Original Article

Inhalation of Human Insulin (Exubera) Augments the Efficiency of Muscle Glucose Uptake In Vivo

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This study assessed the site of increased glucose uptake resulting from insulin inhalation, quantified its effect under steady-state glucose concentrations, and identified the time to onset of effect. Human insulin was administered to 13 beagles via inhalation (Exubera [insulin human (rDNA origin)] Inhalation Powder; $n = 7$) or infusion into the inferior vena cava (Humulin R; $n = 6$) using an algorithm to match plasma insulin levels and kinetics for both groups. Somatostatin and glucagon were infused. Glucose was delivered into the portal vein ($4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and a peripheral vein, as needed, to maintain arterial plasma glucose levels at 180 mg/dl. Hepatic exposure to insulin and glucose and liver glucose uptake were similar in both groups. Despite comparable arterial insulin and glucose levels, hind-limb glucose uptake increased 2.4-fold after inhalation compared with infusion due to increased muscle glucose uptake. Glucose infusion rate, nonhepatic glucose uptake, and tracer-determined glucose disposal were about twice as great compared with intravenous insulin. The effect appeared after 1 h, persisting at least as long as arterial insulin levels remained above basal. Pulmonary administration of insulin increases nonhepatic glucose uptake compared with infusion, and skeletal muscle is the likely site of that effect. *Diabetes* 55:3604–3610, 2006

Therapeutic alternatives to insulin injection for patients with diabetes are currently being developed. One such alternative is pulmonary insulin delivery. Inhaled human insulin (Exubera [insulin human (rDNA origin)] Inhalation Powder) has been approved in the U.S. and European Union for treatment of hyperglycemia in adults with type 1 or type 2 diabetes. Clinical trials comparing inhalation with subcutaneous delivery of insulin have demonstrated reduced fasting plasma glucose levels in patients with diabetes by as much as 40 mg/dl (1–5). This is an intriguing but, as yet, unexplained finding (6).

When insulin inhalation was compared with subcutaneous injection in dogs, despite similar overall arterial and hepatic insulin areas under the curve (AUCs), the glucose required to maintain euglycemia following inhalation was 20% greater (7) and insulin action was twice as great (7a). To determine if these differences were due to the disparity in insulin pharmacokinetics following pulmonary versus subcutaneous administration, or rather an effect associated with the route of insulin delivery, insulin was delivered by infusion into the inferior vena cava (IVC) so that the insulin concentrations and kinetics were matched to those occurring with administration by inhalation (8). In that study, despite delivery of the same glucose load, marked hyperglycemia occurred when insulin was infused but not inhaled. This was due to considerably greater nonhepatic glucose clearance following insulin inhalation, not an increase in hepatic glucose uptake. These observations suggested that there was a unique nonhepatic glucose-lowering effect associated with insulin delivered by inhalation.

The purpose of the present study was to determine the site of increased glucose uptake resulting from insulin inhalation, to quantify its effect under steady-state glucose concentrations, and to establish the time course of the onset of the effect. Using three independent methods, this study demonstrates that administration of insulin into the lungs does indeed increase nonhepatic glucose uptake, that skeletal muscle is the site of that effect, and that the effect manifests by 1 h after insulin inhalation.

RESEARCH DESIGN AND METHODS

Experiments were conducted on 13 healthy, conscious, 18-h fasted, female beagle dogs (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 in order to implant infusion catheters into the jejunal, splenic, and IVC veins. Sampling catheters were implanted into the portal, hepatic, and right iliac veins and left femoral artery. Transonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic and right iliac arteries and the portal vein, as described elsewhere (9). Intraportal catheters (splenic and jejunal) were used for the infusion of glucose (50% dextrose; Baxter Healthcare Corporation, Deerfield, IL). Each animal was used only once.

On the day of the study, intravenous catheters were placed into the cephalic and/or saphenous veins for somatostatin (Bachem California, Tor-

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ATII, angiotensin II; AUC, area under the curve; BK_2, bradykinin receptor subtype 2; GIR, glucose infusion rate; IVC, inferior vena cava; MAP, mean arterial blood pressure.

