Inhalation of Insulin (Exubera) Is Associated With Augmented Disposal of Portally Infused Glucose in Dogs

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The results of the present study, using the conscious beagle dog, demonstrate that inhaled insulin (INH; Exubera) provides better glycemic control during an intraportal glucose load than identical insulin levels induced by insulin (Humulin) infusion into the inferior vena cava (IVC). In the INH group (n = 13), portal glucose infusion caused arterial plasma glucose to rise transiently (152 ± 9 mg/dl), before it returned to baseline (65 min) for the next 2 h. Net hepatic glucose uptake was minimal, whereas nonhepatic uptake rose to 12.5 ± 0.5 mg · kg^{-1} · min^{-1} (65 min). In the IVC group (n = 9), arterial glucose rose rapidly (172 ± 6 mg/dl) and transiently fell to 135 ± 13 mg/dl (65 min) before returning to 165 ± 15 mg/dl (125 min). Plasma glucose excursions and hepatic glucose uptake were much greater in the IVC group, whereas nonhepatic uptake was markedly less (8.6 ± 0.9 mg · kg^{-1} · min^{-1}; 65 min). Insulin kinetics and areas under the curve were identical in both groups. These data suggest that inhalation of Exubera results in a unique action on nonhepatic glucose clearance.
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Insulin delivered by inhalation is an alternative to injected insulin for patients with diabetes. Clinical studies comparing subcutaneous administration of insulin (Humulin) with insulin inhalation (Exubera) suggest that there is a unique glucose-lowering effect associated with inhaled insulin (1). Previous studies in dogs have shown that inhaled insulin has a higher and earlier peak of arterial insulin that clears more rapidly than subcutaneous insulin. Despite the similar arterial and hepatic insulin areas under the curve (AUCs) in the two groups, a 20% greater glucose infusion rate was required to maintain euglycemia in the inhaled insulin group relative to the subcutaneous insulin group (2). This finding was consistent with the unique glucose-lowering effect of inhaled insulin seen in human studies.

The difference in glycemic control resulting from inhalation verses subcutaneous administration of insulin could be explained by the different pharmacokinetic insulin profiles resulting from the two routes of administration, or by some unique aspect of insulin delivery by inhalation. To distinguish between these possibilities, we delivered insulin by inhalation (Exubera) or through infusion (Humulin) into the inferior vena cava (IVC) to create an identical pharmacokinetic profile to that resulting from insulin inhalation. We then examined the effects of both routes of insulin delivery on glucose disposal during a simulated oral glucose load. To further clarify the mechanism by which glucose disposal might be enhanced, we compared the role of the liver and nonhepatic tissues in glucose uptake.

RESEARCH DESIGN AND METHODS

Experiments were conducted on 22 healthy, conscious, 18-h-fasted, male beagle dogs (weight 8–10 kg). Before the study, they were fed a standard diet once a day, and water was provided ad libitum. The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Lovelace Respiratory Research Institute Institutional Animal Care and Use Committee before the start of the study. All dogs underwent a laparotomy 3 weeks before the experiment to implant infusion catheters into the jejunal and splenic veins and IVC and sampling catheters into the portal and hepatic veins and femoral artery. Transonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery and portal vein, as described elsewhere (3). Intraportal catheters (splenic and jejunal) were used for the infusion of glucose (50% Dextrose; Baxter Healthcare, Deerfield, IL). Each animal was used only once.

Recombinant human insulin (Humulin; Eli Lilly, Indianapolis, IN) was used for the intravenous insulin infusion. Dry powder Exubera (currently being developed by Pfizer and Sanofi-Aventis in conjunction with Nektar Therapeutics), a human insulin of recombinant origin and specially formulated for intrapulmonary administration, was used for inhalation. It is assumed that the biological activity and composition of insulin in both preparations were identical. The dry powder was packaged in a foil blister pack, with each blister containing 1.0 mg of human insulin and 0.7 mg of inert mannitol citrate carrier.

On the day of exposure, the dogs were briefly anesthetized. They were then given 0.2 ml of acepromazine subcutaneously; ~15 min later, 5% isofluorane was administered by inhalation until palpebral and pedal reflexes disappeared. The dogs were then intubated with an endotracheal tube, and anesthesia was maintained with 1–2% isofluorane in oxygen. Intravenous catheters were placed into the cephalic and/or saphenous veins to allow somatostatin (Bachem California, Torrance, CA), glucagon (Eli Lilly), and glucose delivery. Insulin was then administered by inhalation or intravenous infusion as described below.

In the inhalation (INH) group, 13 dogs were exposed to the contents of one blister of Exubera using a modified P2.3 device (Inhale Therapeutic Systems, San Carlos, CA) for administration. Compressed breathing air was used to deliver aerosol to the dog via the endotracheal tube. Apnea was induced by ventilating the dog with the anesthesia bag 10–15 times. After apnea was induced, an ~800 ml pulse of compressed breathing air was passed through the device to expose the dog to the insulin. The time of the end of inhalation exposure was considered t = 0. No adverse clinical signs related to the inhalation of insulin were observed during the study.

