Pattern of Postprandial Carbohydrate Metabolism and Effects of Portal and Peripheral Insulin Delivery

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The importance of portal insulin delivery in the regulation of postprandial carbohydrate metabolism is uncertain. To address this question, three groups of dogs were studied: one group in which pancreatic venous drainage was transected and reanastomosed (portal insulin delivery), one in which the pancreatic drainage was transected and anastomosed to the inferior vena cava (peripheral insulin delivery), and one that received only a sham operation. Plasma insulin was greater ($P < 0.05$) during peripheral insulin delivery than in either the portal or sham groups, respectively, before and after meal ingestion. On the other hand, C-peptide concentrations did not differ between groups, resulting in a higher ($P < 0.001$) insulin to C-peptide ratio in the peripheral group. This indicated that the hyperinsulinemia in the peripheral group was due to decreased insulin clearance rather than increased insulin secretion. Isotopically determined splanchnic uptake of ingested glucose, postprandial suppression of hepatic glucose release, incorporation of CO_2 into glucose (a qualitative measure of gluconeogenesis), and total-body glucose uptake were virtually identical in all groups. Similarly, plasma lipid, β-hydroxybutyrate, and lactate concentrations did not differ between groups. Our data indicate that, despite differences in systemic insulin concentration, portal and peripheral insulin delivery comparably regulate hepatic and extrahepatic carbohydrate metabolism after meal ingestion.
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C-peptide	1 nM = 3.02 ng/ml	Glucose	1 mM = 18 mg/dl
Glucagon	1 ng/L = 1 pg/ml	Insulin	1 pM = 0.167 μU/ml

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Determination of the optimal route of insulin delivery required to normalize postprandial carbohydrate metabolism is of physiological interest and, with the advent of human pancreas transplantation, is a pressing clinical question. In nondiabetic subjects, portal insulin concentrations are two- to threefold greater than those in the peripheral circulation (1). For this reason, the liver is exposed to substantially higher insulin concentrations than are peripheral tissues. Because the degree of hepatic insulinization is a determinant of rates of glycogenolysis, gluconeogenesis, and glycogen synthesis (2,3), it may have a major impact on the relative contribution of hepatic and extrahepatic tissues to postprandial carbohydrate tolerance. At least in theory, portal insulin delivery may preferentially regulate hepatic glucose metabolism, whereas systemic insulin delivery may predominantly stimulate glucose uptake by peripheral tissues. However, despite its intuitive appeal, previous investigations have failed to show a clear-cut advantage of portal over peripheral insulin delivery (4–23).

Our experiments were therefore undertaken to determine whether splanchnic uptake of ingested glucose, postprandial hepatic glucose release, CO_2 incorporation into glucose (a qualitative estimate of gluconeogenesis; 24–26), and total-body glucose uptake before and after meal ingestion differ when insulin is secreted into portal or peripheral venous circulation. To do so, animals were studied after transection and reanastomosis of the pancreatic venous drainage to the portal vein (portal insulin delivery) or after anastomosis of the pancreatic venous drainage to the inferior vena cava (peripheral insulin delivery). With this approach, we ensured that the pancreas remained innervated and capable of responding to moment-to-moment changes in arterial glucose concentration. This model also avoids the disruption of exocrine function noted with pancreatic autotransplantation (27). The results in both groups were compared with those in sham-operated animals.

RESEARCH DESIGN AND METHODS

Animals and surgery. Twenty-four healthy, adult, mongrel female dogs weighing 18–29 kg were anesthetized with pentobarbital sodium (32 mg/kg body wt i.v.) after an overnight fast. The dogs were randomly assigned to one of the following procedures.

To study peripheral pancreatic venous delivery, the superior pancreaticoduodenal vein (SPDV) was mobilized and transected from the portal vein and anastomosed end to side to the inferior vena cava in eight dogs as previously described (28). Venous tributaries between the pancreas and duodenum or spleen were transected and/or ligated. The gastroduodenal artery was left intact. All venous connections to the pancreas were therefore disconnected except for the SPDV. One animal died 3 mo postoperatively of anesthetic complications at the time of femoral artery catheter placement.

To study portal pancreatic venous delivery, eight animals received the same surgery as above, except the SPDV was anastomosed end to side to where it was transected from the portal vein.

As controls, we had a sham-operated group. Eight animals had a midline incision made from the processus xiphoideus to the symphysis pubica. The abdomen was explored, and only the intra-abdominal fat pad was removed.