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TABLE 1
Arterial plasma C-peptide and hepatic sinusoidal glucagon levels in overnight-fasted conscious dogs administered insulin by inhalation ($n = 7$) or matched IVC infusion ($n = 6$)

	Arterial plasma C-peptide level (ng/ml)		Hepatic sinusoidal glucagon level (pg/ml)	
t (min)	Inhalation	IVC infusion	Inhalation	IVC infusion
−20	0.34 ± 0.06	0.26 ± 0.03	61 ± 8	49 ± 5
0	0.43 ± 0.13	0.37 ± 0.09	64 ± 9	50 ± 8
5	0.56 ± 0.11	0.44 ± 0.12	72 ± 9	60 ± 5
20	0.17 ± 0.05	0.26 ± 0.18	58 ± 6	60 ± 6
35	0.12 ± 0.05	0.09 ± 0.04	50 ± 5	49 ± 6
65	0.12 ± 0.05	0.05 ± 0.02	44 ± 5	42 ± 4
95	0.11 ± 0.05	0.06 ± 0.03	43 ± 4	38 ± 4
125	0.12 ± 0.06	0.06 ± 0.02	42 ± 4	32 ± 4
185	0.11 ± 0.06	0.06 ± 0.03	39 ± 5	33 ± 3
245	0.11 ± 0.05	0.05 ± 0.03	38 ± 4	33 ± 3

rance, CA), glucagon (Eli Lilly, Indianapolis, IN), and glucose delivery. Each experiment consisted of a tracer equilibration period (−120 to −20 min), a basal period (−20 to 0 min), and an experimental period (0 to 245 min). Exogenous insulin exposure started at 0 min. A priming dose of [3-^{3}H]glucose (8 μCi; DuPont NEN, Boston, MA) was given at −120 min, followed by constant [3-^{3}H]glucose infusion (0.08 μCi/min) until the start of glucose infusion (5 min). During the hyperglycemic clamp, the rate of [3-^{3}H]glucose infusion was varied according to the exogenous glucose infusion rate (GIR) to keep the glucose-specific activity constant. Following the −20-min sample, the dogs were anesthetized (0.2 ml acepromazine and 5% isofluorane) and intubated as previously described (8). Insulin was then administered by inhalation or intravenous infusion as described below.

Exubera (insulin human [rDNA origin] inhalation powder; Pfizer, New York, NY; Nektar Therapeutics, San Carlos, CA) is a dry powder human insulin of recombinant origin and specially formulated for intrapulmonary administration. The insulin is packaged in foil blisters, with each blister containing either 1.0 or 3.0 mg of human insulin. Recombinant human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was used for intravenous insulin infusion. It is assumed that the biological activity of insulin in both preparations was identical.

In the inhalation group, at $t = 0$ min, seven dogs were exposed to the contents of one blister of Exubera using a modified P2.3 device (Nektar Therapeutics, San Carlos, CA), as previously described (8). No adverse clinical signs related to insulin inhalation were observed during the study. In the IVC group, after sham inhalation exposure, at $t = 0$ min, Humulin R (diluted in normal saline [0.9% NaCl; Baxter, Deerfield, IL] with added plasma [3:100 ml]) was infused intravenously into the IVC in six dogs using an algorithm designed to match the arterial plasma insulin kinetic profile of the inhaled insulin group (8). After inhalation the animals were allowed to recover from the anesthesia (this occurred rapidly at ~5–10 min).

Each dog in the inhalation group was administered a 1-mg blister of Exubera. The amount of aerosolized powder delivered to the dogs was ~59%. With the method of exposure used, it is reasonable to assume that all of this material was deposited in the dog's respiratory tract and, based on the size of the aerosolized insulin particles and the method of exposure used, about half of this amount was deposited in the pulmonary region where most absorption of deposited aerosols occurs. Thus, it was estimated that each dog in the inhalation group deposited ~0.59 mg (15 units) of insulin in the total respiratory tract and ~0.29 mg (7.5 units) in the alveolar region. The total amount of insulin administered via intravenous infusion in the IVC group was 1.6 units/kg.

At $t = 5$ min, intravenous somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and glucagon (0.5 $ng \cdot kg^{-1} \cdot min^{-1}$) were infused to inhibit endogenous insulin and glucagon secretion and to replace basal levels of endogenous glucagon, respectively. These infusions were continued throughout the remainder of the experiment. Intraportal glucose infusion (50% dextrose) was delivered at 4 $mg \cdot kg^{-1} \cdot min^{-1}$ from 5 to 245 min. Peripheral intravenous glucose infusion was also given, as needed, to maintain the plasma glucose level at ~180 mg/dl, or matched between groups toward the end of the experiments.