In the IVC group, after sham inhalation exposure, Humulin diluted in saline with added plasma (3:100 ml) was infused intravenously into the IVC in nine dogs

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IVC, inferior vena cava; NHB, net hepatic balance.

TABLE 1
Algorithm for intravenous infusion of insulin for the IVC group

Time (min)	mU · kg^{-1} · min^{-1}
0	1.5
5	2.0
40	1.6
50	1.2
65	0.8
80	0.6
95	0.4
110	0.3
125	0.24
155	0.18
185	0.12
215	0.10
245	0.08
305	0.04
365	0.0

using an algorithm designed to match the arterial plasma insulin kinetic profile of the INH group (Table 1). The start of IVC insulin infusion was considered $t = 0$.

After insulin was administered, the dogs were placed in slings and allowed to recover from the anesthesia (which occurred rapidly, ~5–10 min post $t = 0$). At $t = 5$, intravenous somatostatin (to inhibit endogenous insulin and glucagon secretion; 0.8 μg · kg^{-1} · min^{-1}) and glucagon (0.5 ng · kg^{-1} · min^{-1}) infusions were started. These infusions were continued throughout the remainder of the experiment. Intraportal glucose infusion (50% dextrose) was also started at $t = 5$ using an algorithm designed to mimic glucose absorption from the gut after oral administration (Table 2). At $t = 170$, the intraportal glucose infusion was stopped and, thereafter, glucose was infused intravenously only as needed to maintain euglycemia.

Blood sampling and analytical procedures. Blood samples of ~4 ml were collected from the aorta (femoral artery), and 2-ml samples were collected from the portal and hepatic veins at the following time points: before acepromazine administration (jugular only); after anesthesia (arterial only); just before insulin administration ($t = 0$; arterial only); at $t = 5$ (when somatostatin infusion began); and 10 (arterial only), 15 (arterial only), 20, 35, 50 (arterial only), 65, 80 (arterial only), 95, 125, 155, 185, 215 (arterial only), 245, 305, 335, and 365 min after insulin administration.

Arterial blood glucose was measured in duplicate with a Glucometer Elite and Glucometer Elite blood glucose test strips (Bayer, Pittsburgh, PA); measurements were used to adjust the glucose infusion rate to maintain euglycemia after $t = 170$. Hematocrit, plasma glucose, glucagon, insulin, C-peptide, blood alanine, glycine, lactate, and glycerol concentrations were determined as previously described (3). Hepatic blood flow was measured using implanted transonic flow probes, as described elsewhere (3).

Data analysis. Net hepatic balance (NHB) was calculated with the arterial-venous difference method as NHB = $load_{out} - load_{in}$, where $load_{out} = H \times HF$ and $load_{in}$ = (A × AF) + (P × PF), in which H, A, and P are the substrate concentrations in the hepatic vein, femoral artery, and portal vein blood or plasma, respectively, and HF, AF, and PF are the blood flow in the hepatic vein, hepatic artery, and portal vein, as determined by the ultrasonic flow probes. Using this calculation, a positive value represented net output by the liver, and a negative value represented net hepatic uptake. NHB measurement is most accurate when steady-state substrate concentrations exist. Although plasma glucose concentrations were not at steady state for much of the experiment, the resulting error was probably minimal because the sampling of the blood entering and exiting the liver was timed to account for transit time across the organ. Arterial plasma glucose values were multiplied by 0.74, and portal and hepatic plasma glucose were multiplied by 0.73 to convert them to blood glucose values. The correction factors are based on extensive historical data in the mongrel dog (4) and unpublished data in the beagle (A. Cherrington, Vanderbilt University Medical Center, 2003). The approximate substrate levels in plasma entering the liver sinusoids were calculated as ($A \times$ % AF) + ($P \times$ % PF), where A and P are arterial and portal vein hormone concentrations, respectively, and % AF and % PF are the percent contributions of arterial and portal flow to total hepatic blood flow, respectively.

TABLE 2
Algorithm for intraportal infusion of glucose for Exubera inhalation and Humulin IVC-infused groups

Time (min)	mg · kg^{-1} · min^{-1}
5	12
95	10
110	8
125	6
140	4
155	2
170	0

Nonhepatic glucose uptake was calculated using an established method (5–7) in which the glucose infusion rate was added to the net hepatic glucose balance, and changes in the glucose mass were accounted for when deviations from steady state were present. Nonhepatic glucose clearance was calculated by dividing nonhepatic glucose uptake by the arterial glucose concentration. The AUC was calculated using the trapezoidal rule.

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

RESULTS

After the somatostatin infusion was initiated, the arterial C-peptide levels dropped rapidly (<30 min) in both groups to concentrations below the detection level of the assay (0.1 mg/ml), indicating that endogenous insulin secretion had been quickly and effectively suppressed (Table 3). The arterial and liver sinusoidal glucagon levels were close to basal concentrations and were equivalent in the two groups throughout the experiment (Table 3).

