Small Increases in Insulin Inhibit Hepatic Glucose Production Solely Caused by an Effect on Glycogen Metabolism

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Based on our earlier work, a 2.5-fold increase in insulin secretion should completely inhibit hepatic glucose production through the hormone's direct effect on hepatic glycogen metabolism. The aim of the present study was to test the accuracy of this prediction and to confirm that gluconeogenic flux, as measured by three independent techniques, was unaffected by the increase in insulin. A 40-min basal period was followed by a 180-min experimental period in which an increase in insulin was induced, with euglycemia maintained by peripheral glucose infusion. Arterial and hepatic sinusoidal insulin levels increased from 10 ± 2 to 19 ± 3 and 20 ± 4 to 45 ± 5 μU/ml, respectively. Net hepatic glucose output decreased rapidly from 1.90 ± 0.13 to 0.23 ± 0.16 mg · kg^{-1} · min^{-1}. Three methods of measuring gluconeogenesis and glycogenolysis were used: *1*) the hepatic arteriovenous difference technique (n = 8), *2*) the [^{14}C] phosphoenolpyruvate technique (n = 4), and *3*) the ^2H_2O technique (n = 4). The net hepatic glycogenolytic rate decreased from 1.72 ± 0.20 to −0.28 ± 0.15 mg · kg^{-1} · min^{-1} (P < 0.05), whereas none of the above methods showed a significant change in hepatic gluconeogenic flux (rate of conversion of phosphoenolpyruvate to glucose-6-phosphate). These results indicate that liver glycogenolysis is acutely sensitive to small changes in plasma insulin, whereas gluconeogenic flux is not. *Diabetes* 50:1872–1882, 2001

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AV, arteriovenous; G6P, glucose-6-phosphate; Hep GLY; hepatic release of glycogenolytically derived glucose; Hep GNG; hepatic release of gluconeogenically derived glucose; HGP, hepatic glucose production; HGR, hepatic glucose release; HGU, hepatic glucose uptake; ID, internal diameter; NEFA, nonesterified fatty acid; NHGO, net hepatic glucose output; NHLO, net hepatic lactate output; PEP, phosphoenolpyruvate; PEPCK, PEP carboxykinase; R_a, endogenous glucose production; R_d, endogenous glucose utilization; TGO, total glucose output; UDPG, uridinediphosphoglucose.

Sindelar et al. (1) showed that hepatic glucose production (HGP) can be inhibited by selective increases in the arterial or portal vein insulin concentration. In response to a 14-μU/ml increase in arterial insulin (no change in portal insulin), a >50% reduction in net hepatic glucose output (NHGO) was observed. Likewise, a 14-μU/ml increase in portal insulin (no change in arterial insulin) also resulted in a >50% reduction in NHGO. In addition, the above studies showed that insulin acted directly on the liver, with a rise in hepatic sinusoidal insulin quickly inhibiting HGP by reducing net hepatic glycogenolysis. The indirect effect of insulin on HGP, on the other hand, resulted from a decrease in gluconeogenic flux rate caused by a reduction in the flow of gluconeogenic amino acids and glycerol to the liver and diversion of carbon derived from glycogenolysis to lactate rather than glucose. The reduction in HGP in this group was also, in part, the result of a decrease in net hepatic glycogenolysis, which occurred as a result of a slight rise in the hepatic sinusoidal insulin level, which, in turn, occurred as a result of the rise in hepatic artery insulin. It took 1 h to detect a significant indirect effect of insulin on HGP.

Sindelar et al. (1) created selective changes in the arterial or portal insulin level by infusing somatostatin to inhibit insulin secretion and replacing insulin by infusion through a peripheral and/or portal catheter. Stimulation of pancreatic insulin secretion, on the other hand, results in an increase in both portal and arterial levels of the hormone. Therefore, in the present study, our aim was to determine if a two- to threefold increase in insulin, occurring simultaneously in portal and peripheral blood, would inhibit HGP primarily through an effect on glycogen metabolism. Although Sindelar et al. (1) reported that portally delivered insulin did not affect gluconeogenic flux, their estimate of the latter relied solely on the measurement of the net hepatic uptake (arteriovenous [AV] difference) of gluconeogenic precursors. In the present study, we combined the hepatic AV difference technique, along with two newly developed methods for quantifying gluconeogenesis in vivo, to further address this question (2,3).

RESEARCH DESIGN AND METHODS

Animal care and surgical procedures. Experiments were conducted on eight conscious mongrel dogs (23–29 kg) of either sex that had been fed a once-daily meat and chow diet (34% protein, 46% carbohydrate, 14.5% fat, and

5.5% fiber based on dry weight) (Kal Kan beef dinner; Kal Kan, Vernon, CA; and Purina Lab Canine Diet No. 5,006; Purina Mills, St. Louis, MO). The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

Each dog underwent a laparotomy performed under general anesthesia (15 mg/kg pentothal sodium presurgery and 1% isoflurane inhalation anesthetic during surgery) 2 weeks before the experiment. Using standard sterile techniques that have been described previously (4), silastic infusion catheters (0.03 inches internal diameter [ID]) (Dow Corning, Midland, MI) were placed into a splenic and a jejunal vein for intraportal infusions. Catheters (0.04 inches ID) for blood sampling were placed into the left common hepatic vein, the portal vein, and the femoral artery as previously described (5). The catheters were filled with a saline solution that contained 200 U/ml heparin (Abbott Laboratories, North Chicago, IL), and their free ends were knotted. Doppler flow probes (Instrument Development Laboratories, Baylor College of Medicine, Houston, TX) were placed around the hepatic artery and the portal vein to determine hepatic blood flow as previously described (6). The Doppler leads and the catheters were placed in a subcutaneous pocket before closure of the abdominal skin. The position of the catheter tips was confirmed on autopsy at the end of each experiment.

Only dogs that exhibited a leukocyte count $<18{,}000/mm^3$, a hematocrit >34%, normal stools, and consumption of their daily food ration were used for the study. On the day of the experiment, after an 18-h fast, the catheters and flow probe leads were taken out under local anesthesia (2% lidocaine, Abbott Laboratories). The contents of each catheter were aspirated, and they were flushed with saline. The intraportal catheters (splenic and jejunal) were used for the infusion of insulin (Eli Lilly, Indianapolis, IN). Angiocaths (Deseret Medical, Becton Dickinson, Sandy, UT) were inserted percutaneously into the left cephalic vein for [3-^{3}H]glucose (Du Pont-NEN, Boston, MA), [U-^{14}C]alanine (Amersham, Chicago, IL), and indocyanine green (Sigma, St Louis, MO) infusion. An angiocath was inserted into the right cephalic vein for peripheral glucose infusion. Animals were allowed to rest quietly in a Pavlov harness for 30 min before the experiments started.

Experimental procedure. Each experiment ($n = 8$) consisted of a tracer and dye equilibration period (−140 to −40 min), a basal period (−40–0 min), and an experimental period (0–180 min). At −140 min, a priming dose of [3-^{3}H]glucose (50 μCi) was given, and constant infusions of [3-^{3}H]glucose (0.4 μCi/min) and indocyanine green (0.07 mg/min) were started. Insulin was infused intraportally (600 $\mu U \cdot kg^{-1} \cdot min^{-1}$) throughout the experimental period, with euglycemia being maintained by variable glucose infusion (20% dextrose; Baxter Healthcare, Deerfield, IL) through a peripheral vein.

Whereas the AV difference method was used to measure gluconeogenic flux in all eight dogs in the group, a subset of four animals from this group was also used to compare the AV difference data with data obtained from both [^{14}C]phosphoenolpyruvate (^{14}C-PEP) and ^2H_2O techniques. In this subset, the following modifications were used: oral doses of ^2H_2O (1.5 ml/kg) were given at −200 and −140 min (99.9 atom % D; Sigma), and a constant infusion of [U-^{14}C]alanine (2.2 μCi/min) was started at −140 min.

Arterial blood samples were taken every 10 min during the basal period and every 15 min during the experimental period. Blood samples were drawn from the portal and hepatic veins every 20 min during the basal period, 15 and 30 min after the initiation of the experimental period, and every 30 min thereafter. Arterial plasma glucose levels were monitored every 5 min during the experimental period to facilitate maintenance of euglycemia. The total volume of blood withdrawn did not exceed 20% of the animal's blood volume, and two volumes of saline were given for each volume of blood withdrawn. No significant decrease (~10–15%) in hematocrit occurred with this procedure. In an attempt to compensate for the transit time of glucose through the liver and thus allow the most accurate estimates of net hepatic glucose balance, the arterial and portal blood samples were collected simultaneously ~30 s before the collection of the hepatic venous sample (7).

Immediately after the final sampling time, each animal in the subset of four animals used to compare gluconeogenic measurement techniques was anesthetized with pentobarbital. The animal was then removed from the Pavlov harness while the tracer and hormone infusion continued. A midline laparotomy incision was made, the liver was exposed, and clamps cooled in liquid nitrogen were used to freeze sections of two hepatic lobes simultaneously in situ. The hepatic tissue was then immediately cut free, placed in liquid nitrogen, and stored at −70°C. Approximately 2 min elapsed between the time of anesthesia and the time of tissue sampling. All animals were then killed.