Blood sampling and analytical procedures. Blood samples were collected from the femoral artery and the iliac, hepatic portal, and hepatic veins. Hematocrit, plasma glucose, [3-^{3}H]glucose, glucagon, insulin, C-peptide, blood alanine, lactate, and glycerol concentrations were determined as previously described (9). Hepatic blood flow was measured using transonic flow probes, as described elsewhere (9).

Data analysis. Net hepatic substrate balance (NHB) was calculated with the arterial-venous difference method as $NHB = load_{out} - load_{in}$, where $load_{out} = H \times HF$ and $load_{in} = (A \times AF) + (P \times PF)$, 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. Blood flow in the hepatic artery, portal vein, and iliac artery and mean arterial blood pressure (MAP) were measured using Transonic flow probes and a Transit-time Perivascular Flow Meter (Model T403; Ithaca, NY). Using this calculation, a positive value represents net output by the liver, and a negative value represents net hepatic uptake. Hind-limb balance was calculated as above, using arterial and femoral vein substrate concentrations and hind-limb blood flow. For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using previously published correction factors (ratio of blood to plasma concentrations) (8). Blood glucose concentrations were used for the calculation of net glucose balance because the use of whole-blood glucose ensures accurate balance measurements regardless of the characteristics of glucose entry into the erythrocyte. Nonhepatic glucose uptake was calculated as the GIR plus net hepatic glucose balance, with changes in the glucose mass accounted for when deviations from steady-state were present (10–12). Tracer-determined whole-body glucose utilization (R_d) was measured using an infusion of [3-^{3}H]glucose (13). A two-compartment model with canine parameters was used for data analysis (14). The approximate substrate levels in plasma entering the liver sinusoids were calculated as (A × %AF) + (P × %PF), where A and P are arterial and portal vein hormone concentrations, respectively, and %AF and %PF are the fractional contributions of arterial and portal flow to total hepatic blood flow, respectively. AUC was calculated using the trapezoidal rule.

Statistical analysis. Data are presented as means ± SEM. 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 initiation of somatostatin infusion, the arterial C-peptide levels dropped rapidly (<30 min) in both groups to concentrations near the level of detection of the assay (0.05 ng/ml), indicating that endogenous insulin secretion was quickly and effectively suppressed (Table 1). The arterial and liver sinusoidal glucagon levels were close to basal concentrations and were equivalent in the two groups throughout the experiment (Table 1).

Arterial plasma insulin levels peaked in the inhalation and IVC groups at 62 ± 11 and 67 ± 5 μU/ml (at 35 and 10 min), respectively ($P = 0.42$ for between-group difference; Fig. 1*A*). Hepatic sinusoidal plasma insulin levels peaked at 53 ± 10 and 58 ± 4 μU/ml (at 35 and 20 min, respectively; $P = 0.59$; Fig. 1*B*). The total 245-min AUC for arterial insulin was 5,887 ± 904 and 5,726 ± 246 $\mu U \cdot ml^{-1} \cdot min$ in the inhalation and IVC groups, respectively ($P = 0.70$; Fig. 1*C*). Thus, the insulin levels and kinetic profiles were similar in both groups.

The arterial plasma glucose level increased from basal

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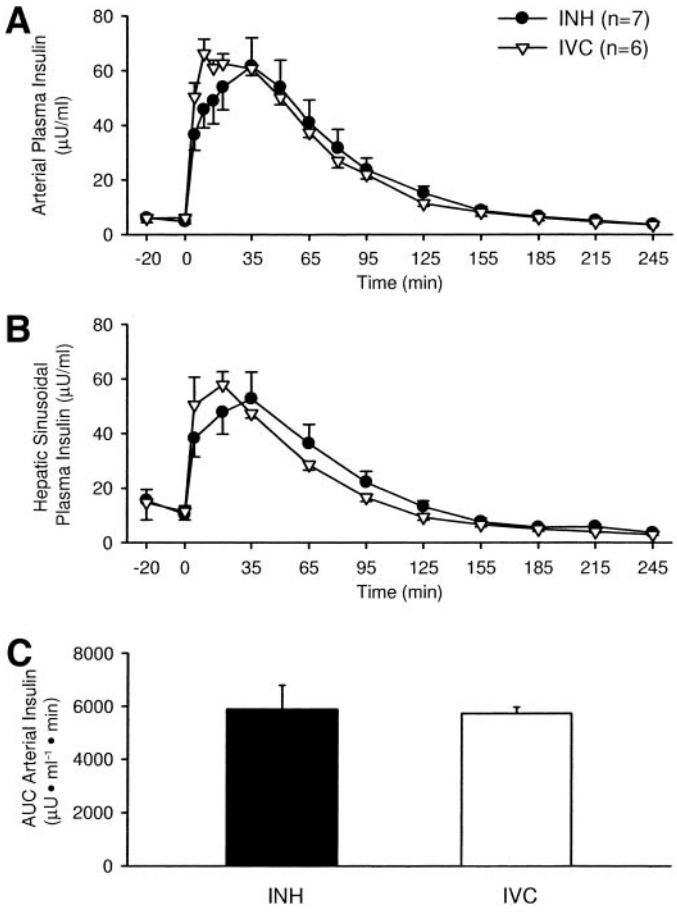

