Suppression of Endogenous Glucose Production by Mild Hyperinsulinemia During Exercise Is Determined Predominantly by Portal Venous Insulin

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Hyperinsulinemia during exercise in people with diabetes requiring exogenous insulin is a major clinical problem. The aim of this study was to assess the significance of portal vein versus arterial insulin to hepatic effects of hyperinsulinemia during exercise. Dogs had sampling (artery, portal vein, and hepatic vein) and infusion (vena cava and portal vein) catheters and flow probes (hepatic artery and portal vein) implanted >16 days before a study. Protocols consisted of equilibration (−130 to −30 min), basal (−30 to 0 min), and treadmill exercise (0–150 min) periods. Somatostatin was infused and glucagon and insulin were replaced in the portal vein to achieve basal arterial and portal vein levels at rest and simulated levels during the first 60 min of exercise. From 60 to 150 min of exercise, the simulated insulin infusion was sustained (C; $n = 7$), modified to selectively create a physiologic increment in arterial insulin (Pe; $n = 7$), or altered to increase arterial insulin as in Pe but with a concomitant increase in portal insulin (PePo; $n = 7$). Euglycemic clamps were performed in all studies. Portal and arterial insulin were 15 ± 2 and 4 ± 1 μU/ml (mean ± SE of all groups), respectively, at $t = 60$ min in all groups. Insulin levels were unchanged for the remainder of the exercise period in C. Arterial insulin was increased from 3 ± 1 to 14 ± 2 μU/ml, whereas portal insulin did not change in Pe after $t = 60$ min. Arterial insulin was increased from 3 ± 1 to 15 ± 2 μU/ml, and portal insulin was increased from 16 ± 3 to 33 ± 3 μU/ml in PePo after $t = 60$ min. Endogenous glucose production (R_a) rose similarly from basal during the first 60 min of exercise in all groups (mean ± SE of all groups was from 2.2 ± 0.1 to 6.8 ± 0.5 mg · kg^{-1} · min^{-1}). The increase in R_a was sustained for the remainder of the exercise period in C. R_a was suppressed by ~40%, but only after 60 min of hyperinsulinemia, and by ~20% after 90 min of hyperinsulinemia in Pe. In contrast, the addition of portal venous hyperinsulinemia caused ~90% suppression of R_a within 20 min and for the remainder of the experiment in PePo. Measurements of net hepatic glucose output were similar to R_a responses in all groups. Arterial free fatty acids (FFAs), a stimulus of R_a, were increased to 1,255 ± 258 μmol/l in C but were only 459 ± 67 and 312 ± 42 μmol/l in Pe and PePo, respectively, by 150 min of exercise. Thus, during exercise, the exquisite sensitivity of R_a to hyperinsulinemia is due entirely to portal venous hyperinsulinemia during the first 60 min, after which peripheral hyperinsulinemia may control ~20–40%, possibly as a result of inhibition of the exercise-induced increase in FFA. *Diabetes* 53:285–293, 2004

In people with insulin- and non–insulin-dependent diabetes, hyperinsulinemia associated with physical activity is an important cause of hypoglycemia (1). This is a problem that has become even more significant in recent years as aggressive insulin therapy and intensive metabolic control are more actively encouraged (2) along with the increase in use of sulfonylureas, agents that stimulate β-cell insulin secretion. The various sites and mechanisms of hyperinsulinemic modulations of glycemia and the body's ability to compensate for these are not completely understood.

Since Prager et al. (3) put forth the notion that insulin regulates endogenous glucose production (R_a) via extrahepatic effects, several groups have attempted to further elucidate the direct and indirect effects of insulin on R_a in both normal (4–14) and diabetic (15–18) humans and dogs. Ader and Bergman (8) concluded by comparison of insulin dose-response experiments in conscious dogs that were given insulin either intraportally or systemically at half the intraportal rate (assuming 50% hepatic insulin extraction) that the peripheral or indirect effects of insulin dominate suppression of R_a. Subsequent studies from these investigators suggested a link between insulin-induced suppression of free fatty acid (FFA) levels and suppression of R_a (9,10). Sindelar et al. (12) found that insulin's direct inhibitory effect (suppression of glycogenolysis) on R_a was more rapid. The indirect effect was equally potent to the direct effect and corresponded to suppression of hepatic FFA and gluconeogenic (GNG) precursor delivery and uptake (13).

Giacca et al. (16) investigated insulin's direct and indirect effects in suppressing R_a in the depancreatized dog. They found that during poor glycemic control, peripheral insulin suppressed R_a more than portal insulin in this model of type 1 diabetes. They later found that when glucagon was replaced in depancreatized dogs to mimic levels actually seen in poorly controlled diabetes, R_a was

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AUC, area under the curve; FFA, free fatty acid; GNG, gluconeogenic; HPLC, high-performance liquid chromatography; NHGO, net hepatic output of glucose.

dominated by portal vein insulin (17). It was concluded from these studies that the main direct hepatic effect of insulin is to counteract the effect of glucagon on glycogenolysis. This group recently showed that insulin inhibits R_a by a direct, hepatic effect in their type 1 diabetes model under euglycemic conditions as well (18). These data have considerable relevance because people with diabetes strive to maintain euglycemia via intensive metabolic control, resulting in increased incidence of hypoglycemia.

The role of increments in portal and peripheral venous insulin will vary based on at least two factors: metabolic state and glucagon levels. With regard to the first, it has been proposed that an increment in peripheral insulin increases in importance when muscle and adipose tissue play a greater role in supporting GNG rate, such as in uncontrolled diabetes, starvation, and exercise (16). With regard to the second factor, it has been suggested that portal vein insulin is more effective in suppressing R_a when glucagon levels are increased (7,11). This is a consideration in previous studies in which glucagon was replaced at 10 times basal levels (8) or not at all (9,10). Exercise gradually increases the GNG rate and while it increases arterial glucagon gradually, portal vein glucagon increases with much faster kinetics (19). The augmentation of peripheral insulin sensitivity by exercise may also influence the hepatic availability of FFAs and GNG precursors (20). Because these factors are modified by exercise, it is possible that the relative importance of increments in peripheral and portal vein insulin in suppression of the exercise-induced increase in R_a may be different. The aim of the present study was to investigate the contributions of portal vein versus arterial hyperinsulinemia in mediating the suppression of R_a during exercise.