After postoperative recovery, animals were fed once daily with 1400 g of standard dog chow (Country Prime, Mankato, MN); water intake was ad libitum.

At least 3 mo postoperatively, the dogs were anesthetized with pentobarbital sodium (32 mg/kg body wt i.v.) after an overnight fast, and a Silastic catheter (no. 602-205, Dow Corning, Midland, MI) was implanted in a femoral artery. The catheter was flushed with 2 ml heparinized saline (50 U/ml), ligated, and sutured subcutaneously. Meal studies were performed 72–96 h later.

Experimental procedures. At the time of the meal studies, all animals had hematocrits >35%, stable weights, normal stools, and good appetites and appeared in healthy condition. Studies were performed after an overnight fast with unanesthetized animals standing quietly in a sling. The arterial catheters were retrieved on the morning of study under local anesthesia (1% lidocaine [1 ml], Invenex, Chagrin Falls, OH) and were used for blood collection. Central venous access was obtained with a 16-gauge 24-inch Intracath (Deseret Medical, Sandy, UT) placed in a saphenous vein on the morning of study and used for isotopic infusion.

The mixed meal contained 472 kcal (45% carbohydrate, 40% fat, 16% protein), consisting of an omelette and jello (made with 50 g dextrose, Fisher, Fair Lawn, NJ) to which 100 μCi [2-^{3}H]glucose was added (all isotopes were from Du Pont-NEN, Boston, MA). All meals were consumed within 15 min.

Primed (~14 μCi) continuous (~0.14 μCi/min) infusion of [3-^{3}H]glucose mixed in 0.9% NaCl was begun 2 h before the mixed meal to allow for isotope equilibration. In addition, five peripheral, five portal, and three sham-operated animals also received a primed (~56 μCi) continuous (~0.56 μCi/min) intravenous infusion of $H^{14}CO_3$. Arterial blood samples for glucose (29) and concentrations of glucose (29), insulin (30), glucagon (31), C-peptide (32), lactate (33), free fatty acid (34), β-hydroxybutyrate (35), cholesterol (36,37), and triglycerides (36) were obtained at regular intervals. When $H^{14}CO_3$ was infused, breath was collected via a tight-fitting rubber glove placed over each animal's snout into 2 ml of a solution containing 0.5 mM hydroxide of Hyamine (Packard, Downer's Grove, IL) and 10 ml Aquasure liquid-scintillation cocktail (Du Pont-NEN) for measurement of breath $^{14}CO_2$ specific activity as previously described (38). After the study, the patency of the venous anastomosis was confirmed at autopsy in all animals except one animal in the peripheral group in which the anastomosis of the SPDV with the inferior vena cava was obstructed. The results from this animal were not included in analysis.

Analysis. [2-^{3}H]- and [3-^{3}H]glucose specific activity were determined as previously described (29) except that samples were counted in a Beckman scintillation counter and H numbers were used for quench correction. Overall, completion of detritiation of [2-^{3}H]glucose was 98.6 ± 0.1% (interassay coefficient of variation [C.V.] 0.3%, intra-assay C.V. 0.2%), whereas 99.7 ± 0.4% (interassay C.V. 2.1%, intra-assay C.V. 2.1%) of [3-^{3}H]glucose remained intact. [^{14}C]bicarbonate loss was documented to be essentially 100% after deproteinization and column chromatography.

Calculations. Rates of total glucose appearance and usage were calculated with the equations of Steele et al. (39) as modified by DeBodo et al. (40) with [3-^{3}H]glucose as the systemic tracer and [2-^{3}H]glucose as the ingested tracer (41,42). Splanchnic uptake of ingested glucose was calculated by subtracting the total mass of ingested glucose reaching the systemic circulation from the amount ingested. The percentage of glucose derived from bicarbonate was calculated by dividing plasma [^{14}C]glucose specific activity of the whole glucose molecule by breath $^{14}CO_2$ specific activity (43). Endogenous hepatic glucose release after meal ingestion was calculated by subtracting the systemic rate of appearance of the ingested glucose from the total systemic rate of glucose appearance (41,42). Integrated responses were calculated as the area under the curve from 0 to 360 min.

The data in the text and figures are presented as means ± SE. Postabsorptive values represent the mean of the values from −30 to 0 min. Statistical analyses were performed with analysis of variance with the Newman-Keuls test for multiple comparisons. $P < 0.05$ was considered to be statistically significant.

