, and 39.8 ± 2.9 in BI, PoI, and PeI, respectively (P < 0.05 for PoI and PeI vs. BI).

Arterial blood lactate and net hepatic lactate balance. The arterial blood lactate concentrations were similar among the three groups. The livers in all groups exhibited net hepatic lactate uptake during the basal period (Fig. 4). After the experimental period began, net hepatic lactate balance changed from uptake to output in all groups. In BI, net hepatic lactate production increased gradually, reaching a plateau between 60 and 120 min, after which it declined slightly. In PoI and PeI, net hepatic lactate production peaked rapidly (60 and 30 min, respectively), after which it fell minimally in PeI but significantly in PoI.

Net hepatic $^{14}CO_2$ production and hepatic glycogen synthesis. Net hepatic CO_2 production was minimal during the basal period in all three groups. It increased in the experimental period, reaching a peak at 120 min in all groups. The average rate of CO_2 production during the experimental period was significantly higher (P < 0.05) in PoI and PeI (1.1 ± 0.3 and 1.0 ± 0.3 μmol glucose equivalents · kg^{-1} · min^{-1}, respectively) than in BI (0.4 ± 0.2 μmol glucose equivalents · kg^{-1} · min^{-1}).

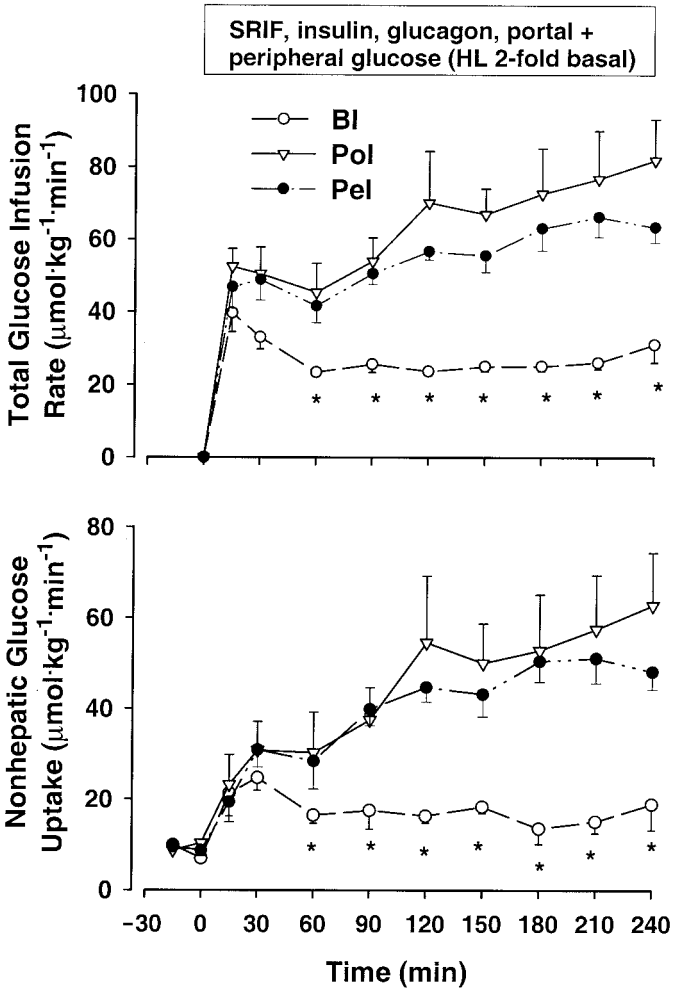

FIG. 3. Rates of total glucose infusion and net nonhepatic glucose uptake in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), and elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. *P < 0.05 for both PoI and PeI vs. BI. There were no significant differences between PoI and PeI. HL, hepatic glucose load; SRIF, somatostatin.