RESEARCH DESIGN AND METHODS

Experiments were performed on a total of 21 overnight-fasted mongrel dogs (mean wt 24.5 ± 0.4 kg) of either sex that had been fed a standard diet (Pedigree beef dinner and Wayne Lab Blox 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry weight). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the Vanderbilt University Animal Care Subcommittee approved the protocols. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg atropine and 15 mg/kg pentobarbital sodium presurgery and 1.0% isoflurane inhalation anesthetic during surgery). Catheters were inserted into the portal vein and hepatic vein for blood sampling purposes. An incision in the neck region allowed the isolation of the carotid artery into which a silastic catheter (0.04 inches inner diameter) was inserted and advanced to the aortic arch for sampling and hemodynamic measurements during experiments. Silastic catheters (0.03 inches inner diameter) were inserted into the vena cava for infusion purposes. Last, a silastic catheter (0.03 inches inner diameter) was inserted into the splenic vein and positioned so that the catheter tip rested just beyond the point where the splenic and portal veins coalesce. This catheter was used for the intraportal infusions of glucagon and insulin. Catheters were filled with heparinized saline and the free ends knotted. Ultrasonic transit time flow probes were fitted and secured to the portal vein and hepatic artery (Transonic Systems, Ithaca, NY). The knotted catheter ends and flow probe leads were stored in a subcutaneous pocket in the abdominal region (except for the carotid artery catheter, which was stored in a pocket under the skin of the neck), so that the complete closure of the skin incisions was possible.

Beginning 7 days after surgery, dogs were acclimatized to running on a motorized treadmill. Blood samples were drawn 3 days before the experiment to determine the leukocyte count and the hematocrit of the animal. Only animals with *1*) a leukocyte count $<18{,}000/mm^3$, *2*) a hematocrit >36%, *3*) a good appetite (consumption of daily food ration), and *4*) normal stools were used.

All studies were conducted in dogs after an 18-h fast. The free catheter ends and flow probe leads were accessed through small skin incisions made under local anesthesia (2% lidocaine; Astra Pharmaceutical Products, Worcester, MA) in the abdominal and neck regions on the morning of the experiment. The contents of the catheters were then aspirated and flushed with saline. The exposed catheters were connected to silastic tubing, which was secured to the back of the dog with quick-drying glue.

Experimental procedures. Experiments consisted of a tracer equilibration period (−130 to −30 min), a basal period (−30 to 0 min), and a moderate exercise period (0–150 min; Fig. 1). A primed (40 μCi) infusion (0.20 μCi/min) of high-performance liquid chromatography (HPLC)-purified [3-^{3}H]glucose was initiated at $t = -130$ min. The infusion of [3-^{3}H]glucose was varied during exercise in proportion to the increased glucose flux normally present during exercise of this intensity (21) in all protocols. A constant-rate indocyanine green ($0.1 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) was also started at $t = -130$ min and continued throughout the study. Indocyanine green was used as a backup method of blood flow measurement (22) when the Doppler probes did not provide a clear signal and as confirmation of hepatic vein catheter placement. All dogs were studied during a pancreatic clamp. A peripheral infusion of somatostatin ($0.8 \text{ μg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was started at $t = -130$ min to inhibit endogenous insulin and glucagon secretion in all groups. Concurrent with the infusion of somatostatin was an intraportal glucagon replacement ($0.5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) designed to re-create basal levels in all groups. Glucagon was infused at rates designed to simulate the exercise response during the exercise period (1.0 and $1.3 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ from 0 to 30 and 30 to 150 min, respectively). An intraportal insulin infusion was also initiated at this time. The insulin infusion rate was adjusted to maintain euglycemia throughout the basal period in all groups. The insulin infusion rate was reduced to 90, 80, and 70% of basal rates in the portal vein from 0 to 10 min, 10 to 30 min, and 30 to 60 min, respectively, to simulate the response of insulin to exercise.

In one group of dogs, the insulin infusion rate remained at 70% of basal rates from 60 to 150 min (C; $n = 7$). In a second group of dogs, the insulin infusion was switched to the inferior vena cava and was increased to 200% the rate at $t = 60$ to selectively create a physiological increment in arterial insulin (Pe; $n = 7$). In a third group of dogs, the insulin infusion rate was increased in the portal vein to 200% the rate at $t = 60$ and was infused in the inferior vena cava at 130% the rate at $t = 60$ to create a concomitant rise in portal insulin equal to the rise in arterial insulin (PePo; $n = 7$). The insulin infusion rates were derived on the basis of the assumption of typical gut (~30%) (12) and liver (~50%) (23) insulin extractions. Glucose was infused in the vena cava to clamp arterial glucose at levels normally seen during exercise in all groups. A transducer connected to the carotid arterial catheter monitored heart rates and blood pressures. Portal vein and hepatic artery blood flows were monitored on-line throughout the experiments.

Blood sample collection and processing. Arterial, portal vein, and hepatic vein blood samples were drawn at $t = -30, -15, 0, 30, 45, 60, 75, 90, 120$, and 150 min in all groups. Plasma glucose concentrations were determined by the glucose oxidase method using a Beckman Instruments Glucose Analyzer (Beckman Instruments, Fullerton, CA). For the determination of plasma glucose radioactivity, samples were deproteinized with barium hydroxide and zinc sulfate, and centrifuged. The supernatant was then evaporated to remove ^3H_2O and reconstituted in 1 ml of water and 10 ml of scintillation fluid (Ecolite (+); ICN Biomedicals, Irvine, CA). Radioactivity was determined on a Packard liquid scintillation counter. Blood samples were deproteinized (1 ml of blood in 3 ml of 4% perchloric acid), and whole-blood lactate, alanine, glycerol, and glutamine concentrations were determined using standard enzymatic methods (24,25) on a Monarch 2000 Centrifugal Analyzer (Lexington, MA). Blood gluconeogenic amino acids (alanine, glycine, serine, and threonine) were determined by HPLC (26). FFAs were measured with the use of the Wako FFA C test kit (Wako Chemicals, Richmond, VA). Immunoreactive insulin was measured with the use of a double-antibody procedure (interassay coefficient of variation [CV] of 16%) (27). Immunoreactive glucagon (3,500 mol wt) was measured in plasma samples that contained 500 KIU/ml Trasylol (FBA Pharmaceuticals, New York, NY) using a double-antibody system (CV of 7%) modified from the method developed by Morgan and Lazarow for insulin (27). Blood samples for norepinephrine and epinephrine measurement were collected into tubes that contained EGTA and glutathione, centrifuged at 4°C, and plasma was stored at −70°C for subsequent HPLC analysis. Catecholamine concentrations were calculated on the basis of linear regression using dihydroxybenzylamine as an internal standard. The CVs using this method were 5 and 7% for norepinephrine and epinephrine, respectively. Plasma cortisol was measured with the Clinical Assays Gamma Coat radioimmunoassay kit (Clinical Assays, Travenol-Genetech Diagnostics, Cambridge, MA) with an interassay CV of 6%.