FIG. 1. Arterial and hepatic sinusoidal plasma insulin levels and 245-min arterial AUC for inhaled (INH; $n = 7$) and IVC-infused ($n = 6$) insulin.

(~110 mg/dl) to ~180 mg/dl in both groups as a result of portal (4 mg · kg^{-1} · min^{-1}) and peripheral (variable rate) glucose infusion (Fig. 2*A*). During the last 2 h, the arterial glucose level increased in the IVC group as a result of insulinopenia, and glucose was infused in the inhalation group to match this rise. The peripheral GIR was similar in both groups during the first 30 min after insulin administration, but by 95 min the GIR was 22 ± 3 mg · kg^{-1} · min^{-1} in the inhalation group compared with 11 ± 1 mg · kg^{-1} · min^{-1} in the IVC group ($P < 0.05$; Fig. 2*B*). It remained significantly greater in the inhalation group until 185 min and also tended to be higher during the last 2 h of the experiment. Thus, the 245-min peripheral GIR AUCs were 2,619 ± 395 and 1,325 ± 165 mg/kg in the inhalation and IVC groups, respectively ($P < 0.05$; Fig. 2*C*).

The hepatic glucose load increased similarly in both groups as a result of portal glucose infusion and matched arterial glucose levels (Table 2). Net hepatic glucose balance was not significantly different in the two groups, switching from net output during the basal period to uptake during the experimental period (Fig. 3*A*). Nonhepatic glucose uptake increased from basal, peaking at 22 ± 2 and 13 ± 1 mg · kg^{-1} · min^{-1} at 95 and 65 min in the inhalation and IVC groups, respectively ($P < 0.05$; Fig. 3*B*). The 245-min changes from basal in nonhepatic glucose uptake AUCs were 2,456 ± 333 and 1,389 ± 277 mg/kg, respectively ($P < 0.05$; Fig. 3*C*). At 95 min, whole-body glucose R_d was 25 ± 3 and 13 ± 4 mg · kg^{-1} · min^{-1} in the inhalation and IVC groups, respectively, and the 245-min changes from basal in R_d AUCs were 2,684 ± 471 and 1,239 ± 567 mg/kg, respectively ($P < 0.05$; Fig. 4).

Hind-limb blood flow tended to increase during hyperinsulinemia in both groups, and hind-limb glucose load increased by twofold (Table 2). Net hind-limb glucose uptake was 15 ± 4 and 7 ± 2 mg/min at 95 min in the inhalation and IVC groups, respectively, and three- and sixfold greater in the inhalation group at 125 and 155 min ($P < 0.05$; Fig. 5*A*). Percent hind-limb glucose extraction was also greater in the inhalation group than in the IVC group between 95 and 155 min ($P < 0.05$; Fig. 5*B*). The changes from basal net hind-limb glucose uptake AUCs were 2,619 ± 395 and 1,325 ± 165 mg 245-min in the two groups, respectively ($P < 0.05$; Fig. 5*C*). MAP tended to be lower after insulin inhalation versus sham inhalation; however, MAP was only available from a limited number of the studies and a high standard error prohibits drawing any conclusion from these data (Table 2).

Arterial blood lactate and alanine levels increased during hyperinsulinemia in both groups (Table 3). Despite an increase in hind-limb glucose uptake, especially in the inhalation group, net hind-limb lactate output did not occur in either group. Furthermore, net hind-limb lactate uptake was similar in the two groups (Table 3). Likewise, net hind-limb alanine balance did not differ between groups (Table 3). Arterial blood glycerol levels decreased similarly in response to hyperinsulinemia in the inhalation and IVC groups, and there was no difference in net hind-limb glycerol output (Table 3).