Measured hepatic glycogen synthesis averaged 7.9 ± 2.0, 14.1 ± 2.2, and 10.8 ± 1.2 μmol glucose equivalents · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively ($P < 0.05$ for PoI vs. BI; $P = 0.17$ for PoI vs. PeI; $P = 0.21$ for PeI vs. BI). The net retention of carbon by the liver is an indicator of the mass of carbon available for deposition as glycogen. Net carbon retention during 0–240 min averaged 4.4 ± 1.2, 12.3 ± 2.5, and 7.1 ± 1.0 μmol glucose equivalents · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively ($P < 0.05$ for PoI and PeI vs. BI and for PoI vs. PeI) (Fig. 4). Whereas the rate of glycogen synthesis is similar regardless of the method used to estimate it, net carbon retention has the advantage of not being dependent upon an estimate of the prestudy glycogen concentrations, which must be obtained from a separate set of 42-h–fasted dogs. Therefore, net carbon retention was used as an index of net glycogen accretion in Fig. 4 and in the DISCUSSION section.

The arterial plasma [^{3}H]glucose specific activity was similar among the three groups (3,686 ± 173, 4,370 ± 175, and 4,353 ± 143 dpm/mg glucose). Glycogen synthesis via the direct pathway averaged 2.8 ± 0.8, 7.6 ± 1.3, and 2.7 ± 0.6 μmol glucose equivalents · kg^{-1} · min^{-1} over the 4 h in

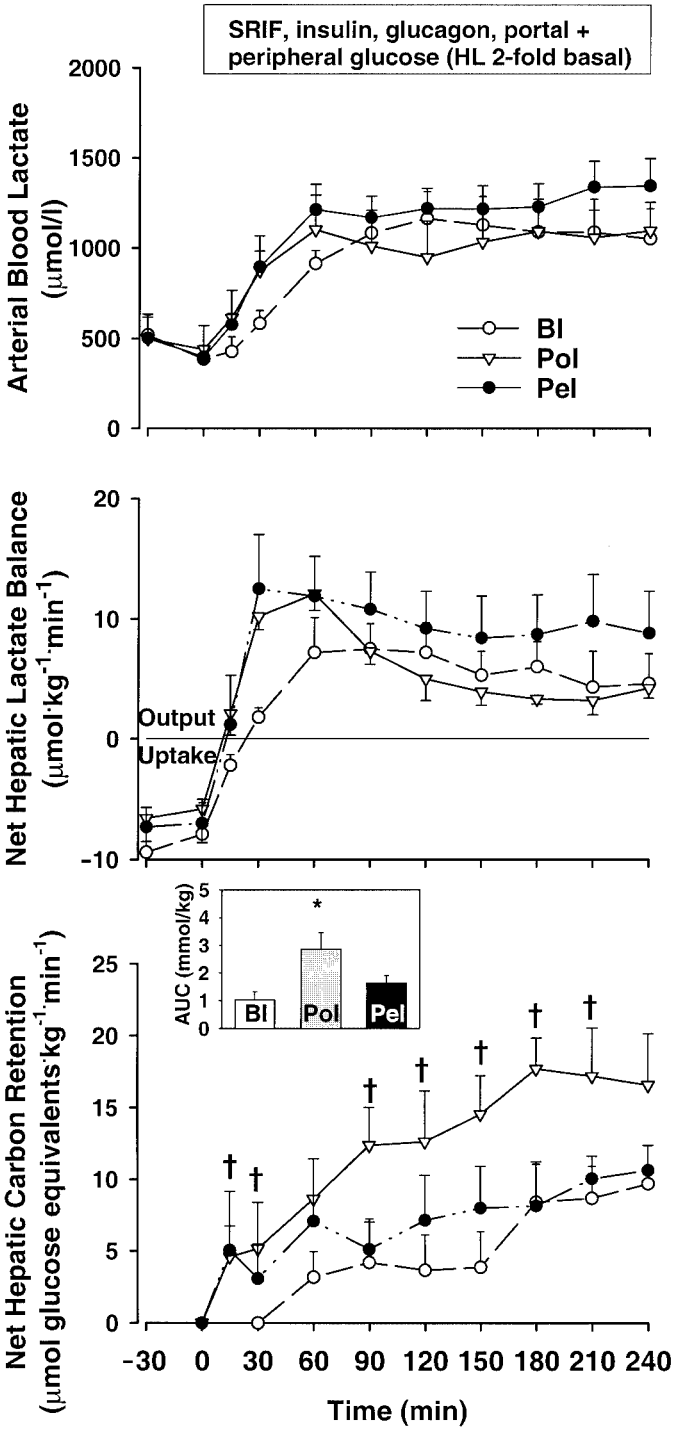

FIG. 4. Arterial blood lactate, net hepatic lactate balance, and net hepatic carbon retention (NHGU minus NHLO and CO_2 release) in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), and elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. *P < 0.05 for PoI vs. both BI and PeI; †P < 0.05 for PoI vs. BI. HL, hepatic glucose load; SRIF, somatostatin.