After Exubera inhalation, the arterial and hepatic sinusoidal plasma insulin levels in the INH group rose rapidly from basal to 68 ± 9 and 55 ± 7 μU/ml, respectively, at 20 min (Fig. 1), after which they declined to 42 ± 4 and 33 ± 3 μU/ml by 65 min. They returned to basal concentrations 2.5 h after administration. IVC insulin infusion (Table 1) was used to simulate the insulin profile produced in the INH group (Fig. 1). Thus the arterial plasma and hepatic sinusoidal insulin levels in the INH and IVC groups were virtually identical.

Portal glucose infusion (algorithm shown in Table 2) caused the arterial plasma glucose level to rise rapidly in the INH group (+32 mg/dl), peaking at 152 ± 9 mg/dl at 20 min (Fig. 2). The plasma glucose level then fell to 111 ± 14 mg/dl at 65 min. During the 2nd and 3rd h, the arterial plasma glucose concentration remained near basal. In the IVC group, the arterial plasma glucose level also rose rapidly (+48 mg/dl), peaking at 172 ± 6 mg/dl at 20 min (Fig. 2). It then fell to 135 ± 13 mg/dl at 65 min, after which it increased to 165 ± 15 mg/dl during the next 2 h. The ΔAUCs in the arterial plasma glucose levels in the INH and IVC groups were 1,039 ± 543 and 1,834 ± 308 mg/dl, respectively, during the 1st hour after insulin administration, and −1,592 ± 984 and 3,306 ± 1,085 mg/dl in the two groups, respectively, during the next 2 h (Fig. 3). During the last 3 h, there was a steady rise in the plasma glucose level in both groups, probably due to the increase in net hepatic glucose output that resulted from insulin deficiency occurring in the presence of basal glucagon. The peripheral glucose infusion rate required to maintain euglycemia after the end of the portal glucose infusion period was significantly greater in the INH compared with in the IVC group (2.14 ± 0.70 vs. 0.11 ± 0.12 mg · kg^{-1} · min^{-1} at 185 min in the INH and IVC groups, respectively; $P < 0.05$) (Table 4).

TABLE 3
Arterial plasma C-peptide levels and arterial plasma and hepatic sinusoidal glucagon levels in overnight-fasted, conscious dogs administered Exubera or matched IVC insulin infusion

	5	10	15	20	35	50	65	95	125	185	245	305	365
Arterial plasma C-peptide level (ng/ml)													
INH	0.37 ± 0.07	0.19 ± 0.03	0.12 ± 0.02	0.09 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.02
IVC	0.52 ± 0.13	0.28 ± 0.08	0.14 ± 0.05	0.13 ± 0.04	0.08 ± 0.03	0.08 ± 0.02	0.09 ± 0.03	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.06 ± 0.02	0.09 ± 0.04	0.06 ± 0.02
Arterial plasma glucagon level (pg/ml)													
INH	40 ± 3	45 ± 3	42 ± 3	43 ± 3	41 ± 3	37 ± 2	35 ± 3	35 ± 2	32 ± 2	32 ± 3	29 ± 2	32 ± 2	32 ± 2
IVC	57 ± 6	61 ± 5	55 ± 3	57 ± 3	51 ± 4	47 ± 4	48 ± 3	38 ± 5	36 ± 5	38 ± 4	38 ± 4	37 ± 4	34 ± 3
Hepatic sinusoidal glucagon level (pg/ml)													
INH	44 ± 4	—	—	44 ± 3	38 ± 2	—	37 ± 2	34 ± 2	29 ± 2	30 ± 3	28 ± 3	32 ± 3	31 ± 2
IVC	56 ± 7	—	—	50 ± 4	47 ± 3	—	42 ± 4	36 ± 4	36 ± 3	39 ± 4	38 ± 4	39 ± 3	33 ± 4

Data are means ± SE. INH group, $n = 13$; IVC group, $n = 9$. Time points are in minutes.

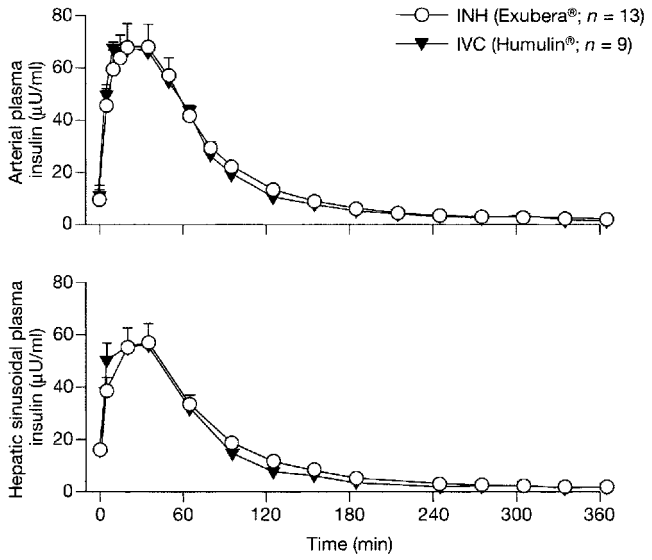

FIG. 1. Arterial and hepatic sinusoidal plasma insulin levels for Exubera inhalation and Humulin IVC-infused insulin.