Analytical procedures. Immediately after each blood sample was drawn, the blood was quickly placed into tubes containing potassium EDTA (1.6 mg/ml) (Sarstedt, Numbrecht, Germany). A 1-ml aliquot of whole blood was removed from the tubes and lysed with 3 ml 4% perchloric acid. The solution was centrifuged, and the supernatant was stored for later determination of whole-blood metabolites (alanine, β-hydroxybutyrate, glycerol, and lactate). Aliquots (90 μl) of the perchloric acid supernatant were placed in 990 μl of 0.2 mol/l sodium acetate buffer, with or without glutaminase, and incubated at 37°C for 1 h. These samples were then stored for later analysis of glutamine and glutamate, respectively. A 1-ml aliquot of whole blood was lysed with 1 ml 10% sulfosalicylic acid and centrifuged, and the supernatant was stored for later analysis of gluconeogenic amino acids glycine, serine, and threonine. A third aliquot (20 μl) of arterial blood was immediately used for the measurement of hematocrit in duplicate using capillary tubes. The remainder of the blood was centrifuged to obtain plasma.

Four 10-μl aliquots of plasma were immediately analyzed for glucose with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA) using the glucose oxidase method. A 1-ml aliquot of plasma received 50 μl of 100,000 KIU/ml Trasylol (FBA, New York) and was stored for analysis of immunoreactive glucagon. The remainder of the plasma was used for analysis of labeled glucose, insulin, nonesterified free fatty acids, cortisol, and [^{2}H]glucose. All samples were kept in an ice bath during the experiment and were then stored at −70°C until the assays were performed.

Plasma [^{3}H]glucose and [^{14}C]glucose were determined by double-label liquid scintillation counting after Somogyi-Nelson deproteinization and ^3H_2O exclusion procedures as described elsewhere (1). Whole-blood metabolite concentrations were determined according to the methods of Lloyd et al. (8) for the Monarch 2000 centrifugal analyzer (Monarch, Lexington, MA). Whole-blood glutamine and glutamate concentrations were determined by the methods described in Wasserman et al. (9). Plasma nonesterified fatty acid (NEFA) levels were determined spectrophotometrically, with an assay adapted to the Monarch analyzer (Wako Chemicals, Richmond, VA). Immunoreactive insulin was measured using a double-antibody radioimmunoassay (10). Immunoreactive glucagon was measured using a modification of the double-antibody insulin method (10). Insulin and glucagon antibodies and ^{125}I tracers were obtained from Linco Research (St. Louis, MO). Blood gluconeogenic amino acids were determined using high-performance liquid chromatography (11). Indocyanine green was measured spectrophotometrically at 810 nm to estimate hepatic blood flow, according to the method of Leevy et al. (12). Hepatic glycogen content was measured as previously described (13). Enzymes and coenzymes for metabolic analyses were obtained from Boehringer Mannheim Biochemicals (Mannheim, Germany) and Sigma.

The ^{14}C-PEP technique of Rossetti et al. (2) requires a hepatic biopsy and uses a radiolabelled gluconeogenic precursor to determine the relative contribution of plasma glucose, net hepatic glycogenolysis, and gluconeogenic flux to the hepatic glucose-6-phosphate (G6P) pool. Analysis of phosphoenolpyruvate (PEP) and uridinediphosphoglucose (UDPG) was carried out in the Rossetti lab using previously described methods (2). Briefly, UDPG and PEP concentrations and specific activities in the liver were measured using sequential chromatographic separations.

The ^2H_2O technique of Landau et al. (3) and Chandramouli et al. (14) makes use of orally ingested ^2H_2O, which labels glucose at carbon five (C5) during gluconeogenesis. Analyses of deuterated glucose in plasma were performed in the Landau lab by previously reported methods (3). Briefly, enrichments in the hydrogens bound to carbon two (C2) and C5 were determined by isolating them in formaldehyde and converting the latter to hexamethylenetetramine by adding ammonia and then assaying for content by mass spectrometry. As pyruvate or glycerol is converted to glucose during gluconeogenesis, the addition of a hydrogen from body water to C5 of glucose occurs. Because of rapid cycling between G6P and fructose-6-phosphate, there is an addition of hydrogen from body water to C2 by both gluconeogenesis and glycogenolysis.

Calculations. The net hepatic balances of labeled and unlabelled glucose and gluconeogenic substrates were calculated using the formula $(H - [0.2*A + 0.8*P]) \times HF$, where H, A, and P are the substrate concentrations in hepatic vein, femoral artery, and portal vein blood or plasma, respectively. HF represents the total hepatic blood flow estimated from indocyanine green, and 0.2 and 0.8 are the approximate contributions of the hepatic artery and the portal vein, respectively (6). With this calculation, a positive value represents net production by the liver, whereas a negative value represents net hepatic uptake. Plasma glucose and [^{3}H]glucose values were multiplied by 0.73 to convert them to blood glucose values as validated elsewhere (13). The data displayed in RESULTS were calculated using indocyanine green–determined blood flows. Both the artery and portal vein Doppler flow probes were simultaneously functional in only three of the eight studies, precluding the use of Doppler-determined flows for the entire database. Nevertheless, the Doppler flow probe data obtained (0.22 ± 0.03 and 0.78 ± 0.03 for hepatic artery and portal vein contributions, respectively) confirmed that the ratio of arterial to portal blood flow that we used was appropriate. The insulin and glucagon levels in plasma entering the liver sinusoids were calculated using the formula $(0.2*[H]_A) + (0.8*[H]_P)$, where $[H]_A$ and $[H]_P$ were arterial and portal vein hormone concentrations, respectively.

Tracer-determined whole-body glucose production (R_a) and utilization (R_d) were measured using a primed, constant infusion of [3-^{3}H]glucose. Because of the underestimation of tracer-determined glucose production seen during a fall in arterial glucose specific activity when the Steele equation is used (15), we chose to use a two-compartment model for data analysis. Data calculation was carried out using the two-compartment model described by Mari (16) and canine parameters reported by Dobbins et al. (17).

For calculation of unidirectional hepatic glucose uptake (HGU), the net [^{3}H]glucose uptake was divided by the arterial [^{3}H]glucose specific activity. The above calculation is based on the assumption that intrahepatic uptake of glucose occurs before glucose production, so as not to dilute the plasma glucose specific activity. Even if this assumption is not correct, the drop in specific activity across the liver is very small; thus, the assumption is of little consequence. Unidirectional hepatic glucose release (HGR) was determined using the formula: HGR = HGU + NHGO.

Gluconeogenic and glycogenolytic methods and calculations. Gluconeogenesis, as classically defined, is the synthesis of glucose from noncarbohydrate precursors. However, not all carbon produced from flux through the gluconeogenic pathway (i.e., PEP to G6P) is released as glucose; therefore, separate estimates of gluconeogenesis, which is the rate of noncarbohydrate-derived glucose release into the blood, and gluconeogenic flux, which is the rate of carbon flux from PEP to G6P, can be made. Likewise, the glycogenolytic contribution to glucose release can be estimated separately from the net hepatic glycogenolytic contribution to G6P and from glycogen degradation, which is the rate of carbon flux from glycogen to G6P, excluding cycling of carbon between G6P and glycogen. Additionally, glycogen synthesis, which is the rate of carbon flux from G6P to glycogen, excluding glycogen cycling, can be estimated.

The ^{14}C-PEP technique (2) can be used to estimate hepatic gluconeogenesis, gluconeogenic flux, glycogenolysis, net hepatic glycogenolysis, glycogen synthesis and glycogen degradation. This method determines the proportion of G6P that is derived from the three sources of input into the G6P pool: plasma glucose, gluconeogenic flux, and glycogen degradation. The fraction of the pool derived from plasma glucose (direct fraction) is determined using the ratio of hepatic ^{3}H-UDPG SA and plasma [^{3}H]glucose specific activity during [3-^{3}H]glucose infusion. The fraction formed through gluconeogenic flux (gluconeogenic fraction) is estimated using the ratio of ^{14}C-UDPG specific activity and two times ^{14}C-PEP specific activity during [U-^{14}C]alanine infusion. It is implicit that little plasma [^{14}C]glucose is taken up by the liver, and indeed, in the present study, the rate of net hepatic [^{14}C]glucose uptake from the plasma was negligible (0.03 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}). Because glycerol enters the gluconeogenic pathway above PEP, the gluconeogenic fraction will be underestimated to the extent that glycerol acts as a gluconeogenic substrate. However, if net hepatic glycerol uptake is known and one assumes that all of the glycerol extracted by the liver is converted to G6P, then the hepatic glycerol uptake rate can be used to determine the extent of underestimation of the gluconeogenic fraction. In the present study, the gluconeogenic fraction was underestimated modestly (21 and 27% in the basal period and at the end of the experimental period, respectively); therefore, we included the fractional contribution of glycerol in the estimate of the gluconeogenic fraction. The remaining glycogenolytic fraction (1 − direct fraction − gluconeogenic fraction) represents the fraction of the G6P pool that is derived from glycogen breakdown, excluding glycogen cycling (the glycogenolytic fraction only represents dilution of the G6P pool by degradation of unlabelled glycogen and therefore does not include molecules that leave the G6P pool and cycle through glycogen). The ^{14}C-PEP technique accounts for glucose cycling, which is the input of extracellular glucose into the G6P pool, followed by the exit of plasma-derived G6P back into the extracellular pool. As described by Rossetti et al. (2), the hepatic gluconeogenic rate can be estimated using the formula:

$$\text{Hep GNG} = \text{TGO} \times \text{GNG fraction}$$

$$\text{TGO} = \text{HGR} + \text{GC}$$

where TGO is total glucose output and equals HGR + glucose cycling (GC) and where:

$$\text{GC} = \text{direct fraction} \times \text{HGR}/(1 - \text{direct fraction})$$

thus:

$$\text{Hep GNG} = \text{HGR} \times \text{GNG fraction}/(1 - \text{direct fraction})$$

where Hep GNG is the hepatic release of gluconeogenically derived glucose and GNG fraction is the gluconeogenic fraction.