BI, PoI, and PeI, respectively ($P < 0.01$ for PoI vs. the other two groups). The mean rates of indirect glycogen synthesis (difference between net hepatic carbon retention and direct glycogen synthesis) were 1.6 ± 0.9, 4.7 ± 3.0, and 4.3 ± 0.7 μmol glucose equivalents · kg^{-1} · min^{-1} in BI, PoI, and PeI, respectively ($P < 0.05$ for PeI vs. BI; $P = 0.15$ for PoI vs. BI).

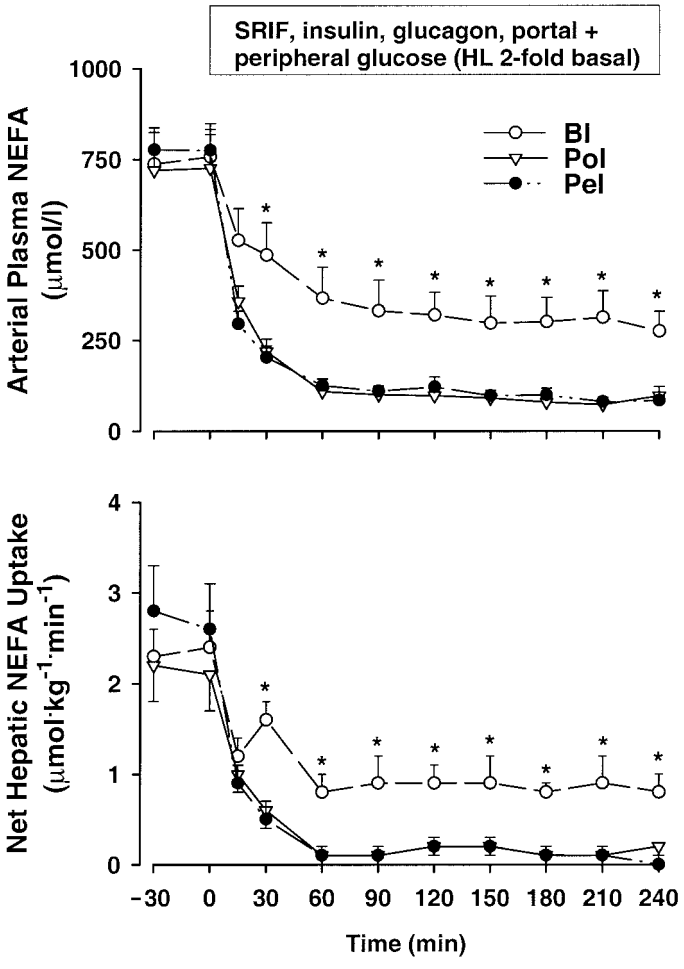

FIG. 5. Arterial plasma NEFA and net hepatic NEFA balance in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), or elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. *$P < 0.05$ for both PoI and PeI vs. BI. HL, hepatic glucose load; SRIF, somatostatin.