RESULTS

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Before meal ingestion, plasma glucose concentrations did not differ in the portal, peripheral, and sham-operated groups (Fig. 1; Table 1). After meal ingestion, the glycemic response was slightly but not significantly greater in the portal and peripheral than in the sham groups.

Both fasting and postprandial arterial plasma insulin concentrations were greater ($P < 0.05$) in the peripheral group than in either the portal or sham groups. Neither fasting nor postprandial C-peptide or glucagon concentrations differed among groups. However, both fasting and integrated postprandial insulin-to–C-peptide ratios were lower ($P < 0.001$)

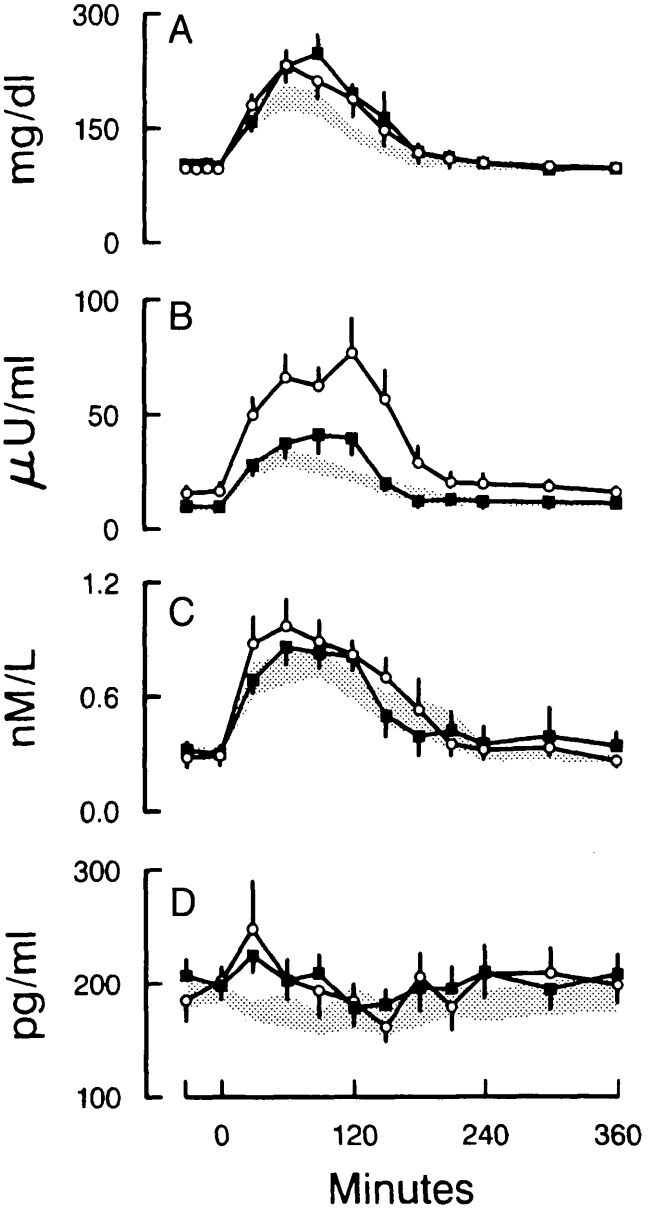

FIG. 1. Plasma glucose (*A*), insulin (*B*), C-peptide (*C*), and glucagon (*D*) concentrations. Mixed meal was ingested at time 0. ■, Portal infusion; ○, peripheral infusion. *Shaded area* (sham) are means ± SE.