This approach estimates the absolute rate of hepatic glucose release that is gluconeogenic in origin. It should be noted that the hepatic gluconeogenic rate calculated using the ^{14}C-PEP technique underestimates whole-body gluconeogenesis by an amount equal to renal glucose production (which is gluconeogenic in origin).

The amount of TGO that is glycogenolytic in origin can be determined using the formula:

$$\text{Hep GLY} = \text{TGO} - \text{Hep GNG} - \text{GC}$$

which is equivalent to:

$$\text{Hep GLY} = \text{HGR} \times \text{GLY fraction}/(1 - \text{direct fraction})$$

where Hep GLY is the hepatic release of glycogenolytically derived glucose.

Because Hep GNG and Hep GLY are derived from fractions representing the unidirectional contribution of gluconeogenic flux and glycogen degradation to the G6P pool, they represent the absolute contribution of carbon from these pathways to glucose production.

When using the ^{14}C-PEP technique to estimate gluconeogenic flux (PEP to G6P), several factors must be considered. To approach total hepatic gluconeogenic flux, all major sources of carbon exiting from the G6P pool must be accounted for, including the contribution of gluconeogenically derived G6P to hepatic glucose oxidation and to hepatic lactate release. Glucose oxidation was assumed to be 0.3 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} throughout the experiment because the process changes little under a variety of conditions, and that value represents a reasonable overall mean (18). Ideally, unidirectional hepatic lactate release would be used to calculate the gluconeogenic flux rate; however, given the complexity of such a measurement, net hepatic lactate output was used instead. Although lactate can be a product or precursor in the liver, we assume that its flux is unidirectional. This approach will underestimate gluconeogenic flux to the extent that simultaneous uptake and release of lactate occurs.

Furthermore, hepatic gluconeogenic flux will be underestimated to the extent that gluconeogenically derived G6P is diverted into the glycogen pool. However, net gluconeogenic carbon deposited in glycogen can also be determined, thus allowing gluconeogenic flux to be calculated as follows:

$$\text{Hep GNG flux} = (\text{TGO} + \text{NHLO} + \text{GO}) \times \text{GNG fraction} + \text{RD}_{\text{GNG}}$$

where GO is glucose oxidation and RD_{GNG} is the net rate of deposition of gluconeogenic carbon in liver glycogen during the experimental period. [^{14}C]glycogen can accumulate directly from entry of [^{14}C]glucose from the plasma or indirectly from the contribution of ^{14}C-labeled gluconeogenic carbon. Direct contribution can be estimated by determining the amount of plasma [^{3}H]glucose deposited in liver glycogen and then calculating the amount of [^{14}C]glucose that was deposited directly. Indirect gluconeogenic contribution can then be estimated by subtracting the direct contribution of [^{14}C]glucose from total [^{14}C]glycogen deposition. The indirect contribution can then be used to calculate the RD_{GNG}. Calculations were carried out according to the following formulas:

Amount of plasma glucose deposited in liver glycogen (direct contribution [DC], milligrams per gram of liver):

$$\text{DC} = \text{liver glycogen content (mg/g liver)} \times \text{liver [}^3\text{H]glycogen SA (dpm/mg)}/\text{arterial plasma [}^3\text{H]glucose SA (dpm/mg)}$$

where SA is specific activity.

Amount of plasma [^{14}C]glucose deposited in liver glycogen (^{14}C DC; dpm/g liver):

$$^{14}\text{C DC} = \text{DC} \times \text{arterial plasma [}^{14}\text{C]glucose SA (dpm/mg)}$$

Total [^{14}C]glucose (^{14}C TC) deposited in liver glycogen (dpm/g):

$$^{14}\text{C TC} = \text{liver glycogen content (mg/g liver)} \times \text{liver [}^{14}\text{C]glycogen SA (dpm/mg)}$$

Gluconeogenic (indirect) contribution of [^{14}C]glucose to liver glycogen deposition (^{14}C IC, dpm/g):

$$^{14}\text{C IC} = {}^{14}\text{C TC} - {}^{14}\text{C DC}$$

Amount of gluconeogenic glucose equivalents deposited in liver glycogen (gluconeogenic carbon, milligrams per gram):

$$\text{GNG C} = {}^{14}\text{C IC}/{}^{14}\text{C PEP SA (dpm/mg)} \times 2 \text{ (to convert to glucose equivalents)}$$

where GNG C is gluconeogenic carbon.

Rate of deposition of gluconeogenic carbon in liver glycogen (RD_{GNG}, milligrams per kilograms per minute):

$$\text{RD}_{\text{GNG}} = \text{GNG C} \times 0.85 \times \text{liver weight (G)/body weight (kg)}$$

× length of experimental period (180 min)

To the extent that ^{14}C-labeled carbon accumulated in liver glycogen during the equilibration and control periods, there would be an overestimation of the contribution of gluconeogenic carbon to the glycogen pool during the test period. Based on previous studies (19), we estimate that this represents ~15% overestimation; therefore, we calculated RG_{GNG} using only 85% of the mass of gluconeogenic carbon deposited in liver glycogen.

Thus, the net hepatic glycogenolytic contribution to G6P can be determined because all major entries and exits of carbon into the G6P pool can be accounted for. The following formula is used:

$$\text{NHGLY} = \text{NHGB} + \text{GO} + \text{NHLO} - \text{GNG flux}$$

where NHGLY is net hepatic glycogenolysis, NHGB is net hepatic glucose balance, and NHLO is net hepatic lactate output.

Glycogen synthesis can be determined using the estimate of RD_{GNG}. Because carbon that cycles through glycogen will not be accounted for by RD_{GNG}, glycogen synthesis represents the flux rate of G6P to glycogen minus that which cycles back to G6P (i.e., not total flux through glycogen synthase). The following formula is used:

$$\text{GLY SYN} = RD_{GNG}/\text{GNG fraction}$$

where GLY SYN is glycogen synthesis.

Glycogen degradation can then be determined using the following formula:

$$\text{GLY DEG} = (\text{TGO} + \text{NHLO} + \text{GO}) \times \text{GLY fraction} + RD_{GLY}$$

where GLY DEG is glycogen degradation and RD_{GLY} is the rate of deposition of glycogenolytic carbon in liver glycogen during the experimental period and is determined using the formula:

$$RD_{GLY} = \text{GLY fraction} \times \text{GLY SYN}$$

where GLY fraction is the glycogenolytic fraction.

Likewise, glycogen degradation does not account for glycogen cycling and does not therefore represent total flux though glycogen phosphorylase but instead is an estimate of the rate of degradation of cold glycogen to G6P. On the other hand, net hepatic glycogenolysis, as calculated above, represents the difference between total flux in and out of glycogen.

Finally, because the ^{14}C-PEP method requires a hepatic biopsy, data were only obtained at the end of the experimental period. Because the PEP and UDPG pools are small and turn over rapidly, their specific activities reach steady state quickly and reflect gluconeogenesis at the time of sampling. For comparison, data obtained using this technique in overnight fasted untreated dogs were obtained from Goldstein et al. (20).

The AV difference technique can be used to estimate hepatic gluconeogenic flux and net hepatic glycogenolysis. This method assumes that there is 100% conversion of gluconeogenic precursors taken up by the liver into G6P and thus provides a maximal estimate of gluconeogenic flux from circulating precursors. It also assumes that intrahepatic gluconeogenic precursors do not contribute significantly to gluconeogenic flux. The errors from these assumptions appear to be small, and they are offsetting to the extent that they occur. Gluconeogenic flux was determined by summing the net hepatic uptake rates of the hepatic gluconeogenic precursors (alanine, glycine, serine, threonine, glutamine, glutamate, glycerol, lactate, and pyruvate) then dividing by two to account for the incorporation of two three-carbon precursors into one six-carbon glucose molecule. Pyruvate uptake was estimated to be 10% of lactate uptake (21). Because lactate can act as a precursor or product in the liver, for the purpose of determining gluconeogenic flux lactate was only included in gluconeogenic uptake in a given dog if net uptake was evident. When net hepatic lactate output occurred, net hepatic lactate uptake was set to zero because under that circumstance lactate becomes a product. The mean lactate data, on the other hand, represent both uptake and output (Fig. 1). Ideally, gluconeogenic flux would be calculated using unidirectional hepatic uptake rates of the gluconeogenic precursors, but this would be difficult because it would require the use of multiple stable isotopes. Net hepatic uptake rates were used instead. Net balance represents a close approximation of unidirectional gluconeogenic amino acid uptake because there is little or no hepatic release of these precursors (including alanine, which is the major amino acid contributor to gluconeogenesis) (22). An exception is glutamine, which can be produced and taken up by the liver; however, quantitatively glutamine balance is very small and thus has little effect on the overall gluconeogenic flux rate. Glycerol has been demonstrated to be simultaneously taken up and released by the liver, with release accounting for 14–30% of uptake (23). In the present study, this would lead to an underestimation of gluconeogenic flux of only 0.02–0.05 mg · kg^{-1} · min^{-1}. On the other hand, significant quantities of lactate can be taken up or released by the liver, but as noted earlier, we assume that appreciable lactate uptake and release do not occur simultaneously. To the extent that they do, however, gluconeogenic flux will be underestimated. The hepatic gluconeogenic flux rate can thus be calculated using the following formula:

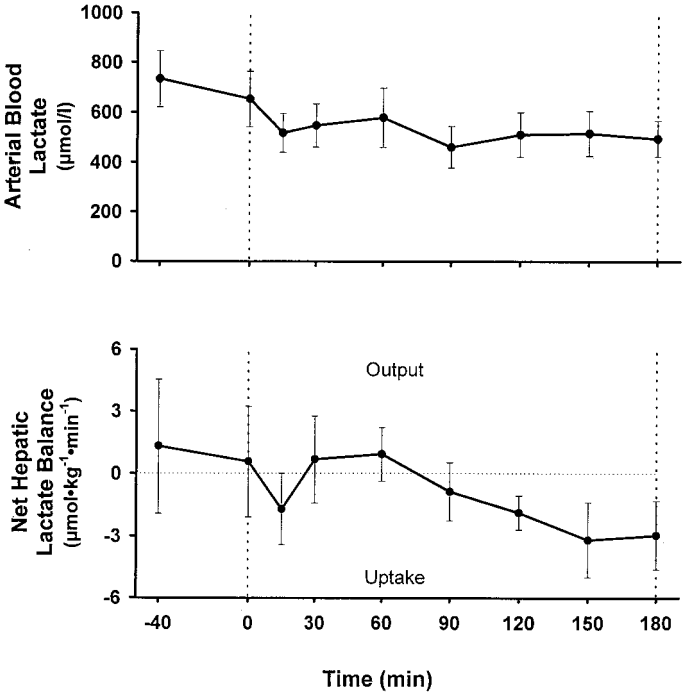

FIG. 1. Arterial blood lactate level and net hepatic lactate balance in 18-h–fasted conscious dogs during the basal period (−40–0 min) and during intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion (0–180 min) (mean ± SE, n = 8).

$$\text{Hep GNG flux} = (\text{net hepatic GNG precursors uptake}/2)$$

Once an estimate of hepatic gluconeogenic flux is obtained, the net hepatic glycogenolytic rate can also be estimated using the AV difference approach according to the formula:

$$\text{GLY DEG} + \text{HGU} + \text{HLU} + \text{NHAAU} + \text{NHGlycerolU}$$

$$= \text{GLY SYN} + \text{HGR} + \text{HLR} + \text{GO}$$

where HGU, HLU, NHAAU, and NHGlycerolU represent the hepatic uptakes of glucose, lactate, gluconeogenic amino acids, and glycerol, and HGR and HLR represent hepatic glucose and lactate release.

The formula can be rearranged as follows:

$$\text{GLY DEG} - \text{GLY SYN} = (\text{HGR} - \text{HGU}) + (\text{HLR} - \text{HLU}) + \text{GO}$$

$$- \text{NHAAU} - \text{NHGlycerolU}$$

which can be simplified to:

$$\text{NHGLY} = \text{NHGB} + \text{NHLB} + \text{GO} - \text{NHAAU} - \text{NHGlycerolU}$$

thus, net hepatic glycogenolytic rate is not affected by the assumption regarding lactate flux.

The ^2H_2O technique (3,24) can be used to calculate whole-body gluconeogenesis and glycogenolysis. This method derives the fraction that whole-body gluconeogenesis contributes to overall endogenous glucose production (R_a) from the ratio of the enrichment of deuterium at C5 of blood glucose to the enrichment at C2. Labeling at C5 occurs during gluconeogenic flux to G6P, whereas labeling at C2 represents labeling of the whole G6P pool. Thus, the C5:C2 ratio provides the fractional gluconeogenic contribution to G6P. Because plasma deuterated glucose is analyzed, there is no discrimination between renal and hepatic gluconeogenesis, and thus the whole-body gluconeogenic fraction is measured. The following formula is used:

$$\text{whole-body GNG} = R_a * \text{C5/C2}$$

This method does not directly assess hepatic gluconeogenic flux; therefore, it cannot be used to estimate net hepatic glycogenolysis. On the other hand,

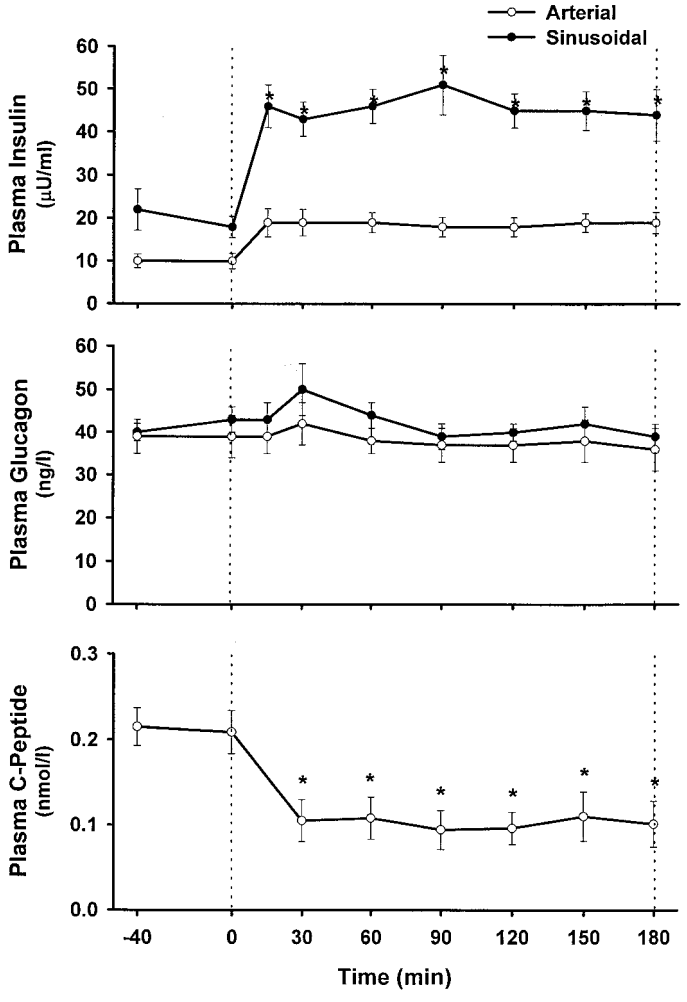

FIG. 2. Arterial and hepatic sinusoidal plasma insulin and glucagon and arterial C-peptide levels in 18-h–fasted conscious dogs during the basal period (−40–0 min) and during intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion (0–180 min) (mean ± SE, n = 8; *P < 0.05 vs. basal).

because R_a = whole-body gluconeogenesis + glycogenolysis, the glycogenolytic contribution to R_a can be estimated according to the formula:

$$\text{WB GLY} = R_a - \text{whole-body GNG}$$

where GLY is the glycogenolytic contribution to R_a.

Whereas the ^{14}C-PEP and AV difference methods estimate gluconeogenesis at the time of blood or tissue sampling, the estimates determined with the ^2H_2O method represent labeling of glucose in the blood formed before the time of blood collection. Based on glucose R_a, glucose mass and the half-life of glucose in blood, the blood samples taken over the last 30 min represent the C5:C2 ratio apparent during a large part of the preceding hyperinsulinemic period.

Statistical analysis. The data were analyzed for differences from baseline values. Statistical comparisons were calculated using one-way analysis of variance (Instat, GraphPad Software). The Student-Newman-Keuls multiple comparisons test was used post hoc when significant F ratios were obtained. When only two values were compared, a paired Student's t test was used. The level of significance was $P < 0.05$ (two-sided test).

RESULTS

The arterial plasma insulin level (Fig. 2) rose from an average of 10 ± 2 μU/ml during the basal period to 19 ± 3 μU/ml during the last 30 min of the study, whereas the liver sinusoidal insulin level rose from an average of 20 ± 4 to 45 ± 5 μU/ml ($P < 0.05$). The arterial and hepatic sinusoidal glucagon levels remained unchanged throughout the study (Fig. 2). The average C-peptide levels were 0.21 ± 0.02 nmol/l during the basal period and fell to 0.10 ± 0.02 nmol/l ($P < 0.05$) during insulin infusion (Fig. 2).

Euglycemia was maintained throughout the study (Fig. 3) as the result of infusion of glucose at an average rate of 1.8 ± 0.6 mg · kg^{-1} · min^{-1} during the hyperinsulinemic period. NHGO decreased rapidly from an average of 1.90 ± 0.13 mg · kg^{-1} · min^{-1} during the basal period to 1.20 ± 0.16 mg · kg^{-1} · min^{-1} at 15 min ($P < 0.05$) and to an average of 0.23 ± 0.16 mg · kg^{-1} · min^{-1} ($P < 0.05$) by the last 30 min (Fig. 3 and Table 1). HGU increased from 0.29 ± 0.09 to 0.64 ± 0.09 mg · kg^{-1} · min^{-1} ($P < 0.05$), HGR decreased from 2.19 ± 0.13 to 0.87 ± 0.15 mg · kg^{-1} · min^{-1} ($P < 0.05$), and R_a decreased from 2.30 ± 0.14 to 1.21 ± 0.15 mg · kg^{-1} · min^{-1} ($P < 0.05$) over the course of the experiment (Table 1).