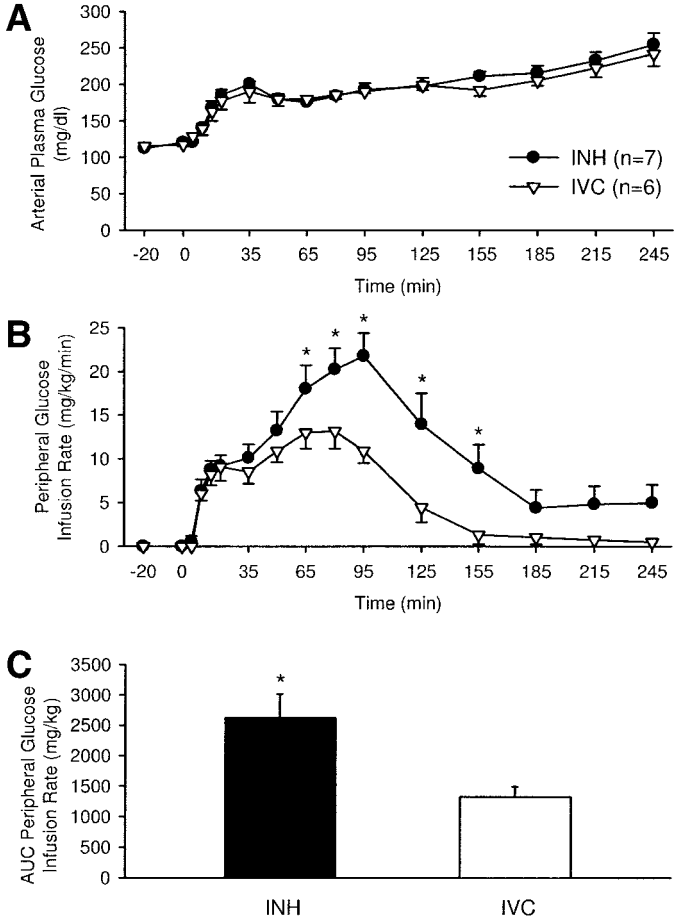

FIG. 2. Arterial plasma glucose levels and peripheral GIR and 245-min AUC for inhaled (INH; $n = 7$) and IVC-infused ($n = 6$) insulin. *$P < 0.05$ INH vs. IVC.

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TABLE 2
Hepatic glucose load and extraction, hind-limb blood flow and glucose load, and MAP in overnight-fasted conscious dogs administered insulin by inhalation ($n = 7$) or matched IVC infusion ($n = 6$)

	Hepatic glucose load (mg · kg^{-1} · min^{-1})		Hepatic glucose extraction (%)		Hind-limb blood flow (ml/min)		Hind-limb glucose load (mg/min)		MAP (mmHg)	
t (min)	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion
−20	31 ± 3	29 ± 2			88 ± 14	85 ± 13	73 ± 11	74 ± 13	96 ± 8	96 ± 4
0	22 ± 3	26 ± 3			81 ± 12	84 ± 10	71 ± 8	73 ± 7	95 ± 8	100 ± 2
5	24 ± 3	29 ± 3			75 ± 9	77 ± 8	67 ± 8	73 ± 8	91 ± 6	97 ± 8
20	46 ± 5	44 ± 5	4 ± 2	6 ± 1	88 ± 10	86 ± 7	120 ± 12	114 ± 15	87 ± 3	103 ± 7
35	51 ± 6	46 ± 6	6 ± 2	5 ± 2	97 ± 9	97 ± 7	145 ± 15	138 ± 18	87 ± 4	98 ± 7
65	48 ± 5	44 ± 3	6 ± 1	8 ± 2	102 ± 18	96 ± 12	133 ± 25	129 ± 18	89 ± 4	96 ± 5
95	55 ± 5	50 ± 6	8 ± 2	4 ± 1	97 ± 20	93 ± 12	140 ± 32	131 ± 16	90 ± 3	100 ± 11
125	57 ± 8	53 ± 4	6 ± 0	4 ± 2	92 ± 20	87 ± 11	130 ± 26	130 ± 18	87 ± 4	102 ± 9
155	59 ± 5	50 ± 4	2 ± 4	4 ± 2	85 ± 14	84 ± 10	132 ± 22	121 ± 17	89 ± 2	102 ± 9
185	58 ± 6	52 ± 2	4 ± 1	3 ± 3	83 ± 11	84 ± 11	132 ± 18	129 ± 19	92 ± 3	100 ± 10
215	60 ± 6	54 ± 3	4 ± 1	1 ± 2	83 ± 14	79 ± 12	143 ± 28	132 ± 24	95 ± 2	100 ± 10
245	65 ± 6	60 ± 5	5 ± 1	3 ± 3	74 ± 16	75 ± 11	138 ± 32	137 ± 25	94 ± 3	97 ± 12

$n = 6$ and 3 for MAP, respectively.