Materials. HPLC-purified [3-^{3}H]glucose was obtained from New England Nuclear (Boston, MA). Glucagon and insulin antisera were obtained from Dr. R.L. Gingerich (Washington University School of Medicine, St. Louis, MO), and the standard glucagon and ^{125}I-glucagon were obtained from Linco Research

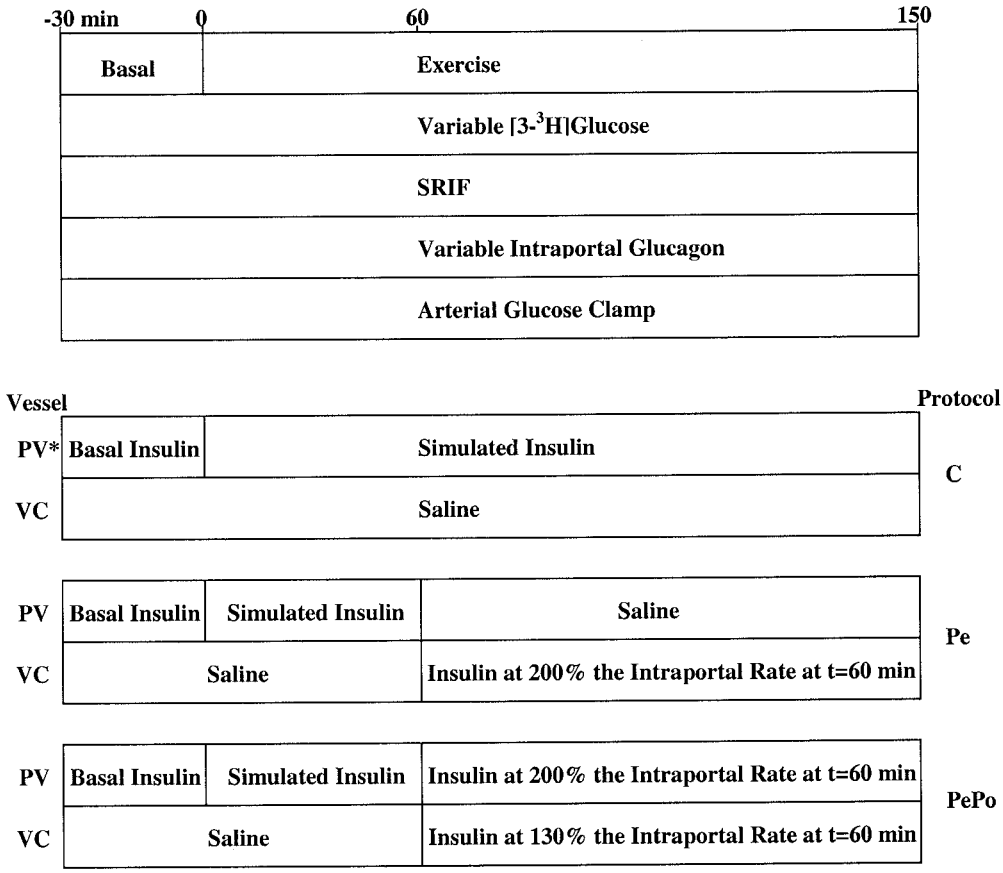

FIG. 1. Experimental protocol. All protocols consisted of a dye and isotope equilibration period (−130 to −30 min), a basal sampling period (−30 to 0 min), and an exercise period in the absence (C) or presence of peripheral (Pe) or both peripheral and portal vein (PePo) hyperinsulinemia. See EXPERIMENTAL PROCEDURES for infusion rates of isotope and hormones. *PV, portal vein; VC, vena cava.

(St. Louis, MO). Indocyanine green, enzymes, and coenzymes for metabolite analyses were obtained from Boehringer Mannheim Biochemicals and Sigma Chemical.

Calculations. Net hepatic uptake of FFA, glycerol, and GNG amino acids, and net hepatic output of glucose (NHGO) and lactate were determined according to the following formula: HAF × ([H] − [A]) + PVF × ([H] − [P]), such that [A], [P], and [H] are the arterial, portal vein, and hepatic vein substrate concentrations, respectively, and HAF and PVF are the hepatic artery and portal vein blood flows. Endogenous R_a and R_d were calculated using the two-compartment approach described by Mari (28).

TABLE 1
Arterial plasma glucagon, norepinephrine, epinephrine, and cortisol levels during rest and exercise in the presence and absence of selective hyperinsulinemia