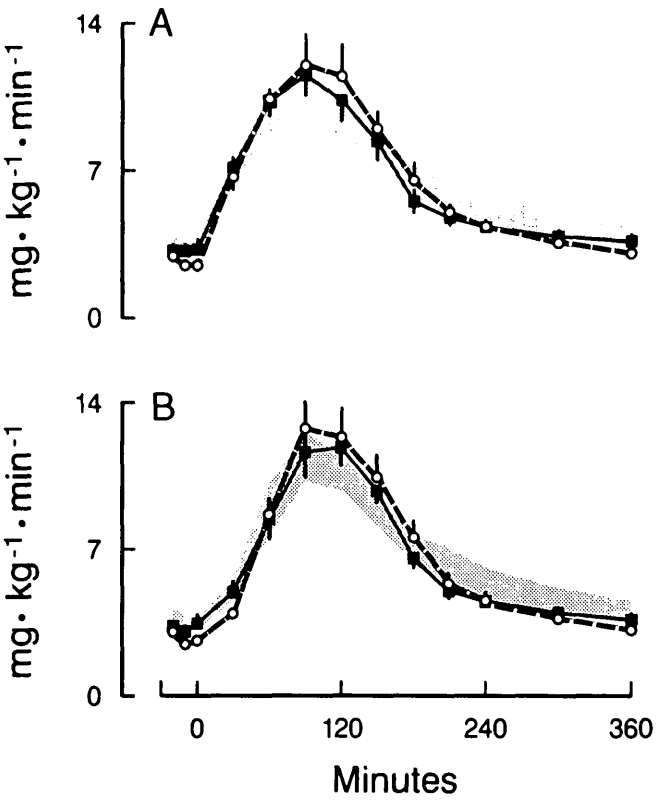

FIG. 2. Rates of glucose appearance (*A*) and disappearance (*B*). Mixed meal was ingested at time 0. ■, Portal infusion; ○, peripheral infusion. *Shaded area* (sham) are means ± SE.

in the peripheral than portal or sham groups, indicating decreased insulin clearance.

Glucose turnover. Rates of glucose appearance and disappearance did not differ among portal, peripheral, and sham groups either before or after meal ingestion (Fig. 2; Table 2). Appearance of ingested glucose, endogenous glucose release, and CO_2 incorporation into glucose also did not differ between groups before or after meal ingestion (Fig. 3; Table 2). Splanchnic uptake of ingested glucose (i.e., the difference between the amount of glucose ingested and the amount of ingested glucose reaching the systemic circulation) was similar in the peripheral, portal, and sham groups

TABLE 1
Hormone concentrations before and after mixed-meal ingestion

	Peripheral	Portal	Sham
Postabsorptive			
Glucose (mg/dl)	95 ± 3	98 ± 2	97 ± 2
Insulin (μl/ml)	15 ± 3	9 ± 1*	8 ± 1*
Glucagon (pg/ml)	192 ± 14	202 ± 13	193 ± 13
C-peptide (pM/ml)	0.28 ± 0.05	0.31 ± 0.04	0.31 ± 0.03
Insulin-to–C-peptide ratio	0.39 ± 0.04	0.22 ± 0.03*	0.19 ± 0.00*
Postprandial†			
Glucose ($g \cdot dl^{-1} \cdot 6\ h^{-1}$)	49.9 ± 3.5	48.7 ± 2.0	44.5 ± 2.0
Insulin ($mU \cdot ms^{-1} \cdot 6\ h^{-1}$)	13.2 ± 1.2	7.1 ± 1.1*	5.9 ± 0.6*
Glucagon ($ng \cdot ml^{-1} \cdot 6\ h^{-1}$)	71.5 ± 5.7	71.5 ± 5.6	63.5 ± 4.9
C-peptide ($pM \cdot ml^{-1} \cdot 6\ h^{-1}$)	200 ± 61	180 ± 29	173 ± 47
Insulin-to–C-peptide ratio	0.49 ± 0.04	0.28 ± 0.04*	0.24 ± 0.01*

*$P < 0.05$ vs. peripheral.
†Total response.

TABLE 2
Glucose flux rates before and after mixed-meal ingestion

	Peripheral	Portal	Sham
Postabsorptive (mg · kg^{-1} · min^{-1})			
Glucose appearance	2.6 ± 0.1	3.2 ± 0.3	3.4 ± 0.3
Glucose disappearance	2.6 ± 0.1	3.22 ± 0.30	3.5 ± 0.3
Ingested glucose appearance	0	0	0
Hepatic glucose release (end rate of appearance)	2.6 ± 0.1	3.1 ± 0.3	3.4 ± 0.3
CO_2 into glucose	0.8 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
Postprandial* (g · kg^{-1} · 6 h^{-1})			
Glucose appearance	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.2
Glucose disappearance	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.2
Ingested glucose appearance	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Hepatic glucose release	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
CO_2 into glucose	0.28 ± 0.03	0.32 ± 0.02	0.26 ± 0.02

There were no significant differences between any groups.
*Total response.

(740 ± 100 vs. 767 ± 119 vs. 666 ± 47 mg · kg^{-1} · 6 h^{-1}, respectively).

