Role of Portal Insulin Delivery in the Disappearance of Intravenous Glucose and Assessment of Insulin Sensitivity

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The contribution of portal insulin delivery to the disappearance of glucose administered intravenously was assessed in the present study. Paired insulin-modified intravenous glucose tolerance tests (IVGTTs) were performed in dogs in which insulin was administered into the portal vein or into a peripheral vein. Peripheral insulin levels were matched in the paired IVGTTs by adjusting the portal insulin dose in proportion to first-pass hepatic insulin extraction. Two sets of IVGTTs were performed. In the first set, hepatic insulin extraction was assumed to be 50% (insulin doses of 0.03 U/kg portal and 0.015 U/kg peripheral; n = 6); in the second set, the assumed extraction rate was reduced to 33% (0.0225 U/kg portal and 0.015 U/kg peripheral; n = 8). In the second set of experiments, a control "zero" dose (no insulin injection) was also performed. For these latter three IVGTTs, the exogenous glucose bolus was labeled with 3-[^{3}H]glucose (25 μCi) to separately assess insulin's effects on the rate of glucose disappearance (R_d) and endogenous glucose production (EGP). For the paired IVGTT based on 33% extraction, the area under the insulin curves after the portal insulin injection was within 2% of that observed with peripheral insulin injection (1,820 ± 711 vs. 1,791 ± 661 μU/ml min; P = 0.79). For these conditions, neither the glucose profiles nor the minimal model estimate of insulin sensitivity (S_I) was significantly influenced by the higher portal insulin delivery (S_I: 3.69 ± 0.56 vs. 3.35 ± 0.60 10^{-4} min^{-1} per μU/ml; portal vs. peripheral; P > 0.05). Analysis of the 3-[^{3}H]glucose tracer dynamics failed to reveal any differences in the portal versus peripheral insulin effect on glucose disappearance or production. We conclude that portal insulin delivery per se does not significantly affect insulin's ability to normalize plasma glucose during acute glucose challenges.
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AUC, area under the curve; EGP, endogenous glucose production; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; HGO, hepatic glucose output; IVGTT, intravenous glucose tolerance test; R_d, rate of glucose disappearance; S_G, glucose effectiveness; S_I, insulin sensitivity.

Insulin is secreted directly into the portal vein, from which a large fraction (~50%) is extracted on first pass (1). The large extraction results in higher insulin concentrations in the portal vein than those observed in the systemic (peripheral) circulation. The physiological significance of the high portal insulin concentration and high hepatic insulin extraction has remained elusive for many years (2–5). It was originally thought that portal insulin delivery was responsible for rapid suppression of hepatic glucose output (HGO); however, several studies have now shown that during euglycemic-hyperinsulinemic clamps, the suppression of endogenous glucose production (EGP; the sum of HGO and glucose production by the kidneys [6]) is primarily determined by peripheral insulin's effect to suppress free fatty acid (FFA) levels (1,7,8), and that higher portal insulin concentrations per se do not further suppress EGP (9,10). It has also been shown that during euglycemic glucose clamps, glucose uptake by the liver is not sensitive to insulin (11).

Unlike the euglycemic glucose clamp, which reflects changes in EGP and rate of glucose disappearance (R_d) in response to peripheral insulin delivery, the minimal model assessment of insulin sensitivity (S_I) was, until recently, based on changes in plasma glucose in response to portally delivered insulin (the endogenous insulin response to either glucose alone or to glucose and tolbutamide). It was reasoned that such a calculation would accurately reflect the effect of endogenous insulin appearance. Recently, we introduced an alternative procedure in which peripheral insulin administration replaced the tolbutamide bolus (12–14) so as to allow S_I to be estimated in subjects without an adequate β-cell response. With the insulin-modified protocol, the estimate of S_I is based on a mixture of endogenous–portally appearing and exogenous–peripherally appearing insulin. Saad et al. (15) have recently shown that the insulin-modified intravenous glucose tolerance test (IVGTT) yields estimates of S_I that are ~30% lower than those obtained with the tolbutamide-modified protocol, raising the possibility that portal insulin delivery per se is quantitatively important to net glucose disappearance during the IVGTT. This could be true if portal insulin delivery has a major effect to suppress EGP. Alternatively, differences in S_I between tolbutamide and insulin IVGTTs could be due to direct effects of tolbutamide itself or differences in the plasma insulin profiles.

In the present study, we examined directly whether the differences between protocols were due to an enhanced effect of portal versus peripheral insulin delivery on the net disappearance of plasma glucose. We performed paired IVGTTs in

which the insulin was given directly into the portal vein or, on a separate experimental day, into a peripheral vein (repeated measures). Peripheral insulin levels were matched by adjusting the portal dose to compensate for first-pass hepatic insulin extraction, which was initially assumed to be 50% but was later reduced to 33%. Paired IVGTTs based on 33% extraction were performed with the addition of 3-[^{3}H]glucose to separately assess insulin's effect to increase R_d from its effect to suppress EGP. That is, any differences in 3-[^{3}H]glucose kinetics between portally and peripherally administered insulin IVGTTs in which the peripheral insulin responses are matched but portal insulin is higher would indicate a portal effect of insulin to increase hepatic glucose uptake; differences in the time course of EGP, which can be estimated from the tracer dilution, would indicate a direct portal insulin effect to suppress HGO.