		Exercise (min)				
				Selective hyperinsulinemia		
	Basal	30	60	90	120	150
Arterial plasma glucagon (pg/ml)						
C	42 ± 5	42 ± 6	50 ± 5	53 ± 2	59 ± 5	70 ± 4
Pe	41 ± 5	48 ± 7	59 ± 10	55 ± 9	54 ± 10	61 ± 9
PePo	49 ± 5	59 ± 9	66 ± 8	71 ± 10	70 ± 10	64 ± 7
Arterial plasma norepinephrine (pg/ml)						
C	104 ± 13	284 ± 64	345 ± 75	352 ± 51	413 ± 62	463 ± 91
Pe	192 ± 25	344 ± 66	363 ± 67	439 ± 76	408 ± 75	455 ± 72
PePo	191 ± 46	274 ± 34	326 ± 36	351 ± 60	372 ± 68	322 ± 54
Arterial plasma epinephrine (pg/ml)						
C	163 ± 18	241 ± 46	296 ± 72	322 ± 68	424 ± 97	516 ± 200*
Pe	141 ± 23	322 ± 63	257 ± 50	366 ± 103	335 ± 96	397 ± 101
PePo	111 ± 25	215 ± 64	233 ± 62	223 ± 64	264 ± 67	215 ± 98
Arterial plasma cortisol (μg/dl)						
C	2.4 ± 0.3	5.0 ± 1.1	8.0 ± 2.0	10.9 ± 1.7	12.6 ± 2.0*	13.4 ± 2.1*
Pe	3.6 ± 1.0	6.4 ± 1.6	8.4 ± 1.7	9.6 ± 2.3	9.8 ± 2.6	10.3 ± 2.8
PePo	1.9 ± 0.2	4.2 ± 0.8	5.5 ± 1.0	6.4 ± 1.1	7.0 ± 1.0	6.0 ± 0.8

Data are means ± SE. *$P < 0.05$ vs. PePo.

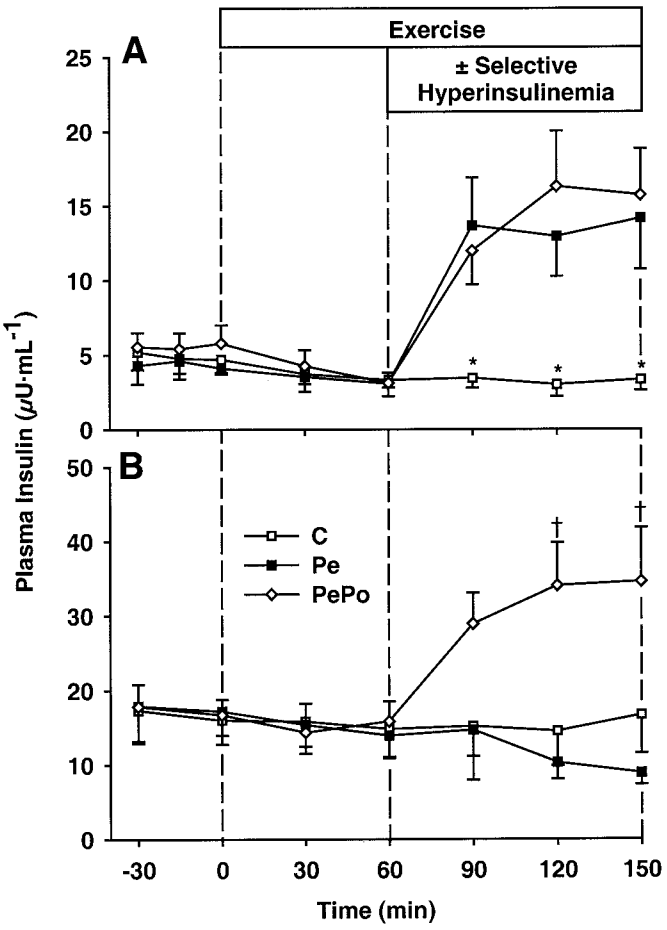

FIG. 2. Arterial (*A*) and portal vein (*B*) plasma insulin in C (open squares), Pe (closed squares), and PePo (diamonds) during the basal period (−30 to 0 min) and treadmill exercise period (0 to 150 min). Data are mean ± SE. *Significantly different compared with corresponding time points in Pe and PePo ($P < 0.05$); †significantly different compared with corresponding time points in C and Pe ($P < 0.05$).

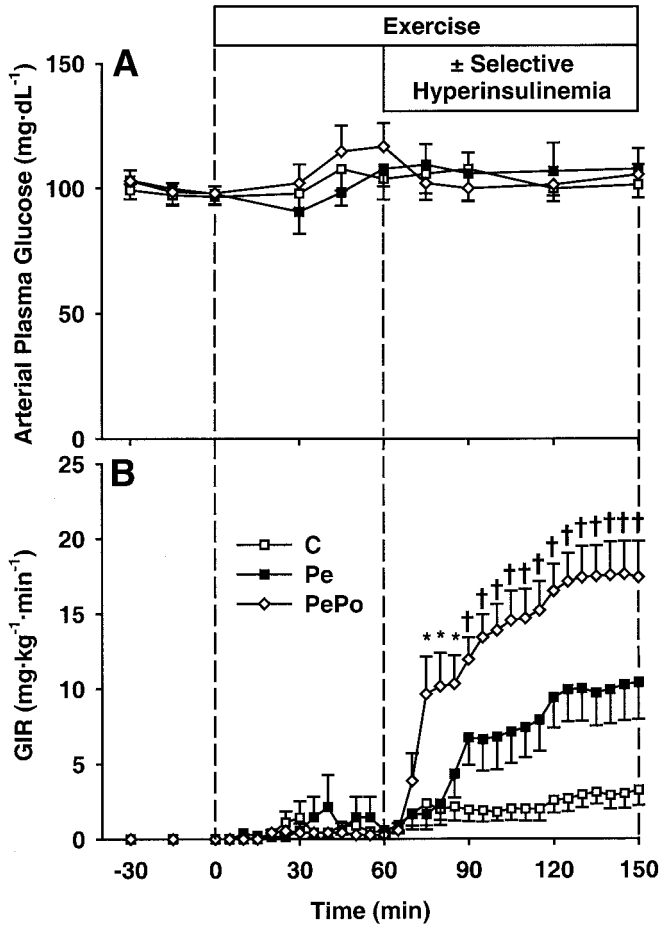

FIG. 3. Arterial plasma glucose (*A*) and glucose infusion rate (*B*) in C (open squares), Pe (closed squares), and PePo (diamonds) during the basal period (−30 to 0 min) and treadmill exercise period (0 to 150 min). Data are mean ± SE. *Significantly different compared with corresponding time points in C and Pe ($P < 0.05$); †significant difference with corresponding time points between all groups ($P < 0.05$).