DISCUSSION

The purpose of this study was to further investigate our observation that insulin inhalation is associated with increased nonhepatic glucose disposal by determining the site where this effect occurs and to establish the time course of the onset of the effect. To do this, the arterial plasma insulin kinetics were closely matched between the groups in which insulin either entered the blood via pulmonary absorption or the IVC, and the plasma glucose levels were clamped at equal concentrations. The data indicate that the pulmonary route of administration was associated with significantly greater glucose disposal, as determined by three independent methods, despite the equivalent arterial plasma insulin and glucose levels. Over a 4-h period, insulin inhalation resulted in twofold or greater increases in nonhepatic glucose uptake, tracer-determined glucose disposal, and hind-limb glucose uptake compared with insulin administered via the IVC. The improvement in glucose disposal following insulin inhalation occurred in the hind limb and, therefore, presumably reflects enhanced skeletal muscle glucose uptake. Liver glucose uptake, on the other hand, was similar in the two groups.

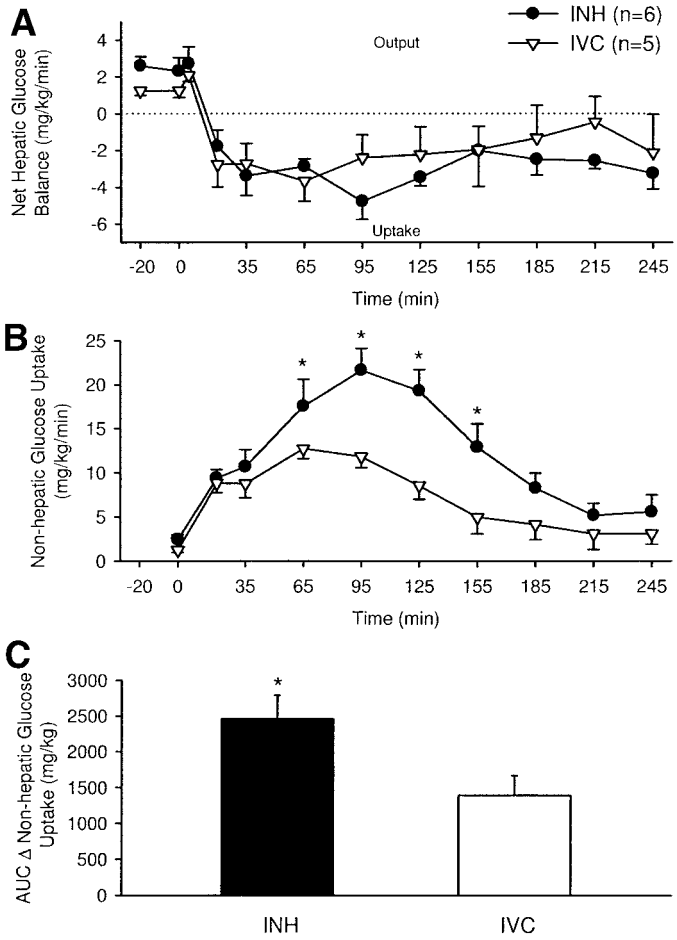

FIG. 3. Net hepatic glucose balance and nonhepatic glucose uptake and 245-min AUC change from basal for inhaled (INH; $n = 6$) and IVC-infused ($n = 5$) insulin. *$P < 0.05$, INH vs. IVC.