The hepatic sinusoidal plasma glucose levels initially increased in the two groups (+74 and +90 mg/dl from 5 to 20 min in the INH and IVC groups, respectively) (Fig. 2). As a result of the elevated arterial glucose levels in the IVC group, the hepatic sinusoidal glucose levels were also higher in the IVC group during the 2nd and 3rd h ($P < 0.05$) (Fig. 2). Consequently, the hepatic glucose load was greater during the 3rd h in the IVC than in the INH group ($P < 0.05$) (Fig. 4).

Due to the rise in the hepatic sinusoidal insulin level and hepatic glucose load, there was a brief switch from net hepatic glucose output to uptake in the INH group (Fig. 4). Despite nearly identical hepatic insulin levels and initially (1st h) similar hepatic glucose loads in the two groups, net hepatic glucose uptake was greater in the IVC group, peaking in 30 min at 4.18 ± 1.37 vs. 1.50 ± 0.73 mg · kg^{-1} · min^{-1} in the INH group ($P < 0.05$). Over the last 3 h of the

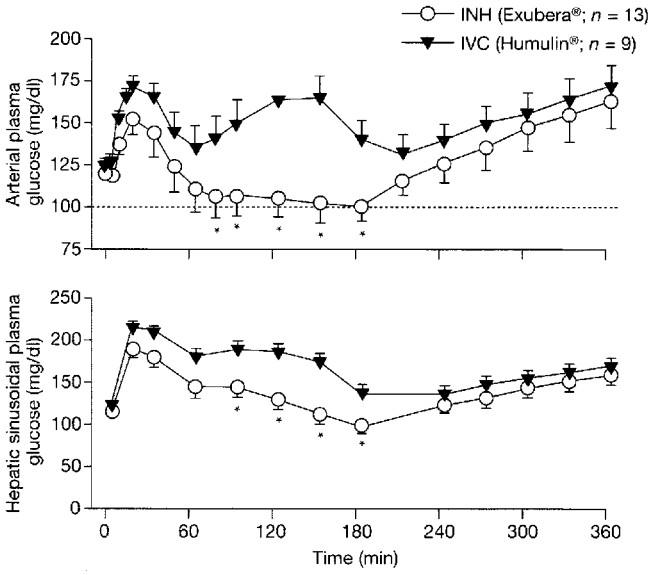

FIG. 2. Arterial and hepatic sinusoidal plasma glucose levels for Exubera inhalation and Humulin IVC-infused insulin. Data are means ± SE. *$P < 0.05$ for INH vs. IVC.

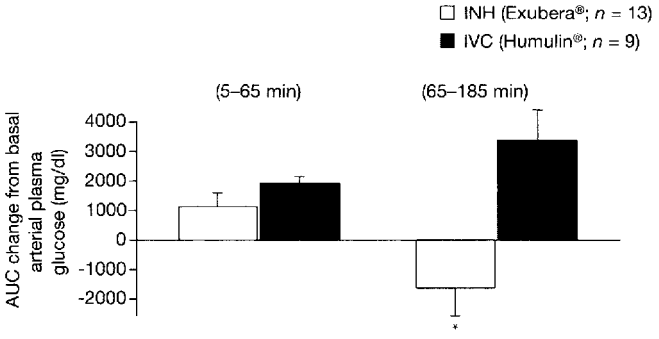

FIG. 3. AUC change from basal arterial plasma glucose for Exubera inhalation and Humulin IVC-infused insulin. Data are means ± SE. *P < 0.05 for INH vs. IVC.

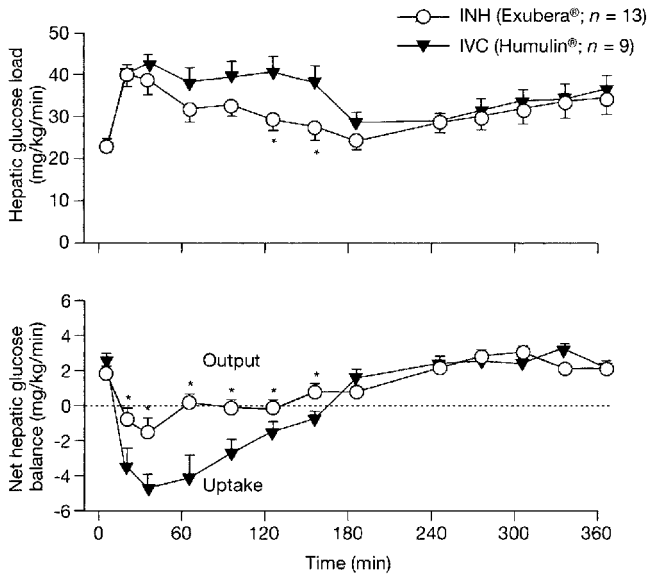

FIG. 4. Hepatic glucose load and net hepatic glucose balance for Exubera inhalation and Humulin IVC-infused insulin. Data are means ± SE. *P < 0.05 for INH vs. IVC.

study, net hepatic glucose output was again similar in the two groups, but the rates were elevated compared with what would be expected in the presence of hyperglycemia, thus clearly reflecting insulin deficiency.