Arterial blood lactate levels fell during the study from 694 ± 111 (basal period) to 504 ± 81 μmol/l (last 30 min), whereas net hepatic lactate balance switched from net output (0.94 ± 2.95 μmol · kg^{-1} · min^{-1}) to net uptake (3.08 ± 1.73 mg · kg^{-1} · min^{-1}) (Fig. 1). The arterial blood glycerol level decreased from 87 ± 12 to 72 ± 13 μmol/l, and the rate of net hepatic glycerol uptake dropped slightly from 1.45 ± 0.29 to 1.18 ± 0.24 μmol · kg^{-1} · min^{-1} (Table 2). The arterial plasma NEFA level dropped from 761 ± 250 to 487 ± 48 μmol/l and remained suppressed,

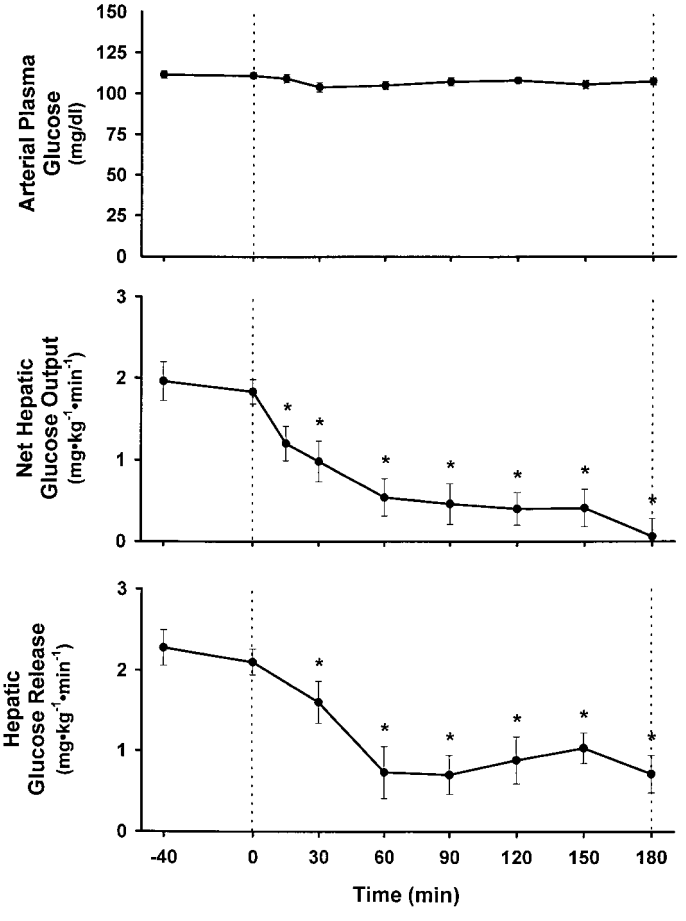

FIG. 3. Arterial plasma glucose level, NHGO and HGR in 18-h–fasted conscious dogs during the basal period (−40–0 min) and during intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion (0–180 min) (mean ± SE, n = 8; *P < 0.05 vs. basal).

TABLE 1
HGU (mg · kg^{-1} · min^{-1}), NHGO (mg · kg^{-1} · min^{-1}), HGR (mg · kg^{-1} · min^{-1}), and endogenous glucose production (R_a) and utilization (R_d) (mg · kg^{-1} · min^{-1}) in 18-h–fasted conscious dogs

Time (min)	−40	0	30	60	90	120	150	180
HGU	0.31 ± 0.13	0.27 ± 0.14	0.63 ± 0.06*	0.19 ± 0.12	0.23 ± 0.11	0.48 ± 0.15	0.62 ± 0.16	0.65 ± 0.12*
NHGO	1.96 ± 0.24	1.83 ± 0.15	0.98 ± 0.25*	0.54 ± 0.23*	0.46 ± 0.25*	0.40 ± 0.20*	0.41 ± 0.23*	0.06 ± 0.22*
HGR	2.28 ± 0.22	2.10 ± 0.16	1.60 ± 0.26*	0.73 ± 0.32*	0.70 ± 0.24*	0.88 ± 0.29*	1.03 ± 0.19*	0.71 ± 0.23*
R_a	2.61 ± 0.12	2.03 ± 0.16	1.91 ± 0.25	1.73 ± 0.25*	1.51 ± 0.22*	1.54 ± 0.22*	1.32 ± 0.20*	1.21 ± 0.20*
R_d	2.61 ± 0.16	2.78 ± 0.13	3.20 ± 0.32*	3.77 ± 0.55*	4.03 ± 0.54*	4.14 ± 0.55*	3.98 ± 0.51*	3.93 ± 0.51*

Data are means ± SE; $n = 8$; *$P < 0.05$ basal period (−40–0 min) vs. experimental period (intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion; 0–180 min).

whereas net hepatic NEFA uptake decreased from 3.25 ± 0.52 to 1.84 ± 0.25 μmol · kg^{-1} · min^{-1} and also remained suppressed ($P < 0.05$) (Table 2). The arterial β-hydroxybutyrate level decreased from 31 ± 12 to 16 ± 4 μmol/l and the rate of net hepatic β-hydroxybutyrate output fell from 1.3 ± 0.5 to 0.9 ± 0.2 μmol · kg^{-1} · min^{-1} (data not shown). Arterial gluconeogenic amino acid levels decreased modestly from 1,984 ± 94 to 1,631 ± 59 μmol/l ($P < 0.05$), whereas total net hepatic gluconeogenic amino acid uptake did not change appreciably (4.4 ± 1.1 and 4.2 ± 0.9 μmol · kg^{-1} · min^{-1} during the basal period and the last 30-min period, respectively) (Fig. 4).

During the basal period, the hepatic gluconeogenic flux rate, as estimated with the AV difference approach using the full group of dogs ($n = 8$), was 0.78 ± 0.10 mg · kg^{-1} · min^{-1} and failed to change in response to hyperinsulinemia (0.88 ± 0.13 mg · kg^{-1} · min^{-1}) (Fig. 5 and Table 3). The net hepatic glycogenolytic rate, on the other hand, decreased sharply from 1.72 ± 0.20 to −0.28 ± 0.15 mg · kg^{-1} · min^{-1} (basal period and last 30-min period, respectively, $P < 0.05$) (Fig. 5 and Table 3), which reflects a complete cessation of net glycogen breakdown.

In the subset of four dogs used to compare the gluconeogenic measurement techniques, neither the AV difference nor the ^{14}C-PEP method showed evidence supporting a significant change in gluconeogenic flux, although the ^{14}C-PEP method provided only an end point estimate. Hepatic gluconeogenic flux, as calculated by the AV difference approach for the subset, was 0.98 ± 0.13 mg · kg^{-1} · min^{-1} during the basal period and 1.14 ± 0.16 mg · kg^{-1} · min^{-1} during the last 30 min of hyperinsulinemia (Table 4). The ^{14}C-PEP method estimated the hepatic gluconeogenic flux rate to be 0.98 ± 0.18 mg · kg^{-1} · min^{-1} by the end of the study; a value similar to that previously determined using this technique (20) in untreated overnight-fasted dogs (0.95 ± 0.14, $n = 8$) (Table 4). Data used to make estimates using the subset ($n = 4$) and data taken from Goldstein et al. (20) ($n = 8$) are provided in Table 5. Specific activities for plasma [^{3}H] and [^{14}C]glucose, hepatic [^{3}H] and [^{14}C]uridinediphosphoglucose, and hepatic ^{14}C-PEP are provided in Table 6.

Whereas hyperinsulinemia did not affect hepatic gluconeogenic flux as determined with either method, the Hep GNG as determined with the ^{14}C-PEP method tended to decrease, although not significantly (26% decrease from 0.78 ± 0.06 to 0.58 ± 0.18 mg · kg^{-1} · min^{-1}) during the basal period and last 30-min period, respectively ($P = 0.21$) (Table 4). Despite a significant decrease in glucose R_a, the contribution of gluconeogenesis to R_a (whole-body gluconeogenesis) was not significantly reduced by insulin (16% decrease from 1.41 ± 0.17 mg · kg^{-1} · min^{-1} during the basal period to 1.18 ± 0.10 mg · kg^{-1} · min^{-1} during the last 30 min of hyperinsulinemia ($P = 0.32$) (Table 4) when measured with the ^2H_2O method.