RESEARCH DESIGN AND METHODS

Animals. Experiments were conducted on nine conscious male mongrel dogs (mean body wt 35.6 ± 1.3 kg, range 20.4–31). Dogs were housed under controlled kennel conditions (12 h light:dark) in the University of Southern California (USC) Medical School vivarium and had free access to standard chow (25% protein, 9% fat, 49% carbohydrate, 17% fiber; Wayne Dog Chow; Alfred Mills, Chicago, IL) and tap water. Dogs were accustomed to laboratory procedures and were used for experiments only if judged to be in good health as determined by visual observation, weight stability, body temperature, and hematocrit. Seven to 10 days before the experiments, chronic catheters were surgically implanted. One catheter was inserted into the jugular vein and advanced to the right atrium for sampling of central venous blood; a second catheter was inserted into the femoral vein for injection of glucose and insulin (peripheral insulin-modified IVGTT only); and a third catheter was inserted into the portal vein for injection of insulin (portal insulin-modified IVGTT only). Catheters were led subcutaneously to the back of the neck and exteriorized. Catheters were flushed with heparinized saline (100 U/ml) at least twice a week, and the exteriorization site was cleaned with hydrogen peroxide (4%).

Experimental protocol. All IVGTTs were performed after an overnight fast (15 h). After a 30-min basal period (–30 to 0 min), glucose (300 mg/kg) was injected into the femoral vein; 20 min after the glucose bolus, insulin was injected over 1–2 min either into the portal vein (portal-insulin modified IVGTT) or, in a paired experiment, into the femoral vein (peripheral-insulin modified IVGTT). Portal and peripheral insulin-modified IVGTTs were initially paired assuming a first-pass hepatic extraction of 50% (portal insulin dose = 0.03 U/kg; peripheral insulin = 0.015 U/kg; n = 6). Based on these preliminary results, the portal insulin dose was reduced so as to allow for only 33% hepatic insulin extraction (portal insulin dose = 0.0225 U/kg; peripheral insulin dose = 0.015 U/kg; n = 8). Six of these latter animals also received a control, glucose-only protocol (no insulin injection). The glucose-only IVGTT and the paired IVGTTs based on 33% hepatic insulin extraction were all performed with the addition of 3-[^{3}H]glucose (25 µCi). Experiments in the same dog were separated by at least 1 week, and the order of experiments was randomized. For all IVGTTs, samples for the glucose, 3-[^{3}H]glucose, insulin, and FFA assays were taken at –20, –10, –2, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 26, 28, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min (32 samples). Samples were immediately centrifuged and the plasma separated into microcentrifuge tubes. Plasma was either kept on ice until processing that day or stored at –20°C. Insulin and [3-^{3}H]-D-glucose samples were collected in tubes containing sodium fluoride, heparin, and lithium (Brinkman, Westbury, NY). Samples for the FFA assay were collected with EDTA and Paraoxon (Sigma, St. Louis, MO) to suppress lipoprotein lipase (16). Samples for the glucagon assay (–20, –2, 3, 5, 8, 12, 16, 22, 26, 30, 40, 50, 70, 90, 120, 160) were collected with Trasylol (aprotinin; 25 µl/ml blood, FBA Pharmaceuticals, New York, NY). The experimental protocol was approved by the USC Institutional Animal Care and Use Committee.

Assays. Immediately after sampling, plasma glucose was measured with an autoanalyzer (YSI 2700; YSI, Yellow Springs, OH; using glucose oxidase). For measurement of [3-^{3}H]-D-glucose concentration, samples were deproteinized with zinc sulfate and barium hydroxide and the supernatant evaporated at 70°C, redissolved in water, and counted in Ready Safe scintillation fluid (Beckman, Fullerton, CA; Beckman liquid scintillation counter). Tracer infusates and plasma samples were processed and counted in an identical manner. Insulin was measured by an enzyme-linked immunosorbent assay (ELISA) originally developed for human serum or plasma by Novo Nordisk and adapted for dog plasma in our laboratory with the assistance of B. Dinesen (17). The ELISA is based on two murine monoclonal antibodies that bind to different epitopes on the insulin molecule; proinsulin is not bound by the antibodies. Materials for the insulin assay were provided by Novo Nordisk (Copenhagen, Denmark). Glucagon was also assayed using a kit obtained from Novo Nordisk. The glucagon kit incorporates a step for ethanolic extraction of plasma and uses antiserum K5563. FFAs were analyzed using a kit from Wako (NEFA C; Wako, Osaka, Japan) that uses the acylation of coenzyme-A.