The clearance and uptake of glucose by tissues other than the liver rose rapidly in both groups after the administration of insulin and glucose. Despite euglycemia and arterial insulin levels that were decreasing rapidly over the 2nd postdosing h in the INH group, nonhepatic glucose clearance continued to rise, peaking at 95 min (17.15 ± 1.71 ml · kg^{-1} · min^{-1}) (Fig. 5). It then fell over the next hour, but remained significantly elevated at 185 min (P < 0.05). Nonhepatic glucose uptake (as opposed to clearance) increased from 1.88 ± 0.39 to a peak of 12.45 ± 0.51 mg · kg^{-1} · min^{-1} at 65 min in the INH group (Fig. 5). The increases in nonhepatic glucose clearance and uptake in the IVC group were markedly less than in the INH group, despite an identical arterial insulin profile and a distinctly higher plasma glucose level (P < 0.05). Nonhepatic glucose clearance rose from 2.81 ± 0.52 to a peak of 8.12 ± 1.09 ml · kg^{-1} · min^{-1} in the IVC group, and nonhepatic glucose uptake increased from 2.56 ± 0.42 to a peak of 8.62 ± 0.96 mg · kg^{-1} · min^{-1} (Fig. 5). Thus, the AUCs for nonhepatic glucose clearance over 5–185 min in the INH and IVC groups, respectively, were 2,111 ± 212 and 1,089 ± 139 ml · kg^{-1} · min^{-1} (P < 0.05), and the nonhepatic glucose uptake AUCs over the same period were 1,645 ± 63 and 1,171 ± 112 mg · kg^{-1} · min^{-1}, respectively (P < 0.05) (Fig. 6).

The total glucose mass delivered by portal glucose infusion (5–170 min) was 17.3 and 16.6 g in the INH and IVC groups, respectively (the masses differed slightly because the glucose was delivered per kilogram of body weight) (Table 5). In the INH group, 1.5 g (8%) was taken up by the liver and 15.8 g (92%), by nonhepatic tissues. In the IVC group, 4.6 g (27%) was taken up by the liver and 12.0 g (73%) by nonhepatic tissues. Thus, in the INH group, significantly more glucose was disposed of by nonhepatic tissues (P < 0.05).

Arterial blood lactate levels declined similarly in the INH and IVC groups over the course of the study (Table 6). Net hepatic lactate balance was similar in the two groups from 0 to 125 min and 245 to 365 min; however, from 155 to 185 min, there was less net hepatic lactate output in the inhalation group (Table 6). This correlated with the difference in net hepatic glucose uptake and undoubtedly reflected increased hepatic glycolytic flux in the IVC group. Arterial blood alanine levels were lower in the INH group over the last 3 h, but the net hepatic alanine balance did not differ between groups during this period (Table 6). As expected, the alanine levels fell in response to a rise in insulin and then tended to drift back up as insulin levels became deficient. The arterial glycerol levels also fell initially in response to the rise in insulin and then rose as insulinopenia developed. The arterial blood glycerol level tended to rebound more quickly in the INH compared with the IVC group, but neither the glycerol level nor the net hepatic balance differed significantly between groups (Table 6).

DISCUSSION

These data show that inhalation of insulin provides an advantage in glycemic control during a simulated oral glucose load when compared with the effects of identical insulin levels resulting from intravenous insulin infusion. Three pieces of evidence support this conclusion. First,

TABLE 4
Peripheral glucose infusion rate required to maintain euglycemia in overnight-fasted dogs 3–6 h after Exubera administration or matched Humulin IVC insulin infusion

	185	215	245	275	305	335	365
INH	2.31 ± 0.73*	1.92 ± 0.63*	0.76 ± 0.38	0.66 ± 0.41	0.63 ± 0.39	0.54 ± 0.35	0.47 ± 0.33
IVC	0.11 ± 0.12	0.09 ± 0.09	0.31 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are means ± SE. INH group, n = 13; IVC group, n = 9. Time points are in minutes. Glucose infusion rate given as mg · kg^{-1} · min^{-1}. *P < 0.05 for INH vs. IVC.