Conversely, both the AV difference and the ^{14}C-PEP methods indicated that the rate of net hepatic glycogenolysis in the subset of four dogs was completely suppressed by the small rise in insulin (Table 4), decreasing by 1.56 and 1.68 mg · kg^{-1} · min^{-1}, respectively (Fig. 6). The Hep GLY also decreased (68% decrease from 1.51 ± 0.09 to 0.49 ± 0.10 mg · kg^{-1} · min^{-1} during the basal period and the last 30-min period, respectively, $P < 0.05$) (Table 4). During the hyperinsulinemic period, the rate of deposition of carbon into hepatic glycogen (glycogen synthesis), excluding glycogen cycling, was 0.70 ± 0.07 mg · kg^{-1} · min^{-1}. This value was not significantly different from the rate of breakdown of hepatic glycogen (glycogen degradation), which was 0.86 ± 0.13 mg · kg^{-1} · min^{-1} during this period (Table 4). The rates of deposition of gluconeogenic (RD_{GNG}) and glycogenolytic (RD_{GLY}) carbon in liver glycogen during the experimental period were 0.28 ± 0.01 and 0.53 ± 0.09 mg · kg^{-1} · min^{-1}, respectively. Whole-body glycogenolysis, as determined with the ^2H_2O method, also decreased significantly between the two periods (60% decrease from 1.00 ± 0.19 to 0.40 ± 0.07 mg · kg^{-1} · min^{-1}, $P < 0.05$) (Table 4).

TABLE 2
Arterial blood glycerol (μmol/l), net hepatic glycerol uptake (μmol · kg^{-1} · min^{-1}), arterial plasma NEFA (μmol/l), and net hepatic NEFA uptake (μmol · kg^{-1} · min^{-1}) in 18-h–fasted conscious dogs

Time (min)	−40	0	30	60	90	120	150	180
Glycerol	87 ± 12	86 ± 12	51 ± 6	67 ± 13	71 ± 14	65 ± 15	74 ± 13	69 ± 12
Net hepatic glycerol uptake	1.22 ± 0.30	1.67 ± 0.27	1.05 ± 0.20	1.18 ± 0.29	1.18 ± 0.25	1.25 ± 0.27	1.21 ± 0.24	1.14 ± 0.24
NEFA	750 ± 244	772 ± 258	483 ± 133	445 ± 48	477 ± 78	399 ± 104	507 ± 94	469 ± 66
Net hepatic NEFA uptake	2.98 ± 0.55	3.52 ± 0.97	1.94 ± 0.36*	1.91 ± 0.27*	2.22 ± 0.43	1.23 ± 0.16*	1.82 ± 0.38*	1.85 ± 0.38*

Data are means ± SE; $n = 8$; *$P < 0.05$ basal period (−40–0 min) vs. experimental period (intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion; 0–180 min).

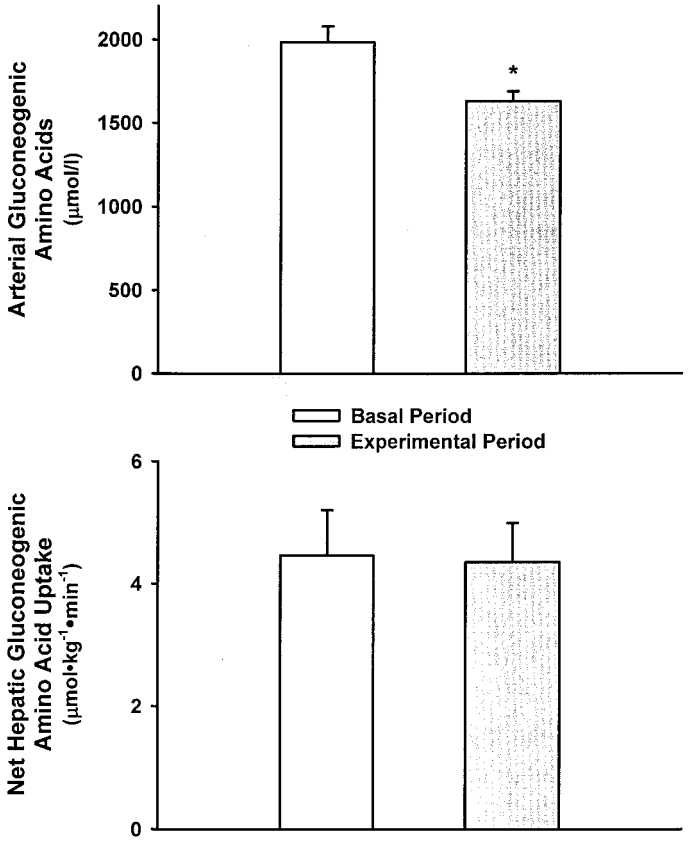

FIG. 4. Arterial blood gluconeogenic amino acid level (alanine, serine, threonine, glycine, glutamate, and glutamine) and net hepatic gluconeogenic amino acid uptake in 18-h–fasted conscious dogs during the basal period (−40–0 min) and during the last 30 min of the intraportal insulin-infusion period (150–180 min) (mean ± SE, $n = 8$, *$P < 0.05$ vs. basal).

DISCUSSION

Insulin is a potent regulator of HGP through both its direct hepatic and indirect peripheral effects. In the present study, we showed that a doubling of insulin throughout the body reduced net hepatic glucose output by inhibiting net hepatic glycogenolysis and diverting gluconeogenic carbon into glycogen. Each independent method of gluconeogenic measurement indicated that hepatic gluconeogenic flux (conversion of PEP into G6P) was not significantly altered by the increase in insulin.

The AV difference method indicated a nonsignificant increase (11%) in gluconeogenic flux over the 3-h hyperinsulinemic experimental period (0.78 ± 0.10 to 0.88 ± 0.13 $mg \cdot kg^{-1} \cdot min^{-1}$, $n = 8$). As fasting progresses, there is a slow increase in the relative contribution of gluconeogenesis to HGP, which probably explains this small rise. In earlier control studies (1) in 18-h fasted dogs in which basal levels of insulin and glucagon were kept constant, there was a nonsignificant increase in gluconeogenic flux of 0.11 $mg \cdot kg^{-1} \cdot min^{-1}$ (14%, $n = 10$) over the same period. Thus, we can conclude that the increase in insulin did not decrease gluconeogenic flux. Hepatic gluconeogenic flux determined with the ^{14}C-PEP method was 0.98 ± 0.18 $mg \cdot kg^{-1} \cdot min^{-1}$ during insulin infusion. Although this method only provides an end point measurement of gluconeogenic flux, basal gluconeogenic flux was estimated to be 0.95 ± 0.14 $mg \cdot kg^{-1} \cdot min^{-1}$ ($n = 8$) in untreated overnight-fasted dogs in earlier studies using the same approach (20), thus confirming that the small increase in insulin had no effect on gluconeogenic flux in the liver. It should be noted that our ability to detect small changes in gluconeogenic flux is limited by the variance inherent in the methods. With a 90% confidence level, our study had the power to detect a change ≥0.27 $mg \cdot kg^{-1} \cdot min^{-1}$ in gluconeogenic flux, had it occurred. Therefore, whereas it is possible that we missed a small inhibition of the gluconeogenic pathway, it is clear that insulin's major effect in the present study was on hepatic glycogenolysis and not on gluconeogenic flux (Fig. 5 and 6, Table 4).

Gluconeogenic flux was not inhibited, yet insulin tended to decrease hepatic (26%, $P = 0.21$) and whole-body gluconeogenesis (16%, $P = 0.32$) (i.e., the amount of gluconeogenically derived carbon released into the blood) (Table 4). This suggests that the insulin-induced decreases in HGP and in the amount of gluconeogenically derived glucose leaving the liver reflected a change in glycogen metabolism and not in overall gluconeogenic flux. Further evidence of redirection of gluconeogenic carbon to glycogen is provided by the hepatic tissue biopsied at the end of the experiment. Assuming a constant deposition during the hyperinsulinemic period, the rate of glycogen synthesis from gluconeogenic carbon accounted for the majority of the decrease in the release of gluconeogenically derived glucose. In overnight-fasted human subjects, Hellerstein et al. (25) reported that carbon derived from the gluconeogenic pathway was diverted into glycogen, and Chiasson

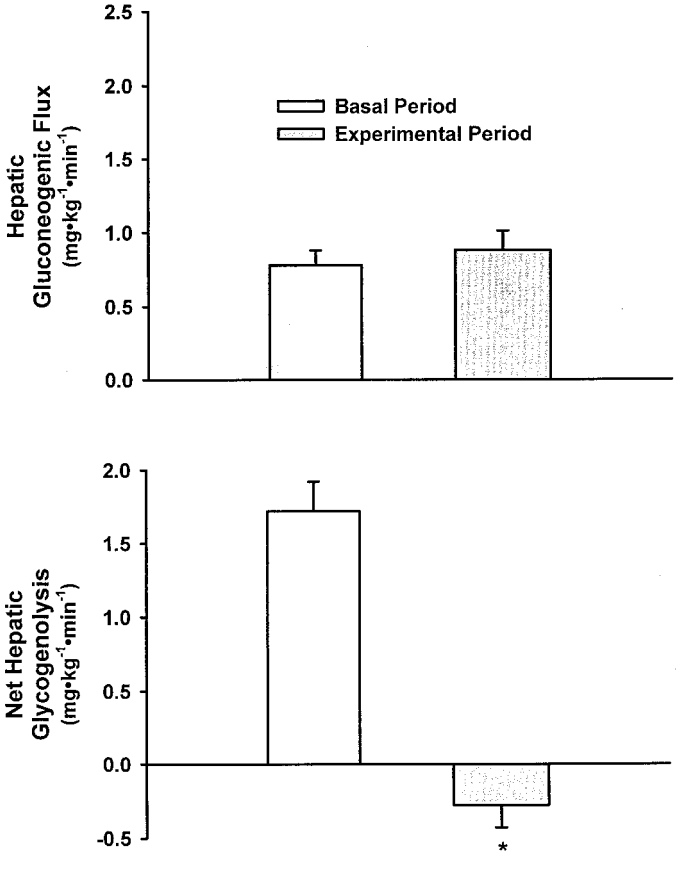

FIG. 5. Hepatic gluconeogenic flux and glycogenolytic rates ($mg \cdot kg^{-1} \cdot min^{-1}$) measured using the AV difference method in 18-h–fasted conscious dogs during the basal period (−40–0 min) and during intraportal insulin infusion (0–180 min) (mean ± SE, $n = 8$, *$P < 0.05$ vs. basal).