Calculations. Minimal model estimates of S_I and glucose effectiveness (S_G) were obtained using the minimal-model identification software (MM3, R.N. Bergman). Percent differences in S_I were calculated as the change relative to the portal IVGTT estimate of S_I (i.e., 100 [$S_{Ipo} - S_{Ipe}$]/S_{Ipo}). Area under the insulin curve after the insulin injections (AUC_{19-180}) was calculated by trapezoidal integration. Two-compartment labeled-IVGTT analysis was performed using the model structure suggested by Caumo, Cobelli, and co-workers (18–21). The model, shown in Fig. 1, has been previously described; briefly, the model incorporates mixing (K_{12}, K_{21}) between the plasma compartment (q_1) and an interstitial fluid compartment (q_2). Insulin-independent fractional glucose uptake is assumed to occur from the plasma compartment alone ($K_p + F_{01}/V_1G_1$), of which one component (F_{01}; brain glucose uptake) is assumed to be saturated. Insulin-dependent fractional glucose uptake [$K_{02} + x(t)$] is assumed to occur from the remote compartment alone (compartment 2). The insulin-independent component is assumed to equal two-thirds of basal glucose uptake. The effect of insulin to increase fractional glucose uptake from compartment 2 [i.e., $x(t)$] is assumed to be proportional to insulin in the remote insulin pool (magnitude and time constant characterized by K_a and K_b, respectively).

Analysis of the two-compartment model was performed in two steps: first, the unknown model parameters were identified from the known model input (tracer bolus) and known model output (plasma 3-[^{3}H]glucose concentration); second, the unknown rate of appearance of endogenous glucose (EGP) was estimated from deconvolution using the known model parameters (identified in step 1) and the known plasma glucose concentration. Parameter identifications were performed using MLAB (Civilized Software, Bethesda, MD) implemented on an IBM-compatible computer. Identifications were obtained by weighted-nonlinear-least-squares (Marquardt-Levenburg algorithm) using inverse variance weights. Variance was estimated by first smoothing the data with a five-point moving average and then taking the squared deviation of the data from the smoothed curve. Deconvolution (step 2) was also performed with MLAB. For the deconvolution step, the plasma glucose response was separated into two components—the theoretical response to the exogenous glucose bolus (termed exogenous glucose in Refs. 18 and 21) and the calculated response to the change in EGP (difference between the theoretical response to the exogenous glucose bolus and the measured glucose response; termed endogenous glucose in Refs. 18 and 21). The calculated plasma glucose response to EGP was smoothed with the five-point moving average before deconvolution to reduce the sensitivity of the estimated EGP dynamics to noise.

Statistical analysis. Statistical analysis was performed with MLAB (paired t tests and power calculations) or with SAS (one-way repeated measures analysis of variance with Tukey's method for multiple comparison). For the unbalanced design in which eight animals received the paired portal/peripheral IVGTTs but only six of the animals had the matching glucose-only protocol, the repeated-measures analysis was performed on the subset of animals that had all three protocols.

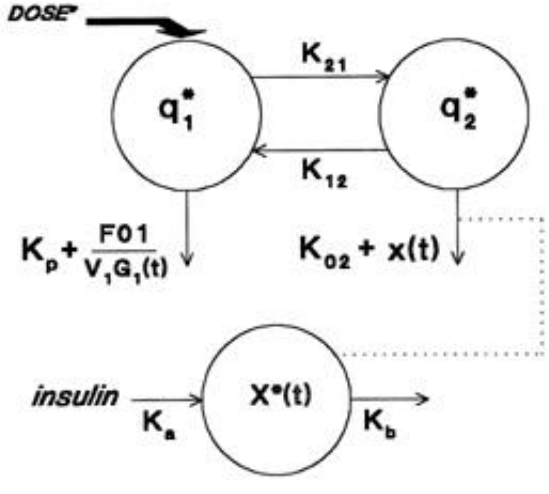

FIG. 1. Two-compartment labeled glucose model used to fit the 3-[^{3}H]glucose tracer data (Fig. 4) and estimate EGP (Fig. 5).

Paired t tests were performed for the eight animals that received the portal/peripheral IVGTTs based on 33% extraction (n = 8) or 50% extraction (n = 6). In one case, these two groups were pooled (n = 14) to increase power and to narrow the confidence interval.

RESULTS

Glucose and insulin time courses for the paired portal-peripheral IVGTTs assuming 50% hepatic insulin extraction (0.03 U/kg portal, 0.015 U/kg peripheral) are shown in Fig. 2. Glucose fell more rapidly, and to a lower nadir, after portal insulin injection (nadir glucose 62 ± 1.5 mg/dl at 40 min) than after peripheral insulin injection (nadir glucose 78 ± 9 mg/dl at 50 min). Despite both glucose and insulin being well matched before the 20 min insulin injection, the mean incremental insulin AUC after the insulin injection ($AUC_{19–180}$) was 28% higher with the portal insulin bolus than with the peripheral insulin bolus (2,469 ± 659 vs. 1,917 ± 602 μU/ml per min; P = 0.17). Although this difference did not achieve statistical significance, it suggested that with rapidly changing portal insulin concentrations the liver did not extract 50% of portally appearing insulin on first pass. Nonetheless, despite the tendency for a difference in plasma insulin, the different routes of insulin appearance injection sites and the different peripheral insulin concentrations did not significantly affect the minimal model estimates of either S_I (2.76 ± 0.52 vs. 2.64 ± 0.30 10^{-4} min^{-1} per μU/ml) or S_G (2.9 ± 0.24 vs. 2.6 ± 0.42 10^{-2} min^{-1}; portal vs. peripheral insulin-modified IVGTTs; P > 0.4 for both).