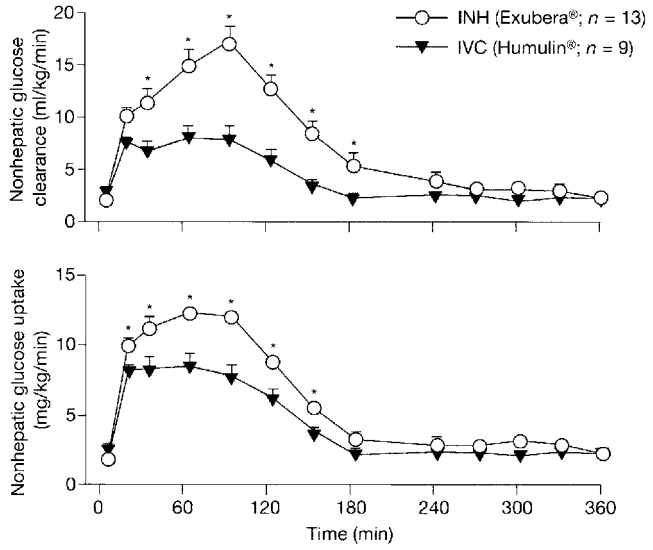

FIG. 5. Nonhepatic glucose clearance and uptake levels for Exubera inhalation and Humulin IVC-infused insulin. Data are means ± SE. *P < 0.05 for INH vs. IVC.

despite identical insulin levels in the INH and IVC groups, during the 2nd and 3rd h euglycemia was present in the INH group, whereas hyperglycemia was observed in the IVC group (the change in the arterial glucose AUC during this period was negative in the INH group and >3,000 mg/dl in the IVC group). In addition, the initial rise in plasma glucose levels tended to be smaller in the INH group (32 vs. 48 mg/dl in the IVC group). Second, the peak increase in nonhepatic glucose clearance was 10 ml · kg^{-1} · min^{-1} greater in the INH group than in the IVC group (Fig. 5). Third, after the portal glucose infusion was turned off, glucose infusion was required to maintain euglycemia in five of the INH dogs, whereas peripheral glucose infusion was still required in only one animal in the IVC group (Table 4). In addition, in three INH animals, glucose infusion was required for the entire study, despite the presence of subbasal plasma insulin levels by 185 min.

The rate of glucose infusion was controlled and was identical in both groups over the first 170 min of the studies. In response to a hyperglycemic, hyperinsulinemic challenge, the liver reacts by switching from net glucose output to net uptake. Previous studies have demonstrated that activation of the portal signal by portally administered

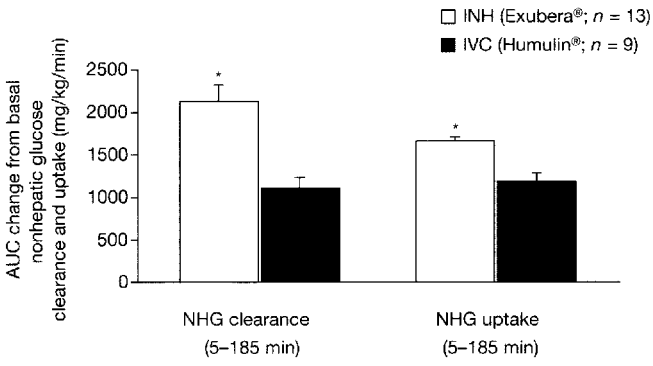

FIG. 6. AUC change from basal nonhepatic glucose (NHG) clearance and uptake for Exubera inhalation and Humulin IVC-infused insulin. Data are means ± SE. *P < 0.05 for INH vs. IVC.

TABLE 5
Distribution of glucose mass in overnight-fasted, conscious dogs administered Exubera or matched Humulin IVC insulin infusion

	Glucose infusion mass (g)	Net hepatic glucose uptake mass (g)	Nonhepatic glucose uptake mass (g)
INH	17.3	1.5 (8)*	15.8 (92)*
IVC	16.6	4.6 (27)	12.0 (73)

Data are means (%). INH group, n = 13; IVC group, n = 9. *P < 0.05 for INH vs. IVC.

glucose in combination with hyperinsulinemia results in hepatic glucose uptake of 4–5 mg · kg^{-1} · min^{-1} (8). Thus, the hepatic response in the IVC group was predictable, with nearly 5 mg · kg^{-1} · min^{-1} of glucose taken up at 30 min. In the INH group, however, net hepatic glucose uptake was only ~1 mg · kg^{-1} · min^{-1} (Fig. 4). This result may be partially explained by the fact that the hepatic glucose load tended to be lower in the INH group (as a result of lower plasma glucose levels). At 20 min, however, the hepatic glucose loads and insulin levels were the same in both groups, yet net hepatic glucose uptake was ~3 mg · kg^{-1} · min^{-1} lower in the INH group. This suggests that there may have been direct inhibition of glucose uptake at the liver immediately after insulin inhalation. The mechanism for this effect remains unclear.

Since the liver response was minimal, yet glucose control was markedly better in the INH group, the improvement was clearly the result of enhanced nonhepatic glucose disposal. Indeed, nonhepatic glucose clearance had increased by ~15 ml · kg^{-1} · min^{-1} by 95 min in the INH group, whereas it had increased by only ~5 ml · kg^{-1} · min^{-1} in the IVC group at the same time point (Fig. 5). Thus, although the INH group had a surprisingly small hepatic response, this was more than compensated for by the magnitude of nonhepatic glucose uptake. The majority of nonhepatic glucose uptake after a glucose load occurs in muscle, as the non−insulin-sensitive tissues (e.g., the central nervous system) saturate their uptake at values close to 100 mg/dl and fat has a limited capacity to store glucose (9–11). Thus, it would appear that the inhalation of insulin is associated with a marked increase in glucose uptake by muscle.