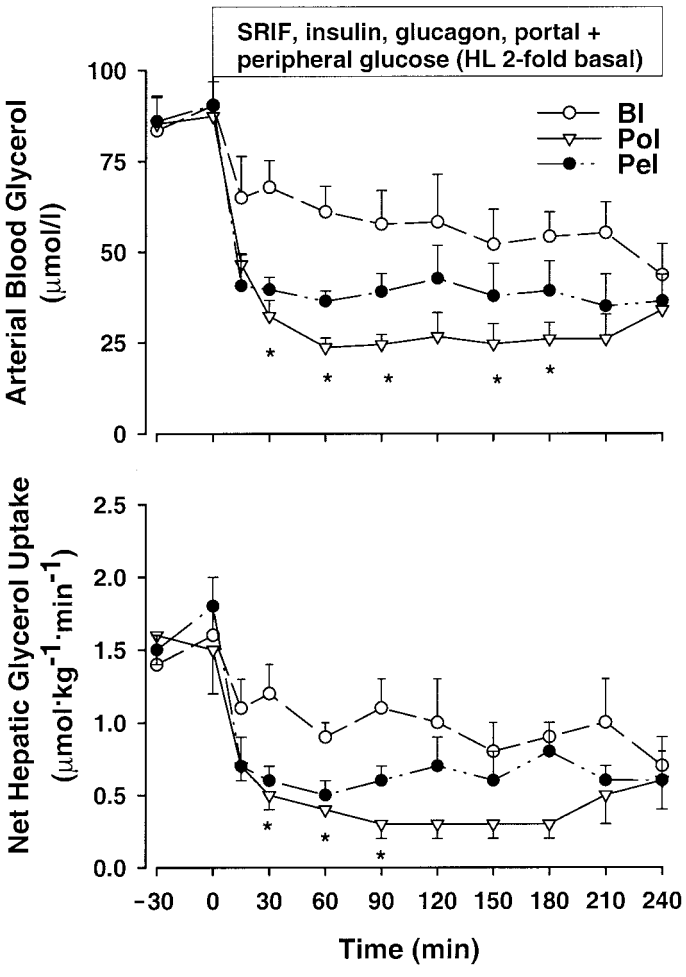

FIG. 6. Arterial blood glycerol and net hepatic glycerol balance in 42-h–fasted conscious dogs under hyperglycemic clamp conditions with portal glucose infusion and basal portal insulin (BI), fourfold basal portal insulin (PoI), or elevated peripheral insulin (PeI). Values are means ± SE; n = 6/group. *$P < 0.05$ for both PoI and PeI vs. BI. HL, hepatic glucose load; SRIF, somatostatin.

The mean hepatic glucose-6-phosphate concentrations at the end of study were 112 ± 16, 97 ± 13, and 63 ± 11 nmol/g wet weight in BI, PoI, and PeI, respectively ($P < 0.05$ for PeI vs. BI; $P = 0.05$ for PoI vs. PeI). Unfortunately, three PoI livers were unavailable for assay; thus, $n = 3$ in that group.

Arterial plasma levels and net hepatic uptake of NEFAs. The arterial plasma NEFA concentrations and net hepatic NEFA uptake decreased from 747 ± 95 μmol/l and 2.3 ± 0.4 μmol · kg^{-1} · min^{-1} during the basal period to 358 ± 72 μmol/l and 1.0 ± 0.2 μmol · kg^{-1} · min^{-1} during the experimental period in BI (Fig. 5). On the other hand, when arterial insulin levels rose in concert with glucose, the plasma NEFA levels and net hepatic uptake of NEFAs fell even further (Fig. 5). Arterial levels and net hepatic uptake of NEFAs fell from 723 ± 68 μmol/l and 2.2 ± 0.3 μmol · kg^{-1} · min^{-1} during the basal period to 137 ± 17 μmol/l and 0.3 ± 0.1 μmol · kg^{-1} · min^{-1} during the experimental period in PoI. In PeI, arterial plasma NEFAs and net hepatic uptake of NEFAs declined from 776 ± 49 μmol/l and 2.7 ± 0.4 μmol · kg^{-1} · min^{-1} to 133 ± 17 μmol/l and 0.2 ± 0.1 μmol · kg^{-1} · min^{-1}.

Arterial blood levels and net hepatic uptake of glycerol. Arterial glycerol levels and net hepatic glycerol uptakes fell from 87 ± 10 μmol/l and 1.5 ± 0.2 μmol · kg^{-1} · min^{-1}, 86 ± 5 μmol/l and 1.6 ± 0.2 μmol · kg^{-1} · min^{-1}, and 88 ± 8 μmol/l and 1.7 ± 0.2 μmol · kg^{-1} · min^{-1} during the basal period to 57 ± 8 μmol/l and 0.9 ± 0.2 μmol · kg^{-1} · min^{-1}, 29 ± 4 μmol/l and 0.4 ± 0.1 μmol · kg^{-1} · min^{-1}, and 39 ± 6 μmol/l and 0.6 ± 0.1 μmol · kg^{-1} · min^{-1} during the experimental period in BI, PoI, and PeI, respectively (Fig. 6). The decrements in both of these values were greater in PoI and PeI than in BI ($P < 0.05$).