Lipids and metabolites. Fasting total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations did not differ among groups (Fig. 4; Table 3). Fasting lactate, free–fatty acid, and β-hydroxybutyrate concentrations also did not differ statistically among groups. After meal ingestion, lactate increased and free–fatty acid and β-hydroxybutyrate concentrations decreased comparably in all groups. The postprandial increase in lactate was slightly but not statistically greater in the portal than the peripheral or sham-operated groups.

DISCUSSION

To determine whether postprandial carbohydrate metabolism differs during peripheral and portal insulin delivery, we

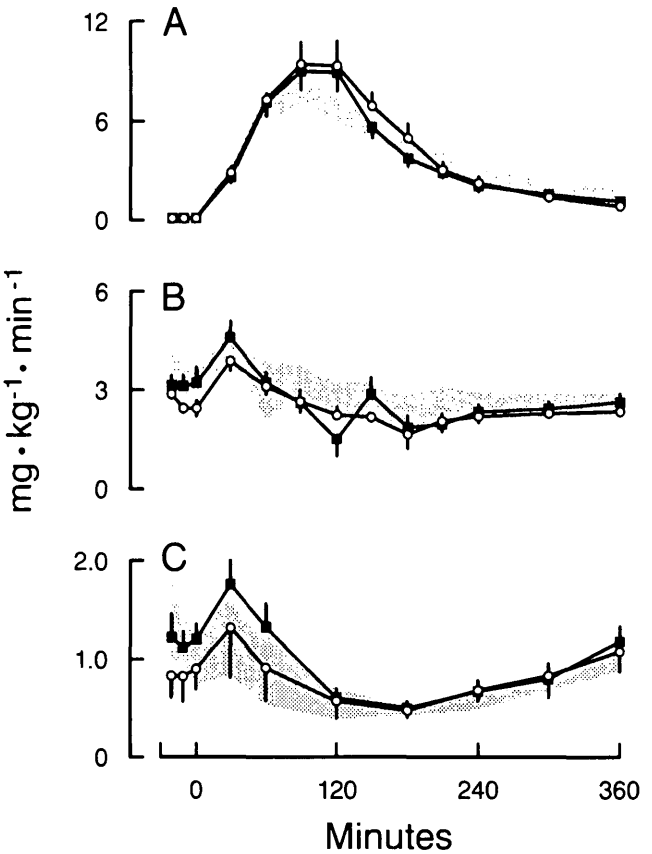

FIG. 3. Appearance of ingested glucose (*A*), endogenous glucose release (*B*), and CO_2 incorporation (*C*) into glucose. Mixed meal was ingested at time 0. ■, Portal infusion; ○, peripheral infusion. *Shaded area* (sham) are means ± SE.

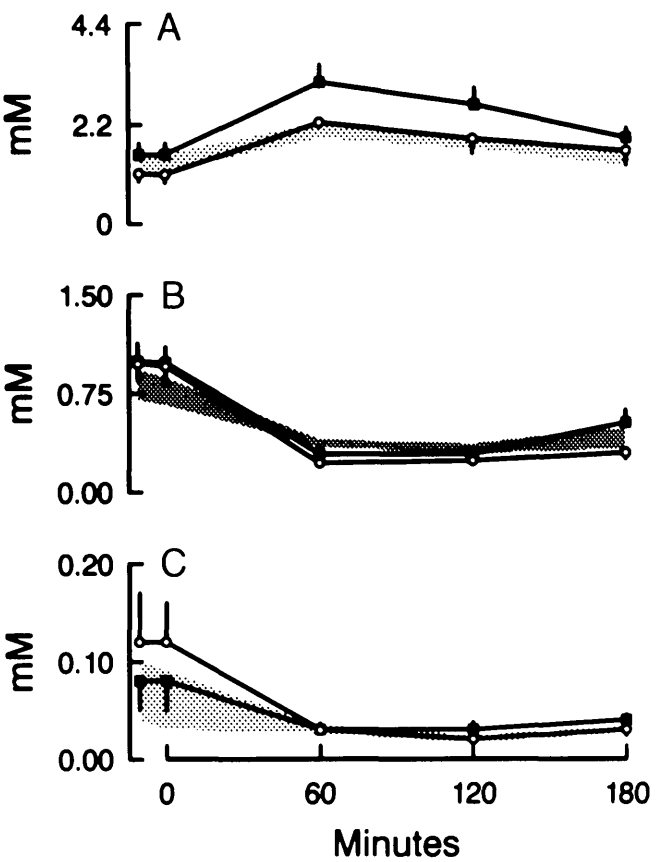

FIG. 4. Plasma lactate (*A*), free fatty acid (*B*), and β-hydroxybutyrate (*C*) concentrations. Mixed meal was ingested at time 0. ■, Portal infusion; ○, peripheral infusion. *Shaded area* (sham) are means ± SE.