The similarity of S_I and S_G estimated from 0.03-portal and 0.015-peripheral insulin-modified IVGTTs suggests that portal delivery did not enhance insulin's ability to normalize plasma glucose. However, because the systemic insulin response curve was higher with portal insulin delivery, the possibility exists that there were both a portal-insulin effect to increase S_I and a saturation effect to decrease S_I, and that the two effects canceled. Thus, a second series of IVGTTs was performed in eight animals in which the portal insulin dose was reduced to 0.0225 U/kg and the peripheral insulin dose left at 0.015 U/kg; that is, the anticipated first-pass hepatic extraction of insulin was taken to be 33% rather then 50%. Reducing the portal insulin dose to 0.0225 U/kg (Fig. 3*B*) resulted in an average portal insulin response that was within 2% of the average peripheral insulin response ($AUC_{19–180}$; 1,820 ± 711 vs. 1,791 ± 661 μU/ml per min; P = 0.79). Matching the systemic insulin response resulted in glucose profiles that were also virtually superimposable (Fig. 3*A*) in regards to both the time to reach the nadir glucose (47.5 ± 5.1 vs. 44.4 ± 5.5 min; P = 0.55) and the nadir glucose concentration (66.6 ± 3 vs. 72.5 ± 3.7 mg/dl; P = 0.14), not different (portal versus peripheral for both comparisons). The magnitude and duration of the exogenous insulin effect, estimated as the difference between the glucose concentration in the "zero-dose" protocol and the glucose concentration during the insulin-modified protocol, were 25.6 mg/dl for the portal IVGTT and 24.0 mg/dl for the peripheral IVGTT. For both insulin-modified protocols, the peak effects occurred at 35 min and had dissipated by 70 min (compare with Fig. 3*A*).

Consistent with superimposable insulin and glucose profiles obtained with the paired 0.0225 U/kg portal and 0.015 U/kg peripheral insulin-modified tests is that neither S_G nor S_I was different (Table 1). Insulin sensitivity ranged from 1.71 to 5.5 for the portal protocol and from 1.6 to 5.7 min^{-1} per μU/ml for the peripheral protocol, with the average values not different (3.69 ± 0.56 vs. 3.35 ± 0.60, portal vs. peripheral; P = 0.51). Similarly, S_G ranged from 1.6 to 4.3 in the portal modified test and from 1.9 to 4.2 in the peripheral test; mean values were not different (3.11 ± 0.41 vs. 3.04 ± 0.41 $10^{-2}min^{-1}$; P = 0.81). Thus, the higher portal insulin delivery (0.0225 vs. 0.015 U/kg) did not affect the estimates of either S_I or S_G. This result suggests that portal insulin appearance per se does not influence the factors responsible for renormalization of glucose after glucose injection.

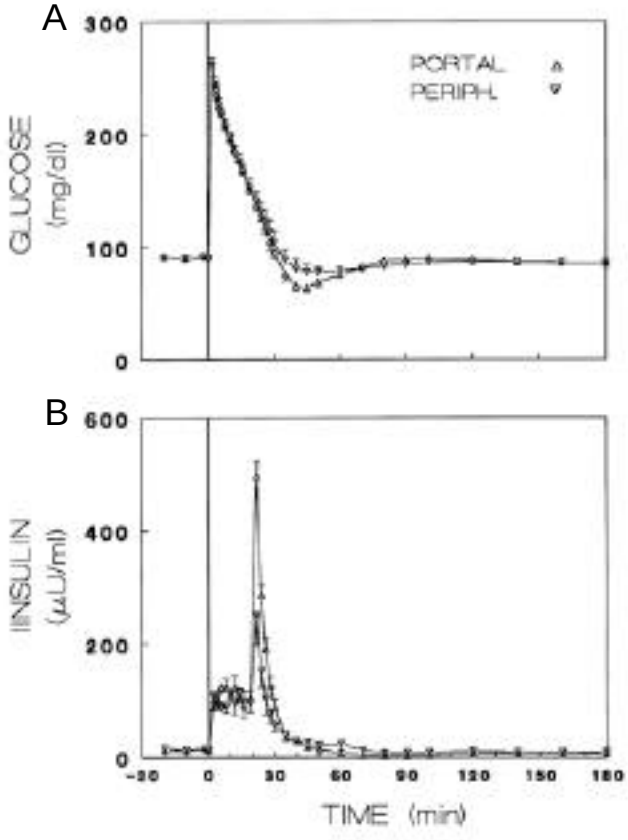

FIG. 2. Glucose (*A*) and insulin (*B*) profiles for either the portal (△) or peripheral (▽) insulin-modified IVGTTs. The portal insulin dose (0.03 U/kg) and peripheral (0.015 U/kg) insulin doses were based on an anticipated first pass hepatic extraction of 50% (i.e., [1-hepatic extraction rate] portal dose = peripheral dose).