DISCUSSION

A twofold increase in the hepatic glucose load in the presence of the portal signal and basal sinusoidal insulin concentrations resulted in a decrease in the rate of net hepatic glucose output but did not result in NHGU within the first 15 min. By the end of the experimental period, NHGU was at a maximal rate of 12.2 ± 2.3 μmol · kg^{-1} · min^{-1}, a value consistent with those observed in earlier studies using similar conditions (9,13). On the other hand, when hyperglycemia and the portal signal were accompanied by a selective rise in the arterial insulin level, there was a more rapid switch from net hepatic glucose output to uptake (5.8 ± 3.5 μmol · kg^{-1} · min^{-1} by 15 min). By the

TABLE 2
AUCs of net hepatic glucose uptake, net hepatic CO_2 production, net hepatic carbon retention, hepatic glycogen synthesis via the direct and indirect pathways, and hepatic glycolysis (mmol glucose equivalents · kg^{-1} · $4\ h^{-1}$) in 42-h–fasted conscious dogs receiving insulin intraportally at 1.8 or 7.2 pmol · kg^{-1} · min^{-1} (BI and PoI groups, respectively) or peripherally at 3.6 pmol · kg^{-1} · min^{-1} (PeI group)

	Group		
	BI	PoI	PeI
Net hepatic glucose uptake	1.64 ± 0.24	3.62 ± 0.64*	2.74 ± 0.55*
Net hepatic CO_2 production	0.09 ± 0.05	0.33 ± 0.10*	0.23 ± 0.06*
Net hepatic carbon retention (glycogen synthesis)	1.03 ± 0.28	2.86 ± 0.59*†	1.67 ± 0.27*
Direct pathway glycogen synthesis	0.68 ± 0.20	1.81 ± 0.31*†	0.66 ± 0.13
Indirect pathway glycogen synthesis	0.39 ± 0.21	1.13 ± 0.73	1.04 ± 0.17*
Net hepatic lactate output	0.57 ± 0.23	0.66 ± 0.10	1.07 ± 0.38
Net hepatic glycolysis‡	0.66 ± 0.28	0.99 ± 0.08	1.30 ± 0.40

Data are means ± SE. $n = 6$/group. *$P < 0.05$ vs. BI; †P 0.05 vs. PeI; ‡net hepatic lactate output + CO_2 production.

end of the experimental period, NHGU was 15.2 ± 3.3 μmol · kg^{-1} · min^{-1}. Addition of an increment in hepatic sinusoidal insulin levels to hyperglycemia and peripheral hyperinsulinemia did not change the initial NHGU response (NHGU 6.0 ± 2.7 μmol · kg^{-1} · min^{-1} by 15 min, $P < 0.05$ vs. BI), but the maximal rate of NHGU (19.7 ± 2.2 μmol · kg^{-1} · min^{-1}; $P < 0.05$ vs. BI) tended to be greater ($P = 0.21$). The increment in NHGU caused by the indirect action of insulin (Table 2) was 1.10 mmol/kg (56% of the total increment), and the increase in NHGU caused by direct insulin action was 0.98 mmol/kg (44% of the total increment). Similarly, the indirect effect of insulin was responsible for ~54% of the difference in fractional extraction between BI and PoI. The enhancement of net hepatic fractional glucose extraction by the fourfold increase in insulin (i.e., PoI vs. BI) in the current investigation was 0.035 ± 0.009. This result was virtually identical to the change in fractional extraction that we previously observed in dogs under similar hormonal and glycemic conditions (insulin-induced Δ in hepatic fractional extraction of glucose = 0.032 with peripheral glucose infusion and 0.035 with portal glucose infusion) (6).