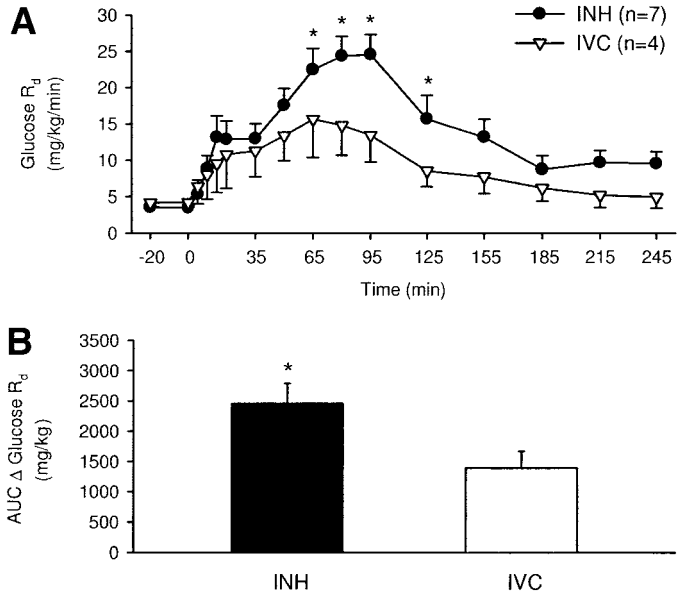

FIG. 4. Glucose utilization and 245-min AUC change from basal for inhaled (INH; $n = 7$) and IVC-infused ($n = 4$) insulin. *$P < 0.05$, INH vs. IVC.

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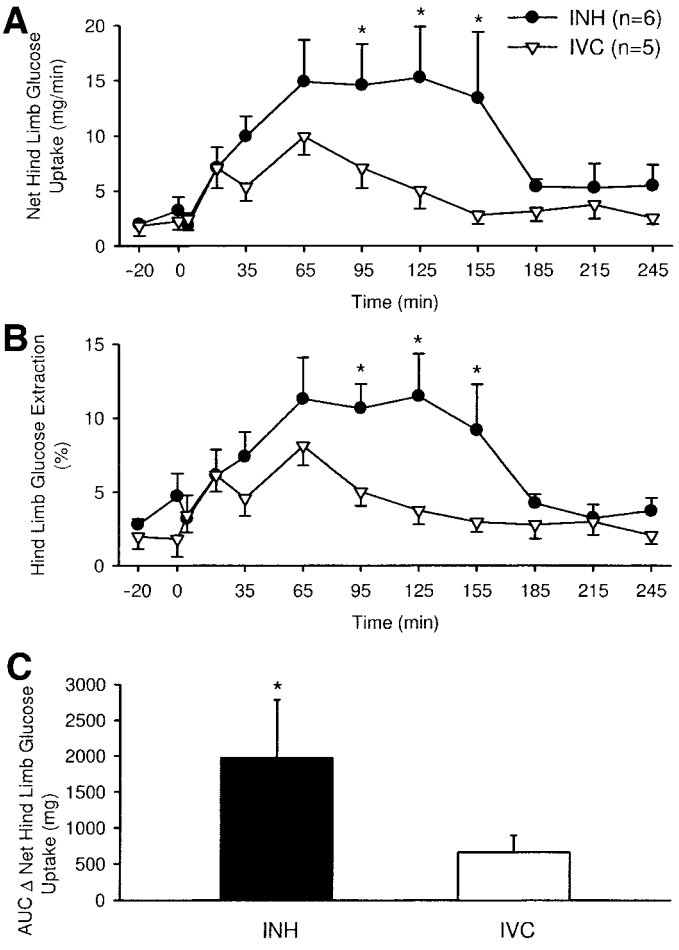

FIG. 5. Net hind-limb glucose uptake, extraction, and 245-min AUC change from basal for inhaled (INH; $n = 6$) and IVC-infused ($n = 5$) insulin. *$P < 0.05$, INH vs. IVC.

As demonstrated by each of the independent methods, the effect associated with insulin inhalation was present after 1 h. The duration of the effect remains unclear, however, since the arterial insulin levels were below basal after 3 h, and thus during the insulin-deficient state there was little stimulation of glucose uptake in either group. Had the arterial insulin level remained elevated, however, the difference in glucose disposal between groups may have persisted.

An unexpected clinical finding is reduced fasting plasma glucose levels in patients with diabetes following premeal treatment with inhaled insulin compared with subcutaneous insulin administration (both groups were also treated with subcutaneous long-acting insulin overnight), a finding observed with dry powder (2–5) and liquid (1) formulations. If the increase in glucose disposal associated with insulin inhalation persists even after the clearance of inhaled insulin from the bloodstream, a prolonged increase in glucose utilization could be responsible for the observed reduction in fasting glucose levels. In the present study, despite insulin deficiency during the last hour, there was still a tendency for the GIR, nonhepatic glucose uptake, glucose R_d, and net hind-limb glucose uptake to be greater in the inhalation group, suggesting that a prolonged increase in glucose utilization may in fact occur.