The minimal model estimate of S_I combines the effect of insulin to enhance peripheral glucose uptake with the effect of insulin to decrease net hepatic glucose balance. Because it might be expected that portal insulin primarily affects the liver by increasing hepatic glucose uptake or decreasing HGO, we separately assessed insulin's effect on the rate of glucose appearance (EGP; hepatic plus kidney glucose output) from its effect on glucose disposal using tracer dilution. This analysis (see METHODS) was performed using the two-compartment model (Fig. 1) developed by Caumo and Cobelli (18). Model parameter estimates are given in Table 2, and the corresponding model fits to the tracer data are shown in Fig. 4. For the glucose-only protocol, the model fit the 3-[^{3}H]glucose data well with no apparent residuals (Fig. 4*D*). For the insulin-modified IVGTTs, the tracer dynamics in the portal and peripheral protocols were nearly identical (Fig. 4*D*), indicating that the portal insulin delivery did not increase total glucose uptake to an appreciable extent. Model analysis for the

TABLE 1
Minimal model estimates for the data shown in Fig. 2

Protocol	S_G [10^2 (min)$^{-1}$]	S_I [10^4(μU/ml)(min)$^{-1}$]
Portal	3.11 ± 0.412	3.69 ± 0.56
Peripheral	3.04 ± 0.412	3.35 ± 0.60

two insulin-modified protocols revealed a small (<5%) residual run immediately before and after the insulin injection (Fig. 4*C*), indicating that the model was unable to completely account for insulin's effect on glucose uptake. The residual runs were, however, similar for the two protocols, and model parameters were not different among the three protocols (Table 2). The similarity of the tracer dynamics and two-compartment model parameters indicates that the higher portal insulin did not significantly affect hepatic glucose uptake.

The estimated time courses for EGP are shown in Fig. 5. For the glucose-only protocol (Fig. 5*B*), basal EGP was estimated to be 3.21 ± 0.49 mg · min^{-1} · kg^{-1} and reached a minimum by ~20 min. A slight, apparently paradoxical increase in EGP was observed immediately after the glucose bolus; however, this increase may have been an artifact arising from experimental errors in estimating the magnitude of the cold glucose bolus (a slight underestimation of the amount of cold glucose injected results in a compensatory overestimation of the initial EGP) or an experimental error in estimating the amount of labeled glucose (an underestimation of the amount of tracer-glucose injected results in an overestimation of the glucose distribution space and, again, an overestimation of the initial EGP). Alternatively, early mixing dynamics could adversely affect the model estimation of V_1 (model error). In any case, for both the portal and peripheral protocols, EGP was reduced to approximately one-third of the basal values within 10 min of the exogenous insulin injection (i.e., by 30 min; Fig. 5*B*). Both the portal and peripheral insulin-modified IVGTTs indicated an increase, or overshoot, in EGP above basal starting at ~50 min (Fig. 5*B*), whereas no overshoot was observed in the glucose-only protocol (Fig. 5*A*). The "overshoot" of EGP observed in both insulin-modified protocols suggests that counterregulation occurred. That a counterregulatory response occurred is supported by the glucagon profile (Fig. 6*A*), which increased after the insulin injection (t = 20 min) and exhibited a similar overshoot to that observed in EGP. The sharp increase in glucagon can be compared with the profile observed in the glucose-only protocol in which the glucagon concentration slowly returns to basal. FFA levels for all three protocols reached a minimum by ~20 min (Fig. 6*B*), after which they also slowly returned to basal. The FFA dynamics were identical for all three protocols.