TABLE 3
Fasting lipid concentrations

	Total cholesterol (mg/dl)	High-density lipoprotein cholesterol (mg/dl)	Triglycerides (mg/dl)
Portal	130 ± 9	94 ± 9	24 ± 2
Peripheral	130 ± 18	94 ± 13	19 ± 2
Sham	133 ± 29	94 ± 9	27 ± 2

There were no significant differences between any groups.

studied dogs that had had their pancreatic venous drainage severed and reconnected to the portal vein or anastomosed to the inferior vena cava. As anticipated, the animals with venous drainage to the systemic circulation had elevated plasma insulin concentrations both before and after meal ingestion (12,13,16,18–21). However, the postprandial pattern of carbohydrate metabolism was the same as that observed in either the portal or sham-operated group. Splanchnic uptake of ingested glucose, hepatic glucose release, and gluconeogenesis, as estimated by CO_2 incorporation into glucose, did not differ among groups.

Compensatory changes in hepatic glucose uptake and release are a major determinant if not the major determinant of postabsorptive and postprandial glucose concentrations (26,41–43). Therefore, it could be anticipated that secretion of insulin into the portal venous system would optimize control of these important metabolic processes. Portal insulin delivery would maximize the rapidity of change of insulin concentrations perfusing the liver while minimizing the insulin concentration to which peripheral tissues are exposed (1). The latter may be important if, as speculated, systemic hyperinsulinemia accelerates atherosclerosis (44) and induces insulin resistance (45). Despite the above line of reasoning, previous studies have failed to demonstrate a clear-cut advantage of portal over peripheral insulin delivery on carbohydrate metabolism. Portal insulin infusion, if anything, produces less hypoglycemia for a given dose than systemic insulin infusion (5,6,12,16). Peripheral insulin infusion with either a computer-controlled infusion device or a predetermined waveform results in the same glycemic excursion as portal insulin infusion (10,15,17,22). Similar to these studies, Kruszynska et al. (19) reported that with the exception of hepatic glycogen content, carbohydrate metabolism did not differ when islets were transplanted beneath the renal or splenic capsule in rats.

The importance of the route of insulin delivery may be diminished if, as has been previously suggested in the presence of adequate insulin concentrations (46–49), glucose rather than insulin is the primary regulator of glycogen synthesis, glycogenolysis, and gluconeogenesis. Heterogeneity of the contribution of the hepatic artery and portal vein to hepatocyte perfusion (50) and similarly of the glucagon-to-insulin ratio perfusing the liver when pancreatic venous effluent is drained in the portal and systemic circulation may further minimize differences in postprandial hepatic response.

Previous studies examining the effects of peripheral insulin delivery on gluconeogenesis have been inconsistent. It has previously been reported that the concentration of the gluconeogenic precursor alanine is lower during peripheral than portal insulin infusion (14). Subsequently, investigators have reported that alanine turnover either increased (51) or decreased (22), Cori cycle activity either decreased (52) or was normal (19), and alanine incorporation into glucose increased (51) during peripheral insulin infusion. Our experiments find no evidence that route of insulin delivery alters the gluconeogenic rate as measured by the rate of incorporation of CO_2. This, as do all in vivo methods for measurement of gluconeogenesis, has limitations (53,54). However, this technique has an advantage over other techniques in that it monitors the contribution of lactate, pyruvate, and alanine to new glucose synthesis (53,54). It also takes advantage of the rapid and extensive equilibration of bicarbonate with intrahepatic metabolic pools (55). Nevertheless, because incorporation of CO_2 into glucose provides a qualitative rather than quantitative measure of new glucose synthesis, our conclusions regarding gluconeogenesis must remain tentative.