It is of interest to consider the time course of insulin's effect on NHGU in the current investigation. Previous work (9) suggested that in the presence of preexisting hyperglycemia but in the absence of the portal signal, the onset of insulin action on NHGU is relatively slow, requiring ~45 min to become significant and ~90 min to achieve a maximal rate. The portal glucose signal itself (in the absence of increased insulin) has a rapid effect, requiring only 15 min to achieve maximal rates of NHGU (9). When hyperinsulinemia was combined with the portal signal, the hormone stimulated NHGU more rapidly than when it alone was increased (9). In the current investigation, both the portal glucose signal alone and the combination of hyperinsulinemia and the portal signal had slower onsets of action than we had previously observed because hyperglycemia did not preexist, as in our previous report (9). Thus, time was required for the plasma glucose level to rise, thereby increasing the time required to see maximal rates of NHGU. Interestingly, the speed of onset of the response to insulin was determined by the peripheral action of the hormone (Fig. 1). Given that the peripheral effects of insulin (i.e., its indirect effects on the liver) are mediated by actions on the adipocyte, the suppression of lipolysis would appear to be important for the early stimulation of NHGU.

The rate of glucose infusion (an index of whole-body glucose disposal) did not differ between PoI and PeI, and the total amounts of glucose infused in those two groups were approximately twofold more ($P < 0.01$) than the amount infused in BI. Similarly, nonhepatic glucose uptake was not significantly different in PoI and PeI, but the AUCs of nonhepatic uptake in both PoI and PeI were more than twofold greater than those in BI ($P < 0.01$ for BI vs. PoI and PeI). These findings are consistent with the comparable circulating insulin concentrations in PoI and PeI.

The current study is the first to compare insulin's direct and indirect actions on the pathways of glycogen synthesis (the direct pathway from plasma glucose and the indirect pathway from gluconeogenic precursors). Hyperinsulinemia and the portal signal have additive effects on glycogen synthesis (9). Whereas insulin acts directly to stimulate glycogen synthase, the portal signal apparently increases NHGU and, subsequently, the intrahepatic level of glucose-6-phosphate via enhancement of glucokinase translocation within the liver (14). Glucose-6-phosphate is an allosteric activator of glycogen synthase, and the portal signal thus probably activates glycogen synthase secondarily (9). Both insulin and the portal signal, therefore, can stimulate hepatic glycogen synthesis through their actions on glycogen synthase. However, the current data indicate that the route of insulin delivery affects both the pathway and total mass of glycogen storage. Adding the indirect effects of insulin to hyperglycemia and the portal signal increased net hepatic carbon retention 61% (PeI vs. BI). None of the increment in net carbon retention was due to glycogen synthesis via the direct pathway; instead, insulin's indirect effects stimulated gluconeogenic flux to glucose-6-phosphate, thus providing additional glucose for glycogen synthesis via the indirect pathway. In contrast, when the direct hepatic effects of insulin were added to the above signals (PoI), they stimulated net hepatic carbon retention by an additional 73%, almost all of which was due to an increase in glycogen synthesis via the direct pathway. This, in combination with the increase in NHGU and the tendency toward an increase in hepatic glucose-6-phosphate levels in PoI versus PeI, raises the possibility that the direct effect of insulin can be attributed in part to enhancement of glucokinase activity, a

rate-determining step for uptake of glucose into the liver. The increased availability of glucose-6-phosphate would stimulate glycogen synthase, as previously mentioned. Alternatively (or additionally), the direct effect could result from a "pull" within the liver, i.e., the activation of glycogen synthase by insulin. Consistent with our findings, previous studies in hyperglycemic rats determined that hyperinsulinemia achieved via peripheral insulin administration did not enhance glycogen synthesis via the direct pathway when compared with basal insulinemia (15).

In all three protocols, the livers rapidly switched from net uptake to net output of lactate after the start of the experimental period. In PoI, net hepatic lactate output (NHLO) reached a peak early in the experimental period (60 min), and then it decreased, reaching ~32% of the peak level by the end of the experiment. In PeI, NHLO increased as in PoI but failed to fall significantly over time. NHLO in BI reached a peak by 90 min but clearly rose more slowly and to a lesser extent. The more rapid efflux of lactate in the PoI and PeI protocols parallels the more rapid increase in NHGU in those two groups. The decline in net hepatic lactate release during the latter part of the experimental period in the presence of elevated sinusoidal insulin levels was consistent with the observed enhancement of glycogen synthesis in the PoI group, i.e., in a net sense, glucose carbon was shifted away from glycolysis and toward hepatic glycogen synthesis in that group.