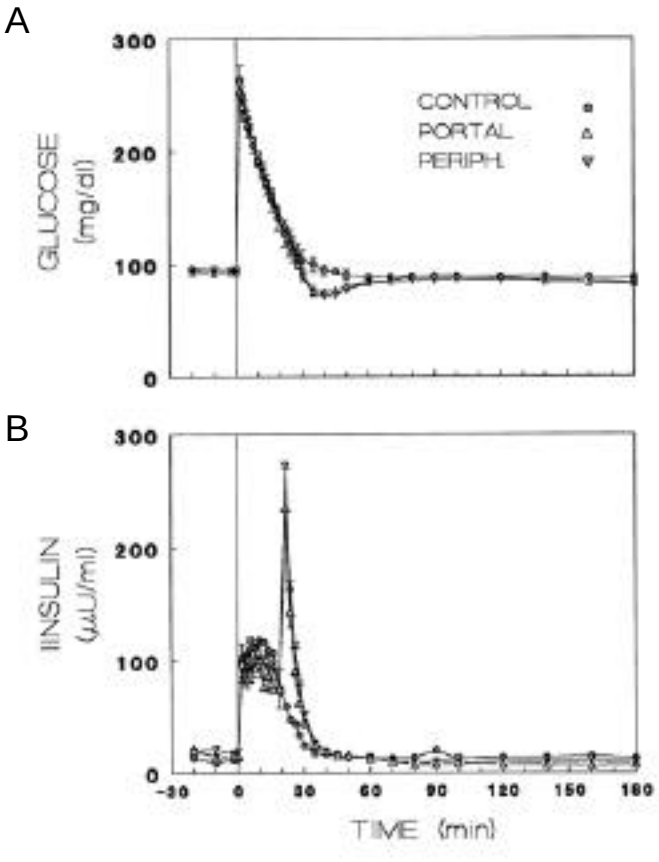

FIG. 3. Glucose (*A*) and insulin (*B*) profiles for either the portal (△) or peripheral (▽) insulin-modified IVGTTs. The portal (0.0225 U/kg) and peripheral (0.015 U/kg) insulin doses were based on an anticipated first-pass hepatic extraction rate of 33% (i.e., [1-hepatic extraction rate] portal dose = peripheral dose).

DISCUSSION

The minimal model estimate of S_I is based on computer analysis of the rate at which plasma glucose returns to basal following an intravenous glucose challenge (IVGTT). Insulin accelerates the rate at which glucose is normalized to basal by increasing the R_d and decreasing the rate of EGP. Insulin is normally secreted directly into the portal vein, from which ~50% is extracted on first pass by the liver. Although the appearance of insulin directly into the portal circulation dramatically elevates the portal insulin concentration and the amount of insulin extracted by the liver is substantial, a physiological effect on EGP has been controversial. In our laboratory, paired euglycemic-hyperinsulinemic clamps performed in the absence of glucagon with insulin infused directly into the portal vein or, in the paired experiment, with

TABLE 2
Two-compartment model parameters (see Fig. 1) for the data shown in Fig. 5

Protocol	V_1 (ml/kg)	K_p (min)$^{-1}$	K_{02} (min)$^{-1}$	K_{12} (min)$^{-1}$	K_{21} (min)$^{-1}$	K_a [(μU/ml)(min)$^{-1}$]	K_b (min)$^{-1}$
Control	157 ± 14	0.01034 ± 0.0023	0.0067 ± 0.0012	0.1473 ± 0.0413	0.1059 ± 0.025	2.65 ± 0.78	0.2275 ± 0.0584
Portal	158 ± 6	0.01129 ± 0.0013	0.0179 ± 0.0018	0.1824 ± 0.0227	0.1111 ± 0.0117	3.51 ± 0.75	0.2624 ± 0.0486
Peripheral	155 ± 5	0.01205 ± 0.0025	0.0102 ± 0.0016	0.2109 ± 0.0426	0.1144 ± 0.0200	2.81 ± 0.80	0.1954 ± 0.0039

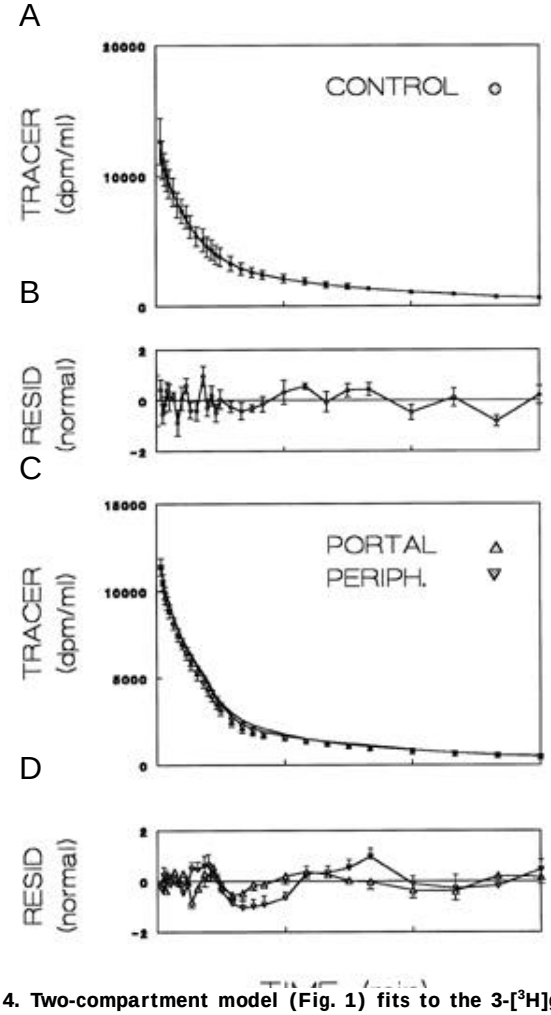

FIG. 4. Two-compartment model (Fig. 1) fits to the 3-[^{3}H]glucose tracer data during the glucose-only protocol (*A*); residual analysis for the glucose-only tracer fit (*B*); tracer concentrations for the portal (△) and peripheral (▽) insulin-modified IVGTTs assuming 33% hepatic extraction and model fits (—— ; *C*); and residual analysis for the portal and peripheral modified IVGTTs (*D*).