To unmask any differences in the glycemic effect due to the route of insulin administration, we chose a fairly large dosage of Exubera. As a consequence of the potent effect of inhaled insulin on nonhepatic glucose uptake, the excursion in plasma glucose level was minimal, thereby limiting the role of the liver in glucose uptake and storage. Had the Exubera dosage and the resulting plasma insulin levels been lower relative to levels in the IVC group, the arterial plasma glucose profiles of the two groups would have been the same. In that case, the hepatic and nonhepatic responses in the two groups would probably have been similar. If our findings apply to humans, the dosage of inhaled insulin could be adjusted so that a normal tissue distribution of glucose could be expected. Thus, in a clinical setting, this increased bioactivity would reduce the amount of insulin that would have to be inhaled for effective glucose control.

In a previous study in humans, when comparable systemic dosages of inhaled insulin (Exubera) or subcutane-

TABLE 6
Arterial blood lactate, alanine, and glycerol levels and net hepatic balance in overnight-fasted conscious dogs after Exubera administration or matched Humulin IVC insulin infusion

	5	20	35	65	95	155	245	305	365
Blood lactate level (μmol/l)									
INH	1,102 ± 90	939 ± 138	837 ± 114	834 ± 102	814 ± 125	620 ± 126	479 ± 80	478 ± 57	472 ± 62
IVC	1,198 ± 198	1,095 ± 234	990 ± 180	771 ± 109	689 ± 98	576 ± 109	552 ± 89	546 ± 84	570 ± 80
Net hepatic lactate balance (μmol · kg^{-1} · min^{-1})									
INH	7.20 ± 2.41	7.11 ± 2.12	11.00 ± 1.99	7.24 ± 1.91	4.81 ± 2.06	0.28 ± 1.65*	0.59 ± 2.40	0.15 ± 1.88	−2.58 ± 1.26
IVC	5.76 ± 2.12	7.47 ± 1.38	9.49 ± 2.81	9.40 ± 2.69	6.03 ± 2.26	5.89 ± 1.88	1.47 ± 1.44	1.17 ± 1.55	−1.71 ± 1.54
Blood alanine level (μmol/l)									
INH	405 ± 20	376 ± 21	330 ± 20	283 ± 18	248 ± 15	214 ± 16*	220 ± 14*	248 ± 17*	270 ± 21*
IVC	372 ± 20	362 ± 28	356 ± 33	299 ± 22	274 ± 24	282 ± 29	321 ± 33	316 ± 30	338 ± 35
Net hepatic alanine balance (μmol · kg^{-1} · min^{-1})									
INH	−1.29 ± 0.25	−2.13 ± 0.47	−1.63 ± 0.26*	−2.01 ± 0.27	−1.63 ± 0.34	−2.05 ± 0.23	−1.75 ± 0.38	−1.42 ± 0.26	−1.33 ± 0.48
IVC	−1.44 ± 0.27	−1.56 ± 0.22	−2.82 ± 0.66	−2.14 ± 0.27	−1.88 ± 0.42	−1.67 ± 0.22	−2.10 ± 0.32	−1.93 ± 0.38	−2.59 ± 0.43
Blood glycerol level (μmol/l)									
INH	21 ± 3	29 ± 5	19 ± 3	31 ± 6	40 ± 11	58 ± 9	62 ± 10	78 ± 12	87 ± 11
IVC	35 ± 8	20 ± 7	16 ± 5	16 ± 3	22 ± 4	42 ± 8	68 ± 6	63 ± 8	81 ± 9
Net hepatic glycerol balance (μmol · kg^{-1} · min^{-1})									
INH	−0.33 ± 0.07	−0.65 ± 0.19	−0.30 ± 0.10	−0.86 ± 0.32	−0.78 ± 0.22	−1.45 ± 0.41	−1.24 ± 0.18	−1.68 ± 0.32	−1.87 ± 0.22
IVC	−0.54 ± 0.17	−0.41 ± 0.17	−0.23 ± 0.17	−0.31 ± 0.08	−0.34 ± 0.14	−0.88 ± 0.24	−1.38 ± 0.15	−1.30 ± 0.25	−1.75 ± 0.29

Data are means ± SE. Time points are in minutes. *$P < 0.05$ for INH vs. IVC. Balance >0 indicates net output and <0 indicates net uptake. INH group, $n = 13$; IVC group, $n = 9$.

ous insulin (Humulin) were administered 10 min before a test meal, inhalation reduced the postprandial glucose rise (0–3 h) at least as effectively as subcutaneous insulin, with less late (4–5 h) glucose reduction below baseline (12). Remarkably, inhaled insulin (Exubera) also reduced fasting glucose in insulin-requiring subjects in the absence of increased overnight dosages of long-acting insulin (1). Although the present study was not long enough to directly explain this unique effect, one possibility is that insulin inhalation amplifies prandial muscle glucose uptake, thereby limiting liver glycogen repletion and resulting in a reduction in fasting glucose production and, therefore, the blood glucose level as well.