Comparing BI and PeI, it is clear that the indirect effect of insulin served to increase hepatic glycogen synthesis 0.64 mmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ 4 h^{-1} and to stimulate glycolysis by an equivalent amount (Table 2). The reduced hepatic glucose-6-phosphate concentrations in PeI versus BI are consistent with an enhancement of glycogen synthesis and glycolysis by insulin's indirect effects. A comparison of PoI and PeI demonstrates that the addition of the direct effect of insulin increased glycogen synthesis by 1.19 mmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ 4 h^{-1} and decreased glycolysis by 0.31 mmol glucose equivalents $\cdot$ kg^{-1} $\cdot$ 4 h^{-1}. There was a strong negative correlation between NHLO and net hepatic NEFA uptake at 30 min ($r = -0.742$, $P < 0.05$), when NHLO was reaching a peak in PoI and PeI. Thus, glucose taken up by the liver was more likely to be diverted to lactate when NEFA uptake was suppressed by insulin's action on adipose tissue. An increase in NHLO in the dogs with the selective increase in peripheral hyperinsulinemia is consistent with our previous findings (2). In that study, an increase in lactate output in dogs with a selective rise in peripheral insulin levels paralleled a decline in arterial NEFA levels and net hepatic NEFA uptake. In contrast, dogs with a selective rise in portal insulin exhibited no fall in NEFA levels or net hepatic NEFA uptake (2). Subsequently, we determined that maintaining basal NEFA levels during a selective increase in peripheral insulin prevented the rise in NHLO (3). Thus, it appears that the fall in the levels and net hepatic uptake of NEFA brought about by an increase in peripheral insulin levels is responsible for directing intrahepatic carbon into glycolysis (3). The shift toward glycolysis could result from a fall in citrate, one of the major inhibitors of phosphofructokinase, the first rate-determining enzyme in the glycolytic pathway. Alternatively, the fall in NEFA could alter the intrahepatic redox state, increasing the NADH/NAD ratio and leading to an activation of lactate dehydrogenase. In the current study, however, NEFA levels and net hepatic NEFA uptake fell to the same levels in PoI and PeI. Thus, changes in NEFA alone cannot explain the manner in which intrahepatic carbon was directed toward glycogen storage in PoI and toward glycolysis in PeI. Apparently, elevated hepatic sinusoidal insulin levels have the ability to shunt carbon away from glycolysis and directly into the glycogen storage pathway.

Arterial NEFA and glycerol concentrations in BI fell ~60% as a result of suppression of lipolysis by hyperglycemia (16,17), peripheral effects of somatostatin (18), or a combination of the two. Net hepatic NEFA uptake followed an unusual pattern in BI, appearing to decline sharply by 15 min and then to rebound somewhat by 30 min, before falling to a plateau of ~1 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1} (Fig. 4). It is likely that the apparent large decline at 15 min represented an overestimate of suppression of NEFA uptake at that point, brought about by non–steady-state conditions (i.e., more rapid decline in arterial than in hepatic vein NEFA concentrations). It follows, then, that the magnitudes of the declines in NEFA uptake in PeI and PoI at 15 min are probably overestimated also, but because net hepatic NEFA uptake was reduced more by hyperinsulinemia, no rebound was observed at 30 min.

Net hepatic $^{14}CO_2$ production was augmented during the experimental period, compared with the basal period, especially in PoI and PeI. Although net hepatic CO_2 production in each group was small compared with NHGU (6, 9, and 8% of NHGU in BI, PoI, and PeI, respectively), it was apparent that a rise in circulating insulin was accompanied by an increase in hepatic glucose oxidation. This stimulation may be due, at least in part, to the antilipolytic effect of the hormone, i.e., increased glucose oxidation when there is a reduced supply of NEFAs available to the liver for oxidation.

Thus, insulin augmented NHGU via both direct and indirect effects. Approximately half of the overall increment in NHGU could be attributed to its indirect effect. This increase was associated with increases in glycolysis and glucose oxidation. On the other hand, insulin also enhanced NHGU via a direct effect on the liver, and this increment in NHGU was accompanied by a significant increase in glycogen storage, especially via the direct pathway. The most striking difference between PoI and PeI was in the fate of glucose carbon taken up by the liver. In a net sense, ~80% of NHGU was stored as glycogen in the PoI group, but hepatic glycogen storage could account for only ~60% of NHGU in PeI. Whereas an increase in peripheral insulin levels per se can stimulate NHGU, a rise in hepatic sinusoidal insulin appears to be important for the efficient storage of glucose in hepatic glycogen.

ACKNOWLEDGMENTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R-01-DK-43706, Diabetes Research and Training Center Grant SP-60-AM-20593, and a grant from the Juvenile Diabetes Foundation International to S.S.

The authors would like to thank Jon Hastings, Melanie Scott, and the members of the Vanderbilt Diabetes Research and Training Center Core Labs (Wanda Snead,

Angelina Penaloza, Eric Allen, and Ying Yang) for technical support.

REFERENCES

1. Prager R, Wallace P, Olefsky JM: Direct and indirect effects of insulin to inhibit hepatic glucose output in obese subjects. *Diabetes* 36:607–611, 1987
2. Sindelar DK, Balcom JH, Chu CA, Neal DW, Cherrington AD: A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes* 45:1594–1604, 1996
3. Sindelar DK, Chu CA, Rohlie M, Neal DW, Swift LL, Cherrington AD: The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* 46:187–196, 1997
4. Ader M, Bergman RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. *Am J Physiol* 258:E1020–E1032, 1990
5. Giacca A, Fisher SJ, McCall RH, Shi ZQ, Vranic M: Direct and indirect effects of insulin in suppressing glucose production in depancreatized dogs: role of glucagon. *Endocrinology* 138:999–1007, 1997
6. Myers SR, McGuinness OP, Neal DW, Cherrington AD: Intraportal glucose delivery alters the relationship between net hepatic glucose uptake and the insulin concentration. *J Clin Invest* 87:930–939, 1991
7. Wilczek H, Gunnarsson R, Felig P, Ostman J, Groth CG, Wahren J: Normalization of hepatic glucose regulation despite systemic insulin delivery: studies in patients with pancreatic transplantation for type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 34:345–349, 1991
8. Kryshak EJ, Butler PC, Marsh C, Miller A, Barr D, Polonsky K, Perkins JD, Rizza RA: Pattern of postprandial carbohydrate metabolism and effects of portal and peripheral insulin delivery. *Diabetes* 39:142–148, 1990
9. Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD: Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the dog. *J Clin Invest* 97:81–91, 1996
10. Moore MC, Pagliassotti MJ, Swift LL, Asher J, Murrell J, Neal D, Cherrington AD: Disposition of a mixed meal by the conscious dog. *Am J Physiol* 266:E666–E675, 1994
11. van de Werve G, Stalmans W, Hers HG: The effect of insulin on the glycogenolytic cascade and on the activity of glycogen synthase in the liver of anaesthetized rabbits. *Biochem J* 162:143–146, 1977
12. Michal G: D-Glucose 6-phosphate and D-fructose 6-phosphate. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. New York, Verlag Chemie Weinheim, 1984, p. 191–198
13. Adkins BA, Myers SR, Hendrick GK, Stevenson RW, Williams PE, Cherrington AD: Importance of the route of intravenous glucose delivery to hepatic glucose balance in the conscious dog. *J Clin Invest* 79:557–565, 1987
14. Cherrington AD: Banting Lecture 1997: Control of glucose uptake and release by the liver in vivo. *Diabetes* 48:1198–1214, 1999
15. Rossetti L, Giaccari A: Insulin does not affect the pathways by which hepatic glycogen is repleted (Abstract). *Diabetes* 40 (Suppl. 1):25A, 1991
16. Shulman GI, Williams PE, Liljenquist JE, Lacy WW, Keller U, Cherrington AD: Effect of hyperglycemia independent of changes in insulin or glucagon on lipolysis in the conscious dog. *Metabolism* 29:317–320, 1980
17. Carlson MG, Snead WL, Hill JO, Nurjhan N, Campbell PJ: Glucose regulation of lipid metabolism in humans. *Am J Physiol* 261:E815–E820, 1991
18. Hendrick GK, Frizzell RT, Cherrington AD: Effect of somatostatin on nonesterified fatty acid levels modifies glucose homeostasis during fasting. *Am J Physiol* 253:E443–E452, 1987