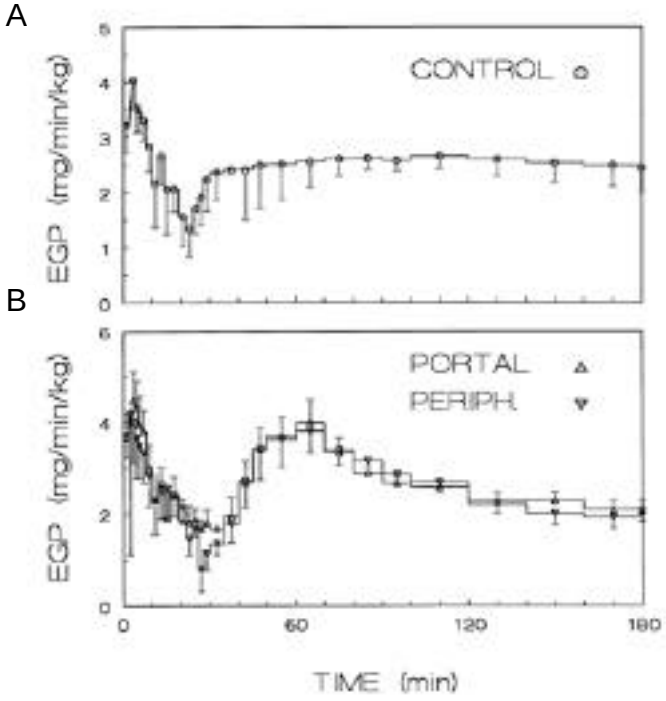

FIG. 5. Estimated EGP based on the tracer fits shown in Fig. 4: glucose-only protocol (*A*) and during the paired portal (△) and peripheral (▽) insulin-modified IVGTT protocols (*B*).

insulin infused into a peripheral vein at one-half the portal vein infusion rate failed to reveal an effect of portal insulin on either R_d or EGP (1). Although these results confirmed the earlier results obtained by Ader and Bergman (9) and Giacca et al. (10) in studies in which glucagon was present, Sindelar et al. (22) have provided evidence that if glucagon is maintained at basal, a selective increase in either portal or peripheral insulin will affect net hepatic glucose balance. Further, the Sindelar study (22) indicated that a selective increase in portal insulin resulted in a more rapid effect than a selective increase in peripheral insulin; the authors speculated that this was due to a direct effect of portal insulin on glycogenolysis. The contention that there is both a direct portal and an indirect peripheral action of insulin to suppress HGO is also supported by Lewis et al. (23) in humans. To further resolve this issue, Mittelman et al. (24) recently performed a comprehensive glucagon dose-response study in which paired euglycemic clamps were again performed using portal and peripherally administered insulin. In this latter study, the direct effect of portal insulin was evident at high glucagon concentrations, but the indirect effect remained dominant at all physiological doses.

The presence or absence of a portal insulin effect during euglycemic conditions cannot be used to infer what the effect would be during the rapidly changing portal hyperglycemic-hyperinsulinemic conditions during an IVGTT. In the present study, we reasoned that if a portal insulin effect exists, it should be reflected by minimal model analysis of the rate of glucose normalization during IVGTTs in which insulin is injected directly into the portal vein. That is, if portal insulin delivery is quantitatively important for normalizing glucose, then the minimal model estimate of S_I should be higher with an insulin-modified IVGTT in which the insulin is delivered portally rather than peripherally. That S_I was nearly identical in the paired portal and peripheral insulin-modified IVGTTs supports the contention that portal insulin appearance per se is relatively unimportant to the flux changes that occur after glucose injection (i.e., suppression of EGP and enhancement of glucose uptake by liver and/or periphery).

In the present study, the portal insulin bolus was adjusted to obtain equivalent peripheral insulin levels; to do this, the anticipated hepatic extraction rate had to be reduced from 50% (0.03 U/kg portal and 0.015 U/kg peripheral) to 33% (0.0225 U/kg portal and 0.015 U/kg peripheral). Paired IVGTTs in which the portal dose was twice the peripheral dose resulted in higher peripheral insulin levels with portal delivery than with peripheral delivery (Fig. 2*B*), whereas those based on the lower extraction were precisely matched (Fig. 3*B*; AUC_{19-180} after portal and peripheral insulin injection within 2%). Matching the peripheral insulin levels resulted in plasma glucose responses that were virtually identical despite the 50% higher dose of insulin injected in the portal-insulin modified protocol (Fig. 3*A*, △, ▽). For both sets of paired IVGTTs, S_I was not affected by portal insulin delivery; that is, in both the paired portal-peripheral IVGTTs based on 50% extraction and 33% extraction, the estimates of S_I were