Statistical analysis. SigmaStat (SPSS, Chicago, IL) software installed on a Gateway PC was used to perform statistical analyses. Statistical comparisons between groups and over time were made using ANOVA designed to account for repeated measures. Specific time points were examined for significance using contrasts solved by univariate repeated measures. Statistics are reported in tables and figures. Data are presented as mean ± SE. Statistical significance was defined as $P < 0.05$.

RESULTS

Plasma insulin and glucagon concentrations. Arterial insulin was similar between groups during the basal period and first 60 min of exercise (Fig. 2*A*). Arterial insulin did not change after $t = 60$ min in C but rose similarly in Pe (from 3 ± 1 μU/ml at $t = 60$ min to 14 ± 3 μU/ml at $t = 150$ min; $P < 0.05$) and PePo (from 3 ± 1 μU/ml at $t = 60$ min to 16 ± 3 μU/ml at $t = 150$ min; $P < 0.05$). Portal vein insulin was similar between groups during the basal period and first 60 min of exercise (Fig. 2*B*). Portal vein insulin did not change in C or Pe after $t = 60$ min but rose in PePo (from 16 ± 3 μU/ml at $t = 60$ min to 35 ± 7 μU/ml at $t = 150$ min; $P < 0.05$). Arterial glucagon was similar between groups throughout the study (Table 1).

Plasma cortisol, epinephrine, and norepinephrine concentrations. Arterial plasma cortisol, epinephrine, and norepinephrine were similar between groups during the basal period and rose similarly with exercise, except that cortisol (at $t = 120$ and 150 min) and epinephrine (at $t = 120$ min) were significantly greater in C versus PePo toward the end of the exercise period (Table 1).

Arterial plasma glucose concentration and kinetics. Arterial plasma glucose (Fig. 3*A*) was similar in all groups during the basal period and throughout exercise. The mean rate of exogenous glucose infusion required to clamp at euglycemia during the final 60 min of exercise (Fig. 3*B*) was 2.5 ± 0.2, 9.6 ± 0.6, and 15.8 ± 0.5 mg · kg^{-1} · min^{-1} in C, Pe, and PePo, respectively ($P < 0.05$ between each group). NHGO was similar during the basal period and rose similarly during the first 60 min of exercise in each group (Fig. 4). NHGO remained elevated in C and Pe but was rapidly and significantly suppressed in PePo (5.4 ± 0.8 at $t = 60$ min to 1.2 ± 0.4 mg · kg^{-1} · min^{-1} at $t = 150$ min; $P < 0.05$).

R_a was similar during the basal period and rose similarly during the first 60 min of exercise in each group (Fig. 5*A*). R_a remained elevated in C after $t = 60$ min. R_a was significantly suppressed from 5.9 ± 0.4 mg · kg^{-1} · min^{-1} at $t = 60$ min to 4.2 ± 0.8 mg · kg^{-1} · min^{-1} at $t = 120$ min and 3.4 ± 0.9 mg · kg^{-1} · min^{-1} at $t = 130$ min in Pe. R_a was rapidly and nearly completely suppressed in PePo (7.3 ± 1.1 to 0.4 ± 1.2 mg · kg^{-1} · min^{-1} at $t = 150$ min; $P < 0.05$).

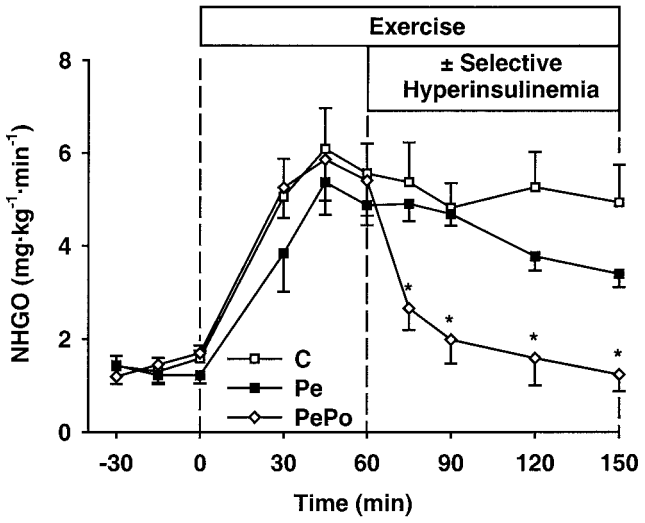

FIG. 4. NHGO in C (open squares), Pe (closed squares), and PePo (diamonds) during the basal period (−30 to 0 min) and treadmill exercise period (0 to 150 min). *Significantly different compared with corresponding time points in C and Pe ($P < 0.05$).

R_d was similar during the basal period and rose similarly during the first 60 min of exercise between groups (Fig. 5*B*). R_d was significantly greater in PePo and Pe than C by $t = 80$ and 120 min of exercise, respectively. R_d was significantly greater in PePo than Pe from $t = 90$ to 130 min of exercise.

Arterial concentrations and net hepatic uptake of FFAs, glycerol, lactate, and gluconeogenic amino acids. Arterial FFA levels (Table 2) were similar during the basal period and first 60 min of exercise between groups. Beyond this point, arterial FFA continued to rise in C but declined in Pe and PePo ($P < 0.05$). Arterial glycerol levels were similar during the basal period and first 60 min of exercise between groups. Arterial glycerol continued to rise in C but declined in Pe and PePo ($P < 0.05$). Arterial concentrations and net hepatic uptake of alanine, glutamine, glycine, lactate, serine, and threonine were similar between groups throughout the study, except that net hepatic alanine uptake was significantly greater in Pe versus PePo toward the end of the exercise period (Tables 2 and 3).