TABLE 3
NHGLY, NHGO, estimated hepatic oxidation (GO), NHLO, and gluconeogenic flux (GNG flux) (mg · kg^{-1} · min^{-1} in glucose equivalents) measured using the AV difference method in the full set of 18-h–fasted conscious dogs

	NHGLY	=	NHGO	+	GO	+	NHLO	−	GNG flux
Basal period	1.72 ± 0.20	=	1.90 ± 0.13	+	0.30	+	0.30 ± 0.11	−	0.78 ± 0.10
Hyperinsulinemic period	−0.28 ± 0.15*	=	0.23 ± 0.16*	+	0.30	+	0.06 ± 0.03	−	0.88 ± 0.13

Data are means ± SE; $n = 8$, *$P < 0.05$ basal period (−40–0 min) vs. last 30-min period (intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion; 0–180 min).

and associates (26,36) demonstrated that insulin treatment resulted in diversion of newly formed glucose into glycogen in people and dogs.

The ^2H_2O method estimates of gluconeogenesis are somewhat higher than those of the ^{14}C-PEP method (Table 4). This can, at least in part, be explained by the contribution of renal gluconeogenesis to R_a because the ^2H_2O method does not distinguish between sites of gluconeogenesis and therefore provides a whole-body estimate. Assuming that renal gluconeogenesis contributes ~12% to glucose production in the overnight-fasted dog (≅0.3 mg · kg^{-1} · min^{-1}) (27), half of the difference between the methods can be accounted for. The remaining 0.3 mg · kg^{-1} · min^{-1} is likely the result of the fact that the ^{14}C-PEP and ^2H_2O techniques incorporate independent methods for the assessment of glucose production (HGR and R_a, respectively). Therefore, any errors in these methods would contribute to differences in gluconeogenic estimates. This is clear from Table 1, in which the average difference between HGR and R_a, beyond the estimated 12% renal contribution, is 0.27 mg · kg^{-1} · min^{-1}. Although HGR and R_a are not statistically significantly different from each other, there is greater divergence during the early part of the experimental period, when non–steady-state issues would be more likely to contribute to an overestimation of R_a. Clearly, the two gluconeogenic methods are in close agreement; however, as shown by the similarity in the magnitude of decline in the gluconeogenic rate during the experimental period (decrease of 0.20 and 0.23 mg · kg^{-1} · min^{-1} as estimated with the ^{14}C-PEP and ^2H_2O methods, respectively), the two methods would be expected to yield similar glycogenolytic estimates because the liver is the only important source of glycogenolytic glucose production. Although the basal ^{14}C-PEP estimate taken from Goldstein et al. (20) is greater than the basal ^2H_2O estimate in the present study, the two methods are in close agreement in respect to the rate of glycogenolysis within the same group of animals at the end of the experimental period (0.49 and 0.40 mg · kg^{-1} · min^{-1}, respectively), and both methods indicate a similar decrease in glycogenolysis after insulin treatment (68 and 60%, respectively).

Contrary to the previous findings of this lab and others (28), it has been hypothesized that the primary means by which insulin maintains control over HGP is extrahepatic (indirect) in origin (29). In conjunction with earlier work by Sindelar et al. (1), which showed that a selective increase in portal insulin directly inhibited glycogenolysis, the present finding that a 25-μU/ml increase in liver sinusoidal insulin completely inhibits net hepatic glycogenolysis clearly demonstrates that liver glycogenolysis is exquisitely sensitive to even small increases in hepatic sinusoidal insulin and supports the conclusion that insulin acts directly on the liver. On the other hand, when Sindelar et al. (1) selectively raised the arterial insulin level by 14 μU/ml (i.e., no change in portal insulin level), a 24% decrease in gluconeogenic flux was brought about and only a slight decrease in net hepatic glycogenolysis occurred (11%) (1). The latter was caused by the insulinization of the liver sinusoids that resulted from the contribution of arterial blood to liver blood flow. In the present study, we predicted that our arterial insulin increase of 9 μU/ml would bring about approximately half of the gluconeogenic response seen by Sindelar et al (1) (~12% reduction). We, in fact, saw no decrease in gluconeogenic flux. This may, in part, be explained by the fact that lipolysis was only inhibited to one-third the extent seen by Sindelar et al. (1). In other studies, Sindelar et al. (1) showed that when plasma NEFA levels were prevented from decreasing during selective arterial hyperinsulinemia, over half of insulin's inhibition of NHGO was eliminated (30). As expected in the present study, arterial NEFA levels and net hepatic NEFA uptake decreased slightly in response to the increase in peripheral insulin level. However, unlike the study of Sindelar et al. (1), in

TABLE 4
Hepatic and whole body gluconeogenic GNG, glycogenolytic GLY, gluconeogenic-flux (GNG flux), and NHGLY rates (mg · kg^{-1} · min^{-1}) measured using the AV difference, ^{14}C-PEP, and ^2H_2O methods in four 18-h–fasted conscious dogs

	^{14}C-peptide (hepatic)		AV difference (hepatic)		^2H_2O (WB)	
	Basal period (−40 to 0 min)	Last 30-min period	Basal period (−40 to 0 min)	Last 30-min period	Basal period (−40 to 0 min)	Last 30-min period
GNG	0.78 ± 0.06*	0.58 ± 0.18			1.41 ± 0.13	1.18 ± 0.17
GLY	1.51 ± 0.09*	0.49 ± 0.10†			1.00 ± 0.19	0.40 ± 0.07†
GNG flux	0.95 ± 0.14*	0.98 ± 0.18	0.98 ± 0.13	1.14 ± 0.16		
NHGLY	1.59 ± 0.21	−0.08 ± 0.11†	1.31 ± 0.18	−0.25 ± 0.25*		
GLY SYN	NM	0.70 ± 0.07				
GLY DEG	NM	0.86 ± 0.13				

Data are means ± SE; $n = 4$. *Data taken from Goldstein et al. (20) ($n = 8$); NM, not measured; †$P < 0.05$ basal vs. last 30 min (intraportal 600 μU · kg^{-1} · min^{-1} insulin infusion; 0–180 min).

TABLE 5
NHGO, HGR, R_a, TGO, plasma hepatic GC (mg · kg^{-1} · min^{-1}), and fractional contributions of GNG, GLY, and plasma glucose (direct) to the G6P pool in the subset of 18-h–fasted conscious dogs during the last 30 min of intraportal insulin infusion (600 μU · kg^{-1} · min^{-1}, 150–180 min)

	Basal period		Last 30-min period
	Edgerton et al.	Goldstein et al. (20)	Edgerton et al.
NHGO	1.91 ± 0.13	1.99 ± 0.11*	0.59 ± 0.13
HGR	2.18 ± 0.18	2.29 ± 0.13*	1.07 ± 0.16
R_a	2.40 ± 0.17	2.49 ± 0.12*	1.58 ± 0.18
TGO		2.91 ± 0.14*	1.44 ± 0.48
GC		0.62 ± 0.07*	0.37 ± 0.22
GNG Fraction		0.27 ± 0.02*	0.41 ± 0.04
GLY Fraction		0.51 ± 0.02*	0.37 ± 0.05
Direct Fraction		0.22 ± 0.02*	0.22 ± 0.06

Data are means ± SE; $n = 4$. *Data taken from Goldstein et al. (20) ($n = 8$).

which higher peripheral insulin led to a greater decrease in NEFA and to a surge in net hepatic lactate production, the decrease in NEFA (from 761 to 487 μmol/l) in the present study apparently was not great enough to redirect G6P carbon to lactate. This is consistent with the observation by Sindelar et al. (1) that an initial drop in NEFA from 610 to 407 μmol/l did not result in an increase in NHLO, whereas a further decrease in NEFA to 240 μmol/l did result in an increase in NHLO (1). In addition, it is possible that a 12% change in gluconeogenic flux did occur, but we did not detect it.

Insulin can indirectly affect liver glucose production not only through an effect on fat but also through inhibition of glucagon secretion. Glucagon potently stimulates glycogenolysis; therefore, an insulin-mediated reduction in plasma glucagon would provide an indirect means of inhibiting HGP. The small increase in arterial insulin level that we induced, however, was not large enough to produce an effect on the α-cell; therefore, the plasma glucagon level did not decrease. C-peptide levels decreased by 50% in response to the exogenous insulin infusion, indicating that endogenous insulin secretion decreased by about half in response to hyperinsulinemia. This undoubtedly caused the insulin level in the vasculature of the pancreas to decrease rather than increase, as it would have if endogenous secretion had increased. Therefore, it is important to realize that had there been a true increase in insulin secretion, the insulin level in the local environment around the α-cell would have increased (rather than decreased) and might have caused a suppression of glucagon secretion. In the study by Lewis et al. (31), which used normal human subjects whose insulin secretion was stimulated by tolbutamide, it was estimated that a twofold increase in insulin secretion would decrease the plasma glucagon level by ~5–10 pg/ml. We estimate that this decrease would result in a 0.2–0.4 mg · kg^{-1} · min^{-1} decrease in HGP. Because glucagon increases glycogenolysis (32), this change would also have affected glycogen metabolism. Thus, any decrease in HGP caused by a decrease in glucagon would be small and redundant, considering the potent inhibitory effect of the increase in sinusoidal insulin on liver glycogen breakdown.