Despite higher insulin concentrations in the peripheral group, glucose uptake did not differ before or after meal ingestion from that observed in the portal group. If anything, postabsorptive glucose uptake in the peripheral group was slightly (but not significantly) lower than in the other groups. Because glucose concentrations were comparable in both groups, these findings suggest insulin resistance. Most (56–58) but not all (59–61) studies demonstrated that short-term hyperinsulinemia is associated with insulin resistance. However, Wardzala et al. (61) pointed out that the effects of hyperinsulinemia on insulin action change with time. In contrast to previous short-term studies, these experiments assessed carbohydrate metabolism after >3 mo of pre- and postprandial hyperinsulinemia. Although these studies strongly suggest that the peripheral glucose uptake was not appropriate for the prevailing insulin concentrations, they do not necessarily establish hepatic insulin resistance. Because insulin was secreted directly into the inferior vena cava in the peripheral group, systemic and portal venous insulin concentrations presumably did not differ. Therefore, the two-fold greater systemic insulin concentrations in the peripheral group likely approximated the portal venous insulin concentrations present in the portal group. This could account for the similar splanchnic uptake of ingested glucose and similar postprandial suppression of hepatic glucose release and incorporation of CO_2 into glucose. In any case, these experiments strongly suggest that drainage of insulin into the systemic circulation, as is commonly performed during human pancreas transplantation, will result in inappropriately low insulin-stimulated glucose uptake.

The higher systemic insulin concentrations observed during peripheral insulin delivery could have been caused by increased insulin secretion and/or decreased insulin clearance. C-peptide concentrations were measured to distinguish between these two possibilities (62). C-peptide concentrations did not differ in the three groups before or after meal ingestion. On the other hand, the integrated insulin-to–C-peptide ratio was higher ($P < 0.001$) in the peripheral than the portal or sham groups. These data indicate that the increased circulating insulin concentrations in the peripheral group were due to decreased insulin clearance rather than increased insulin secretion (62). Decreased insulin clear-

ance presumably resulted from the fact that the insulin secreted directly into the vena cava bypassed initial hepatic extraction. The lack of change in insulin secretion when insulin is drained into the systemic concentration has implications for human pancreas transplantation. If insulin resistance due to systemic hyperinsulinemia had caused a compensatory increase in insulin secretion, then peripheral insulin drainage may have predisposed to β-cell exhaustion. On the other hand, because hyperinsulinemia resulted from decreased insulin clearance, demands on the transplanted β-cells presumably were not increased.

Various studies have suggested a relationship between hyperinsulinemia and atherogenesis (for review, see ref. 44). Acute changes in insulin can alter lipolysis, lipogenesis, and triglyceride metabolism (63–66). However, in our studies, the route of insulin delivery had no detectable effect on plasma lipids. These results are similar to those previously reported by Kruszynska et al. (67) in rats and Goriya et al. (68) in dogs. Of interest in our experiments, the plasma lactate concentrations tended to be higher in the portal group than the peripheral or sham-operated group. In contrast, Goriya et al. (68) observed increased lactate concentrations when insulin was infused into either the portal or the peripheral venous system. However, it should be noted that the 7-h square wave employed by Goriya et al. resulted in systemic hyperinsulinemia regardless of whether insulin was infused into the portal or peripheral venous system. It seems unlikely that the slightly higher lactate concentrations observed in our portal group were due to the route of insulin delivery, because the sham group also secreted insulin into the portal vein. However, because the apparent difference in lactate concentration between groups did not reach statistical significance, we cannot exclude chance alone. In any case, our data and those of Kruszynska et al. (58) suggest that portal and peripheral insulin delivery have similar effects on lipid and carbohydrate metabolism.

In summary, this study demonstrates that the pattern of pre- and postprandial carbohydrate metabolism is similar during portal and peripheral insulin delivery. Splanchnic uptake of ingested glucose, postprandial suppression of hepatic glucose release, incorporation of CO_2 into glucose, and total-body glucose uptake were virtually identical. Because the pancreases were left in situ with innervation intact, our experiments were able to directly address the effects of peripheral versus portal venous drainage. However, it remains to be determined whether these results pertain to the denervated pancreas used for transplantation. Furthermore, our experiments only examined carbohydrate turnover; although the route of delivery had no effect on plasma lipid concentrations, our experiments did not assess the effects of portal versus peripheral venous drainage on amino acid or fat turnover or on the potential deleterious effects of long-term hyperinsulinemia on atherogenesis. Such data will be required before the risk-to-benefit ratio of pancreas transplantation procedures designed to achieve portal insulin drainage can be properly assessed.

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