DISCUSSION

In previous studies, subjects with type 1 diabetes received a constant peripheral venous infusion of insulin at a fixed rate during 45 (29) or 60 min (30) of exercise. This resulted in a mild peripheral hyperinsulinemia relative to what normally occurs during exercise. Despite the relative peripheral hyperinsulinemia, R_a and R_d were matched and blood glucose was constant. These results in humans with type 1 diabetes contrasted with studies in the exercising dog, in which the fall in insulin was prevented with an intraportal venous insulin infusion instead of a peripheral venous infusion (31). This relative portal venous hyperinsulinemia rapidly alleviated >50% of the exercise-induced R_a in the dog model. This effect was due to an attenuation of the hepatic glycogenolytic response. The results of the present study provide the most likely explanation for the discrepancy in R_a data between the aforementioned studies. The presence of a physiological increase in peripheral

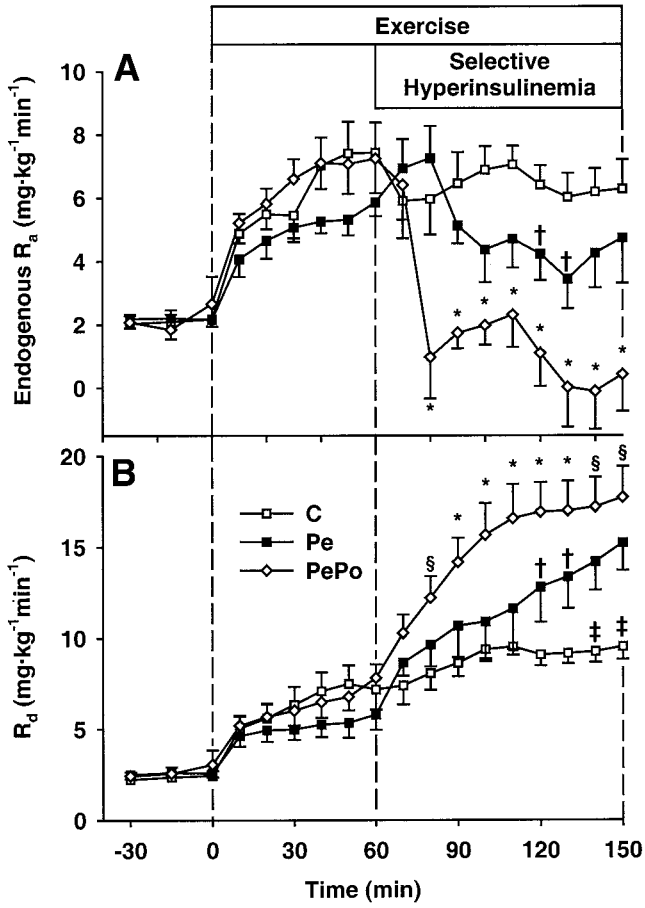

FIG. 5. Endogenous glucose production (*A*) and glucose disappearance (*B*) in C (open squares), Pe (closed squares), and PePo (diamonds) during the basal (−30 to 0 min) and treadmill exercise period (0 to 150 min). Data are mean ± SE. *Significantly different compared with corresponding time points in C and Pe ($P < 0.05$); †significantly different compared with corresponding time points between all groups ($P < 0.05$); ‡significantly different compared with corresponding time points in Pe and PePo ($P < 0.05$); §significantly different compared with corresponding time points in C ($P < 0.05$).

insulin alone had no effect on R_a for the first 60 min of exposure (i.e., 120 min of exercise). This is consistent with the results in type 1 diabetes studies described above, in which insulin was administered only peripherally. After 60 min of exposure to mild hyperinsulinemia, R_a was modestly suppressed. This reduction in R_a may have been related to the marked reduction in arterial FFA concentrations, because this has been reported to act as a signal to the liver to suppress R_a during rest (7,9,10,13). As opposed to the delayed and partial reduction of R_a with peripheral insulin alone, a physiological increase in portal insulin equal to the increment in peripheral insulin rapidly and nearly completely suppressed R_a.

The increments in portal vein and arterial insulin used in this study were chosen to reflect mild hyperinsulinemia within the physiological range. A selective increase in portal vein insulin (i.e., peripheral insulin concentration unchanged from fasting basal) can be created only by infusing insulin peripherally in the basal state and tapering it reciprocally when increasing portal vein insulin infusion. Accomplishing this requires either a basal period of portal venous hypoinsulinemia or peripheral venous hyperinsulinemia, which may alter the response to subsequent

TABLE 2
Arterial levels and net hepatic uptake of FFAs, glycerol, and lactate during rest and exercise in the presence and absence of selective hyperinsulinemia

		Exercise (min)				
				Selective hyperinsulinemia		
	Basal	30	60	90	120	150
Arterial FFA (μmol/l)						
C	656 ± 56	527 ± 71	702 ± 114	792 ± 112	1,066 ± 195	1,212 ± 196
Pe	700 ± 81	689 ± 116	877 ± 127	529 ± 84	505 ± 92*	459 ± 67*†
PePo	501 ± 29	576 ± 74	680 ± 75	464 ± 58	378 ± 56*	312 ± 42*†
Net hepatic FFA uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	3.5 ± 0.2	3.2 ± 0.4	3.5 ± 0.5	4.4 ± 0.7	5.8 ± 1.9	6.2 ± 1.3
Pe	3.3 ± 0.3	3.7 ± 1.0	4.9 ± 1.4	3.5 ± 1.4	4.2 ± 1.0	3.9 ± 1.3
PePo	2.9 ± 0.4	4.7 ± 1.3	4.6 ± 1.0	3.1 ± 1.0	2.8 ± 0.9	2.7 ± 0.2*
Arterial glycerol (μmol/l)						
C	74 ± 7	179 ± 29	211 ± 27	223 ± 26	303 ± 47	355 ± 66
Pe	78 ± 7	174 ± 25	168 ± 28	147 ± 29	156 ± 35*	149 ± 32*
PePo	57 ± 4	152 ± 13	170 ± 16	117 ± 25	128 ± 29*	106 ± 23*
Net hepatic glycerol uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	1.1 ± 0.1	2.3 ± 0.3	3.3 ± 0.4	3.8 ± 0.5	3.8 ± 0.5	5.2 ± 0.8
Pe	1.5 ± 0.2	2.9 ± 0.3	3.3 ± 0.5	3.3 ± 0.8	3.4 ± 0.8	3.2 ± 0.8*
PePo	1.0 ± 0.1	2.6 ± 0.4	3.1 ± 0.6	3.0 ± 0.5	3.2 ± 0.9	2.5 ± 0.6*
Arterial lactate (μmol/l)						
C	869 ± 57	1,122 ± 149	1,121 ± 151	1,214 ± 207	1,421 ± 427	1,445 ± 438
Pe	737 ± 49	1,065 ± 142	1,144 ± 155	1,195 ± 199	1,106 ± 185	919 ± 175
PePo	591 ± 28	926 ± 181	867 ± 151	788 ± 185	764 ± 190	758 ± 154
Net hepatic lactate uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	−7.3 ± 1.7	−19.7 ± 4.2	−15.4 ± 2.9	−6.5 ± 1.9	−4.0 ± 3.3	−0.5 ± 3.2
Pe	−4.3 ± 1.8	−6.6 ± 1.9	−8.0 ± 3.3	−8.4 ± 2.3	−8.4 ± 3.9	−8.0 ± 4.7
PePo	−5.5 ± 1.1	−11.7 ± 4.1	−12.0 ± 3.8	−5.2 ± 3.6	−6.1 ± 3.1	−5.7 ± 2.2