The explanation for the unique effect of inhalation of Exubera on nonhepatic glucose disposal is not readily apparent, but a variety of hypotheses exist. First, it is possible that a signal is generated by the lung or that a substance is released from the lung after insulin inhalation that stimulates nonhepatic glucose uptake by acting directly on target tissues, increasing the sensitivity of those tissues to insulin, and/or triggering a neurally mediated response. One such candidate molecule is nitric oxide (NO), which has been shown to have both insulin-dependent and -independent effects on glucose metabolism in muscle (13,14). In addition, NO synthase is activated by insulin in both the lung and muscle (13,15,16). However, NO produced and released local to skeletal muscle in response to hyperinsulinemia cannot explain the enhanced nonhepatic glucose clearance in the INH group, as the level of insulin in muscle was similar in both groups. Furthermore, NO has a half-life that is generally thought to be around a few milliseconds in the presence of erythrocytes; therefore, NO released from the lung presumably would be rapidly bound and inactivated by hemoglobin. Recent studies, however, have suggested the possibility that some NO may escape inactivation by traveling in a plasma compartment in the blood and act as an endocrine factor distal to the site of its release (17,18). Furthermore, it has been suggested that under hyperinsulinemic conditions, NO action in the liver can alter the release of a humoral factor, which increases nonhepatic glucose disposal (19,20). This mechanism may involve parasympathetic input as the effect is blocked by surgical liver denervation and atropine and is restored by acetylcholine administration (21,22). Thus, it is conceivable that an NO-mediated mechanism might be involved in response to insulin inhalation. However, other factors originating in the lung may also be involved in mediating the observed effects. Future studies are needed to identify candidate substances that could be released after inhalation of insulin.

Other possible indirect effects of insulin inhalation on glucose metabolism exist as well. For example, inhalation of insulin might affect pulmonary neurogenic pathways and associated metabolic regulatory circuits. In our study, we saw no direct evidence for this occurring, although glucose utilization and peripheral insulin sensitivity have been shown to increase at high altitudes as a result of differences in oxygen sensing (23,24). Although this effect may result from a direct action on target tissues due to lower oxygen tension in the blood, it could also be the result of changes in oxygen sensing in the lungs. In addition, the chemoreceptor carotid bodies are sensitive to various signals, including hypoxia, hypoglycemia, ex-

cess carbon dioxide, NO, and pH (25,26). NO and carbon dioxide appear to have inhibitory effects on carotid body function (25), and denervation of the area surrounding the carotid bodies results in inhibition of glucose production and enhanced glucose disposal (27). On the other hand, stimulation of carotid bodies produces sympathetic activation and has been demonstrated to produce hyperglycemia in vivo (26,27). Thus, if carotid body signaling is reduced after inhalation, possibly through NO or CO_2, greater glucose disposal would be expected, as was observed in the present study.

Several other explanations for the unique effect of Exubera exist, but these are less likely. For example, degradation of insulin in the lung could have resulted in modification of the hormone such that it was still biologically active but was not recognized by the antibodies in the insulin assay. If this had been the case, the level of active insulin in the inhalation group would have exceeded the level in the IVC group, even though the levels appeared similar in the radioimmunoassay. This explanation is unlikely, however, as the fall in blood glycerol levels in the INH and IVC groups paralleled each other, suggesting that the biologically active insulin levels were indeed properly assessed and similar. Another mechanism by which nonhepatic glucose uptake might be increased after insulin inhalation is by increased insulin-stimulated lung glucose uptake. Based on studies in the rat, however, it appears that although lung glucose utilization can increase in response to insulin, the basal rates of lung glucose uptake are small and increase only to a plateau that is approximately twofold of basal levels (28–30). Based on comparison with the rat, lung glucose uptake in the dog might be predicted to be 0.4–0.6 $mg \cdot kg^{-1} \cdot min^{-1}$ during hyperinsulinemia, which is not enough to account for the difference in nonhepatic glucose uptake in the INH and IVC groups. The rates of rat lung glucose uptake were determined in the euglycemic state; however, they might be somewhat greater during hyperglycemia. Finally, although the composition of the insulin was identical in both groups, it is conceivable that the additional effect seen in the INH group was the result of a direct or indirect effect of the mannitol citrate carrier. Although mannitol citrate is considered to be inert and there is no evidence to suggest this otherwise, this was not controlled for in the protocol.

In summary, this study demonstrates that inhalation of Exubera results in a unique effect on nonhepatic glucose clearance. This effect is manifested within 15 min, peaks at about 90 min, and lasts for up to 4 h. The likely site of this effect is muscle, but the cellular mechanism by which it occurs requires further study.

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