By the end of the experimental period, net hepatic glycogenolysis (as estimated with the ^{14}C-PEP and AV difference methods) was completely inhibited (Table 4). Independent evidence for the effect of insulin on the net process was obtained by using labeled-carbon deposition in glycogen. At the end of the experimental period, the difference between the rate of carbon being stored as glycogen (glycogen synthesis) (0.70 ± 0.07 mg · kg^{-1} · min^{-1}) and the rate of glycogen being broken down (glycogen degradation) (0.86 ± 0.13) was not significantly different from zero (Table 4). Although there was no net hepatic glycogenolytic breakdown at the end of this period, glycogenolysis was contributing 0.49 mg · kg^{-1} · min^{-1} to HGP (Table 4), indicating that glycogen cycling was occurring during the hyperinsulinemic state.

Given the marked sensitivity of glycogenolysis to insulin, it is clear that small increases in insulin secretion inhibit NHGO by limiting net glycogenolysis and directing gluconeogenic carbon to glycogen, whereas larger increases in insulin secretion (threefold increases or greater) produce additional direct effects on gluconeogenesis or hepatic glucose uptake. Insulin's effect on gluconeogenesis seems to occur mainly via the hormone's peripheral (fat and muscle) actions (1). When tracer methods are used to measure glucose production, insulin's effect on HGU cannot be seen, and the additional reduction in tracer-determined HGP as a result of higher levels of insulin would result only from gluconeogenic inhibition. The finding that insulin's peripheral effects appear to dominate the reduction of HGP provides an explanation for the conclusions of others (33–35). In those studies, peripheral insulin infusion was associated with elevated hepatic insulin concentrations that themselves resulted in a pronounced glycogenolytic inhibition. With glycogenolytic inhibition already substantial, the additional increases in sinusoidal insulin level that resulted from portal insulin infusion could not further inhibit glucose production (28). Thus, in those studies, the suppression of HGP correlated with the increasing peripheral insulin level and was probably due to an inhibition of gluconeogenesis, rather than a direct action of insulin on the liver (because glycogenolysis was most likely already completely inhibited).

These results raise the question of why the inhibitory effects of insulin on gluconeogenic flux are evident in vitro

TABLE 6
Plasma [^{3}H]- and [^{14}C]-glucose, hepatic [^{3}H]- and [^{14}C]-UDPG, and hepatic ^{14}C-PEP specific activities (dpm/mg) in the subset of 18-h–fasted conscious dogs during the last 30 min of intraportal insulin infusion 150–180 min

^{3}H-PG	^{14}C-PG	^{3}H-UDPG	^{14}C-UDPG	^{14}C-PEP
12,516 ± 1,395	7,699 ± 1,125	3,212 ± 554	16,291 ± 1,514	27,273 ± 2,844

Data are mean ± SE; $n = 4$.

but not in vivo. One explanation for the hormone's apparent lack of effect on gluconeogenic flux in vivo is a poor sensitivity of the process to insulin. In humans and dogs, Chiasson and associates (26,36) showed that an arterial plasma insulin level of 100 μU/ml suppressed HGP, in part by directing gluconeogenic carbon into glycogen (but not by decreasing gluconeogenic alanine flux), whereas an insulin level of 421 μU/ml (a value >20 times higher than in the current experiments) significantly inhibited gluconeogenic alanine flux. However, these results are complicated by the fact that concomitant with the increase in insulin, glucagon decreased by 33%. The latter may have accounted for the decrease in gluconeogenesis or may have made possible the suppressive effect of high insulin on the process. An apparent effect of insulin on gluconeogenesis was reported in several early studies (37,38) that used the isolated perfused rat liver model, but the insulin doses used were 300- to 400-fold higher than the dose administered in this experiment and at least 100-fold higher than the concentrations to which the liver would be exposed under physiological conditions. However, it should be noted that in rat hepatoma cells, physiological levels of insulin can inhibit transcription of PEP carboxykinase (PEPCK), which is a key gluconeogenic regulatory enzyme (39). Nevertheless, the physiological relevance of this finding is unclear.

Another possibility is that an effect of insulin on gluconeogenic flux may be masked by the hormone's overriding effect on glycogen breakdown. The decrease in G6P resulting from insulin's inhibitory effect on glycogen breakdown and flux through glycolysis might cause an upregulation of the gluconeogenic pathway, thus offsetting the direct inhibitory effect of insulin. In the perfused-liver studies cited above, Lombardo et al. (37) used glycogen-depleted livers from fasted diabetic rats, which may have increased the sensitivity of the gluconeogenic process to insulin and facilitated the detection of an inhibition of gluconeogenesis by the hormone. On the other hand, Jefferson et al. (38) demonstrated an insulin-induced reduction of incorporation of ^{14}C into glucose after infusion of [^{14}C]lactate in perfused livers from fed rats. It is not clear, however, whether insulin had a true inhibitory effect on gluconeogenic flux or whether the reduction of [^{14}C]glucose was the result of diversion of gluconeogenic carbon into glycogen.

Finally, it is also possible that the in vivo effects of physiological increments in insulin on hepatic gluconeogenic flux are only visible chronically, and that our 3-h experimental period was simply not long enough to see a change. Because of PEPCK's important role in controlling gluconeogenesis, the rate of change in the amount of PEPCK protein could be expected to have an effect on the overall gluconeogenic rate. Cell culture studies have demonstrated that PEPCK gene transcription is inhibited by insulin with a $t_{1/2}$ of 12–15 min and that PEPCK mRNA levels also decrease rapidly, with a $t_{1/2}$ of about 30 min (40). In vivo studies in the diabetic mouse and rat and in the nondiabetic dog confirm that pharmacological doses of insulin reduce hepatic PEPCK gene expression within several hours (41–43). In alloxan-treated hyperglycemic diabetic rats, PEPCK protein synthesis was shown to be inhibited by a pharmacological level of insulin with a half-life of 40–60 min (44). These data suggest that our 3-h hyperinsulinemic period should have been long enough to see a PEPCK-associated effect on gluconeogenesis.

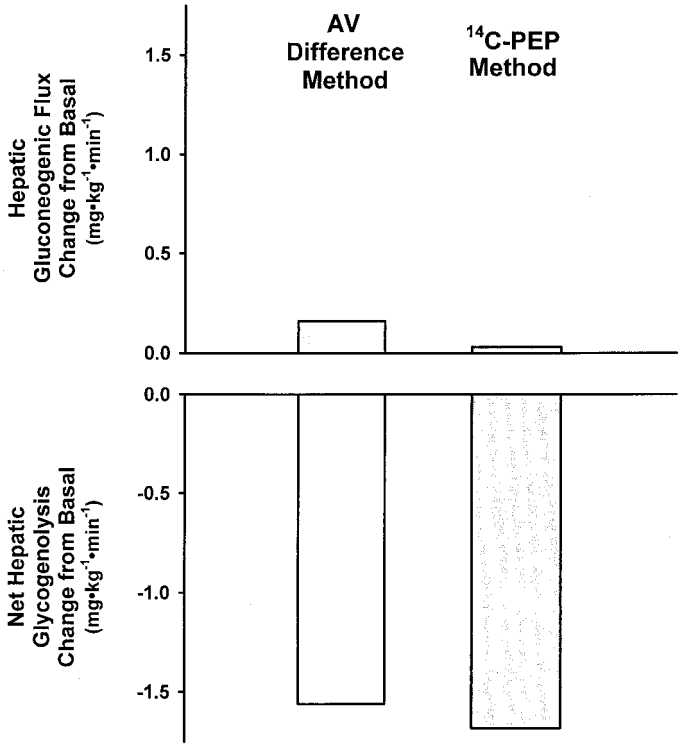

FIG. 6. Average values for the change from basal value in hepatic gluconeogenic flux and glycogenolytic rates (mg · kg^{-1} · min^{-1}) during the last 30 min of the intraportal insulin-infusion period (150–180 min) measured using the AV difference and ^{14}C-PEP methods in 18-h–fasted conscious dogs (mean ± SE, n = 4, *P < 0.05 vs. basal).

In summary, the gluconeogenic techniques are in agreement: a doubling of insulin levels in the periphery and liver had no effect on hepatic gluconeogenic flux, whereas it significantly decreased net hepatic glycogenolysis (~1.5 mg · kg^{-1} · min^{-1}). When the contribution of gluconeogenesis to glucose production (rather than gluconeogenic flux) is considered, both the ^{14}C-PEP and the $^{2}H_2O$ techniques suggested a slight decrease, but this was the result of a diversion of gluconeogenic carbon to glycogen. Thus, the results of the present study confirm that glycogen metabolism in the liver of the dog after an overnight fast is exquisitely sensitive to small changes in plasma insulin, whereas gluconeogenic flux is not. Furthermore, they suggest that small increases in insulin secretion exert acute action on the liver in the normal dog selectively through changes in glycogen metabolism.

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