Data are means ± SE. A negative number represents a net output. *$P < 0.05$ vs. C; †$P < 0.05$ vs. $t = 60$ min.

portal venous hyperinsulinemia. For this reason, we used the approach of creating portal venous hyperinsulinemia on the background of a mild peripheral venous hyperinsulinemia, which was matched in Pe and PePo. The background of mild peripheral venous hyperinsulinemia had only a delayed and relatively small effect on R_a and therefore left an adequately large R_a from which the effect of portal insulin could be assessed.

Peripheral hyperinsulinemia did not result in a measurable increase in estimated insulin levels within the liver sinusoids, calculated by the equation $(HAF \times [I]_A) + (PVF \times [I]_P)$, where $[I]_A$ and $[I]_P$ are arterial and portal vein insulin concentrations. It is possible to selectively increase peripheral insulin without substantially altering insulin levels in the hepatic sinusoid because the hepatic artery supplies only ~28% of liver blood flow, and ~30% of plasma insulin is extracted by the gastrointestinal tract before it enters the portal vein.

Previous studies have shown that insulin's extrahepatic effect to suppress glucose production is linked to suppression of glucagon secretion and lipolysis (7,9,10,13,17) and more recently to hypothalamic insulin signaling (32). The experimental design used in this study re-created the exercise-stimulated increase in glucagon and was done so equivocally in all groups, excluding this as a factor in peripheral insulin's suppression of glucose production. In the presence of peripheral hyperinsulinemia, arterial FFA levels did not fall as rapidly as has been previously reported for similar peripheral hyperinsulinemia under sedentary conditions (12,13). This could possibly be explained by an increased lipolytic stimulation as a result of the presence of elevated catecholamines during moderate exercise. Net hepatic GNG precursor (GNG amino acids, glycerol, and lactate during net uptake) uptake was similar between groups throughout the study (data not shown). Because GNG precursor uptake was similar, one could conclude that the decrease in hepatic glucose production is attributable to an inhibition of glycogenolysis (33). However, an effect of FFA on the fraction of GNG precursors converted to glucose within the liver cannot be ruled out. Because hepatic GNG precursor uptake, glucagon, and arterial FFA were similar in Pe and PePo during the first 60 min of hyperinsulinemia, we conclude that suppression of R_a in PePo during this initial period was due entirely to portal vein hyperinsulinemia.

The addition of an equal increment in portal venous insulin rapidly (within 20 min) and nearly completely suppressed R_a during exercise. This result was confirmed by arteriovenous difference measurements, which showed rapid and significant suppression of NHGO in PePo. Although R_a was lower in Pe than in C at two points, significance between Pe and C is lost when the data are expressed as an area under the curve (AUC) for the suppression of R_a. The AUCs were 101 ± 82, 111 ± 65, and 510 ± 98 mg/kg for C, Pe, and PePo, respectively ($P < 0.05$, PePo vs. C and Pe). AUCs for the suppression of NHGO

TABLE 3
Arterial levels and net hepatic uptake of alanine, glutamine, glycine, serine, and threonine during rest and exercise in the presence and absence of selective hyperinsulinemia