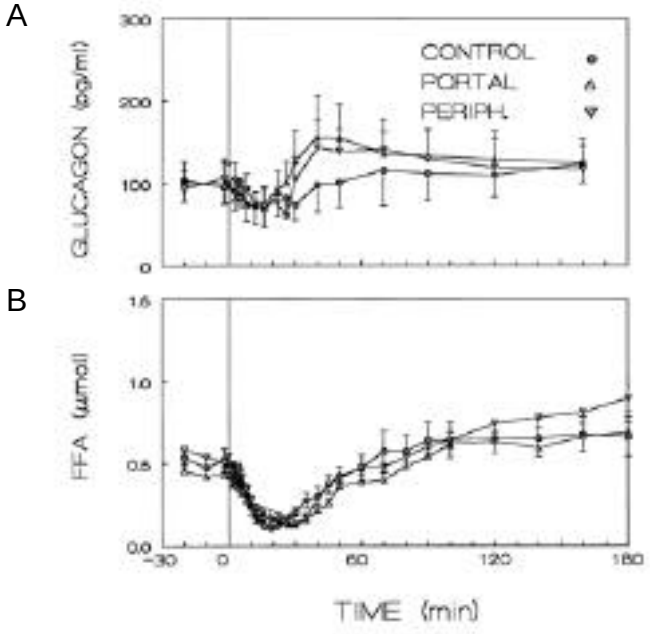

FIG. 6. Glucagon (*A*) and FFA (*B*) concentrations during the glucose-only (○) and paired portal (△) and peripheral (▽) insulin-modified IVGTTs.

similar (RESULTS and Table 1). Thus, in the present study, neither the magnitude of the insulin response nor the route of insulin delivery significantly affected S_I.

There are two important caveats to the conclusion that portal insulin delivery is unimportant for normalizing glucose after an intravenous challenge: *1*) the reduction in the portal dose may have reduced our power to discern a portal insulin effect; and *2*) the conclusion is based on comparing protocols in which the liver is preinsulinized with endogenous portal insulin for 20 min before the exogenous portal or peripheral insulin boluses. The first caveat relates to statistical power: with matched plasma insulin (paired IVGTTs based on 33% extraction), we failed to observe any difference in S_I with eight animals. However, mean S_I was 11% higher in the portal protocol, with a 95% CI between –9 and 31%. Combining the results of the paired IVGTTs based on 50% extraction (n = 6) with those based on 33% extraction (n = 8) reduced the magnitude of the effect to virtually zero (mean difference +1%; NS, P = 0.46) and narrowed the confidence interval to between –16 and 18%. Nonetheless, this CI still does not allow us to absolutely rule out a portal insulin effect. This is because the minimal model estimate of S_I includes both a peripheral and a hepatic component, with approximately two-thirds of S_I being due to the peripheral component and one-third being due to the hepatic component. Thus, in the present protocol, where we administered 50% more insulin portally than peripherally, one could argue that the net effect on S_I would only have been 16.7% even if the suppression of HGO had been in direct proportion to the increase in portal delivery. However, results from the two-compartment, labeled IVGTT analysis indicated that this was not the case; that is, EGP was not suppressed to a greater extent by the portal insulin bolus (Fig. 5), supporting the contention that there was no direct portal effect of insulin on HGO suppression (all the insulin effect was mediated via the peripheral insulin concentration). Tracer dynamics in the paired portal/peripheral IVGTTs were also superimposable (Fig. 4), and no differences were detected in the two-compartment model parameters (Table 2), indicating that insulin's effect to increase hepatic glucose uptake was also not enhanced with portal insulin delivery. Taken together, all the analyses show that portal insulin delivery did not enhance either hepatic glucose uptake or the suppression of EGP.

The second caveat relates to the experimental design: in the present design, portal insulin-modified IVGTTs were compared with peripheral insulin-modified IVGTTs. For this design, the first 20 min of both IVGTTs have identical portally delivered endogenous insulin, during which time the plasma glucose response returns to near basal conditions and EGP is already partially suppressed. Thus, although the exogenous insulin had a further glucose-lowering effect—glucose fell a further 25.6 mg/dl for the portal IVGTT and 24.0 mg/dl for the peripheral IVGTT compared with the IVGTT in which insulin was not administered (Fig. 3*A*)—this glucose-lowering effect was countered by a dramatic increase in glucagon (Fig. 6*A*) and EGP (Fig. 5*B*). Although it could be argued the glucagon response may have masked any portal insulin effect to further suppress EGP (or maintain suppression), this reasoning is opposite to what was observed in our clamp studies with glucagon replaced at various doses (24); that is, under clamp conditions, the direct effect of insulin is increased rather than masked with increasing glucagon. The data in the present study is, however, consistent with our previous studies using euglycemic-hyperglycemic clamps that have shown that the peripheral effect of insulin to suppress HGO is mediated via FFAs (1,7), in that FFA levels in the portal and peripheral insulin-modified IVGTTs were well matched (Fig. 6*B*).

In summary, the present study demonstrated that under the hyperglycemic-hyperinsulinemic conditions after an intravenous glucose challenge, portally and peripherally delivered insulin are equally effective in normalizing the glucose, provided the plasma insulin response is matched (conditions in which 50% more insulin is delivered portally than peripherally). Paired insulin-modified IVGTTs in which the peripheral insulin levels were matched resulted in virtually identical glucose response curves, despite the higher portal injection used to compensate for first-pass extraction (33% first-pass hepatic extraction). Minimal model analysis resulted in identical estimates of S_I for the paired IVGTTs, and the two-compartment model analysis indicated no difference in glucose uptake or suppression of EGP. We conclude that portal insulin delivery is unimportant for the normalization of plasma glucose after an intravenous glucose challenge.

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