		Exercise (min)				
				Selective hyperinsulinemia		
	Basal	30	60	90	120	150
Arterial alanine (μmol/l)						
C	439 ± 20	449 ± 36	428 ± 41	367 ± 43*	339 ± 49	316 ± 39
Pe	440 ± 24	426 ± 53	400 ± 43	370 ± 42*	336 ± 41	301 ± 33
PePo	398 ± 18	363 ± 34	322 ± 26	245 ± 19	225 ± 25	205 ± 18
Net hepatic alanine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	2.1 ± 0.3	1.9 ± 0.5	2.7 ± 0.4	2.8 ± 0.5	3.0 ± 0.4	3.0 ± 0.5
Pe	2.8 ± 0.3	3.5 ± 0.5	3.8 ± 0.7	4.3 ± 0.6*	4.6 ± 0.6*	4.2 ± 0.8*
PePo	1.9 ± 0.2	2.1 ± 0.4	2.4 ± 0.5	2.3 ± 1.0	1.8 ± 0.6	1.7 ± 0.8
Arterial glutamine (μmol/l)						
C	734 ± 44	778 ± 48	748 ± 45	704 ± 48	715 ± 48	731 ± 43
Pe	849 ± 31	835 ± 65	788 ± 77	756 ± 67	746 ± 65	613 ± 105
PePo	829 ± 22	731 ± 80	742 ± 53	714 ± 49	668 ± 51	662 ± 63
Net hepatic glutamine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	−0.8 ± 0.4	−0.2 ± 0.3	0.6 ± 0.6	0.0 ± 0.5	0.4 ± 0.6	1.5 ± 0.5
Pe	0.2 ± 0.4	1.7 ± 0.6	1.0 ± 1.0	1.3 ± 0.6	2.4 ± 0.7	0.9 ± 0.9
PePo	−0.7 ± 0.3	0.1 ± 0.3	0.9 ± 0.6	0.9 ± 0.4	1.1 ± 0.4	1.3 ± 0.8
Arterial glycine (μmol/l)						
C	454 ± 18	455 ± 61	419 ± 35	378 ± 31	371 ± 43	318 ± 37
Pe	435 ± 30	436 ± 68	412 ± 64	388 ± 67	342 ± 56	295 ± 45
PePo	451 ± 14	443 ± 45	427 ± 40	419 ± 47	379 ± 50	291 ± 26
Net hepatic glycine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	6.3 ± 0.4	5.3 ± 0.6	5.7 ± 0.9	5.9 ± 0.9	5.9 ± 0.7	4.6 ± 0.7
Pe	7.5 ± 0.4	6.8 ± 1.0	6.6 ± 1.2	7.0 ± 1.3	6.2 ± 1.0	6.2 ± 1.1
PePo	7.6 ± 0.4	7.3 ± 0.8	7.6 ± 0.8	7.0 ± 1.1	6.1 ± 0.5	5.1 ± 0.2
Arterial serine (μmol/l)						
C	271 ± 16	245 ± 47	237 ± 35	205 ± 21	225 ± 31	206 ± 30
Pe	271 ± 23	254 ± 45	242 ± 42	237 ± 39	201 ± 33	191 ± 32
PePo	256 ± 10	262 ± 22	240 ± 14	226 ± 25	236 ± 24	228 ± 26
Net hepatic serine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	3.7 ± 0.2	3.0 ± 0.3	3.3 ± 0.6	3.5 ± 0.6	3.4 ± 0.5	3.0 ± 0.5
Pe	4.2 ± 0.3	3.7 ± 0.7	3.6 ± 0.8	4.0 ± 0.8	3.5 ± 0.7	3.5 ± 0.7
PePo	4.1 ± 0.3	3.8 ± 0.5	4.1 ± 0.4	3.7 ± 0.3	3.4 ± 0.4	3.1 ± 0.4
Arterial threonine (μmol/l)						
C	461 ± 36	456 ± 81	419 ± 74	358 ± 37	402 ± 65	346 ± 57
Pe	446 ± 36	442 ± 61	440 ± 71	437 ± 84	378 ± 61	338 ± 63
PePo	466 ± 21	495 ± 59	516 ± 56	549 ± 59	541 ± 85	440 ± 86
Net hepatic threonine uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)						
C	6.4 ± 0.4	4.7 ± 0.9	5.0 ± 0.8	5.3 ± 0.9	5.6 ± 0.7	4.5 ± 0.7
Pe	7.1 ± 0.5	7.0 ± 1.0	6.8 ± 1.6	7.8 ± 1.7	6.4 ± 1.3	6.6 ± 1.4
PePo	7.9 ± 0.5	7.6 ± 0.8	9.1 ± 1.2	9.6 ± 1.2	8.9 ± 1.5	8.2 ± 2.0

Data are means ± SE. A negative number represents a net output. *$P < 0.05$ vs. PePo.

showed similar results, again reaffirming the exquisite sensitivity to portal venous insulin in suppressing glucose production during exercise.

The predominance of portal vein insulin to suppress R_a shown in the present study is consistent with recent work using liver-specific insulin receptor knockout mice (34). These mice are incapable of having any direct hepatic effect of insulin to suppress glucose production and thus represent a unique model to investigate direct versus indirect hepatic insulin action. High-dose insulin chosen to maximize extrahepatic insulin action failed to suppress glucose production, suggesting the absence of an indirect glucose production–suppressing effect of insulin in this model. They found that glucagon was not suppressed, and even with marked suppression of FFA, there was no suppression of glucose production. However, when considering these data, it must also be noted that the livers of these mice were smaller, accompanied by an increase in liver enzymes and lipid accumulation, enlarged mitochondria as a result of oxidative stress, and hyperplastic hepatocytes. If FFA flux to the liver is indeed an extrahepatic regulator of R_a, then it may require the presence of an intact insulin-signaling network.

It is interesting that the addition of portal vein hyperin-

sulinemia to peripheral hyperinsulinemia during exercise resulted in a significantly greater R_d. This is in contrast to studies by Sindelar et al. (12) that showed no difference between R_d with a selective increment in peripheral insulin or equal increments in peripheral and portal vein insulin (14) in sedentary dogs. The difference in R_d with the addition of portal vein hyperinsulinemia seen in the current study may be evident only during exercise as a result of the presence of large glucose fluxes, which are not seen during rest. The sum of the exogenous glucose infusion rate and NHGO provides an estimate of nonhepatic glucose uptake and was also increased by the addition of portal insulin. By 150 min of exercise, the estimated nonhepatic glucose uptake was 8.1 ± 0.7, 14.1 ± 2.4, and 19.6 ± 2.7 mg · kg^{-1} · min^{-1} in C, Pe, and PePo, respectively, which corresponds to and supports the R_d values. The precise mechanism behind this enhanced R_d during exercise in the presence of portal vein hyperinsulinemia is unknown but may be due to a neural (35) or humoral signal (36–38) that influences muscle metabolism. A portal signal has been hypothesized to coordinate regulation of tissue glucose metabolism at the level of both the liver and the skeletal muscle (39).

Changes in [3-^{3}H]glucose specific activity specific activity potentially create errors in the calculation of R_a (40). It is difficult to clamp specific activity without previous knowledge or assumptions relating to R_a response. It is not necessary to keep specific activity perfectly constant to avoid significant modeling errors when calculating R_a (41). Other measures were taken in the present study to minimize errors inherent in tracer calculations (42,43). HPLC-purified tracer was used to avoid potential errors in the evaluation of glucose turnover associated with impurities (44). A two-compartment model for calculation of R_a, which has been shown to have less non–steady-state error than that of the Steele model, was used (28). Finally, R_a data were confirmed by hepatic arteriovenous difference measurements of glucose.

In summary, the results show that during exercise, *1*) the rapid and nearly complete suppression of R_a in the presence of portal and peripheral venous hyperinsulinemia is due largely to the presence of portal venous hyperinsulinemia, *2*) an increment in arterial insulin that suppresses FFA has a small suppressive effect on R_a that becomes evident only after 60 min of exposure, and *3*) the addition of an equal increment in portal venous insulin on a background of mild peripheral hyperinsulinemia increases R_d compared with peripheral hyperinsulinemia alone. These findings have important implications in developing strategies for islet implantation and use of insulin delivery systems in people with diabetes. With the sensitivity and rapidness of portal insulin's suppression of R_a comes increased risk of hypoglycemia during exercise in individuals in whom therapies that result in portal venous hyperinsulinemia are used.

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