The present study, however, does not rule out the possibility that the increased glucose disposal associated with insulin inhalation is restricted to the period of hyper-

TABLE 3
Arterial blood lactate, alanine, and glycerol levels and net hind-limb balance in overnight-fasted conscious dogs after insulin administration by inhalation ($n = 7$) or matched IVC infusion ($n = 6$)

	Blood lactate level (μmol/l)		Net hind-limb lactate balance (μmol · kg^{-1} · min^{-1})		Blood alanine level (μmol/l)		Net hind-limb alanine balance (μmol · kg^{-1} · min^{-1})		Blood glycerol level (μmol/l)		Net hind-limb glycerol balance (μmol · kg^{-1} · min^{-1})	
t (min)	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion	Inhalation	IVC infusion
−20	616 ± 77	691 ± 149	0.6 ± 1.9	−1.6 ± 3.0	348 ± 53	404 ± 6	1.2 ± 0.6	−0.4 ± 0.6	74 ± 6	59 ± 3	2.2 ± 1.0	1.5 ± 0.5
0	1,168 ± 190	1,014 ± 305	−4.7 ± 3.7	−4.2 ± 4.6	544 ± 98	523 ± 84	0.9 ± 1.3	0.5 ± 1.0	13 ± 6	29 ± 9	1.3 ± 0.3	1.7 ± 0.7
5	1,176 ± 127	1,456 ± 252	−5.5 ± 3.7	−9.8 ± 3.5	615 ± 76	624 ± 40	0.8 ± 1.1	−0.4 ± 0.9	19 ± 3	17 ± 2	1.2 ± 0.4	0.6 ± 0.1
20	1,017 ± 109	1,107 ± 264	−4.5 ± 3.3	−3.1 ± 3.8	499 ± 79	551 ± 35	0.6 ± 0.6	−0.1 ± 1.0	30 ± 3	32 ± 7	1.0 ± 0.2	0.7 ± 0.3
35	1,043 ± 125	996 ± 172	−6.4 ± 3.3	−1.8 ± 2.8	446 ± 67	514 ± 35	0.8 ± 0.5	0.8 ± 0.8	35 ± 5	26 ± 6	0.8 ± 0.3	0.7 ± 0.2
65	959 ± 115	853 ± 132	−2.6 ± 3.0	−1.9 ± 1.9	353 ± 50	412 ± 33	0.7 ± 0.7	1.0 ± 1.0	21 ± 5	20 ± 5	0.5 ± 0.3	0.4 ± 0.2
95	916 ± 110	862 ± 138	−1.2 ± 2.6	−1.5 ± 2.9	310 ± 43	372 ± 27	1.7 ± 0.8	1.8 ± 1.0	19 ± 6	21 ± 1	0.9 ± 0.3	0.6 ± 0.4
125	841 ± 100	787 ± 127	−2.4 ± 1.7	−2.2 ± 2.6	295 ± 49	343 ± 23	0.8 ± 1.4	0.7 ± 0.9	22 ± 7	31 ± 3	0.8 ± 0.4	1.5 ± 0.7
155	813 ± 140	661 ± 93	−0.1 ± 2.5	−0.8 ± 2.8	277 ± 45	320 ± 17	1.4 ± 0.8	1.5 ± 1.2	32 ± 13	41 ± 8	1.4 ± 0.5	1.8 ± 1.2
185	998 ± 262	705 ± 108	−1.5 ± 2.7	−1.7 ± 2.9	318 ± 47	335 ± 16	0.7 ± 0.6	1.2 ± 0.5	54 ± 21	48 ± 9	1.0 ± 1.1	0.5 ± 0.4
215	870 ± 155	787 ± 141	−3.6 ± 2.1	−4.4 ± 3.4	303 ± 43	374 ± 27	0.4 ± 0.5	0.2 ± 0.6	41 ± 9	55 ± 14	1.2 ± 0.4	1.3 ± 0.4
245	1,001 ± 197	856 ± 178	−6.2 ± 3.0	−3.4 ± 2.1	349 ± 51	418 ± 30	0.4 ± 0.6	0.4 ± 0.7	51 ± 7	65 ± 9	1.9 ± 0.7	0.8 ± 0.2

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insulinemia following inhalation. In a previous study, IVC insulin infusion was used to match the arterial insulin levels and kinetics following inhalation, and intraportal glucose was delivered at the same load. Plasma glucose levels were left to vary as a result of insulin action just as in clinical practice and unlike the present study (8). Greater nonhepatic glucose clearance occurred after insulin inhalation, thus reducing the hepatic glucose load and, as a result, liver glucose uptake. Therefore, by this mechanism, patients treated with Exubera may experience decreased hepatic glycogen deposition and thereby a subsequent reduction in overnight glucose production. Thus, the decrease in fasting plasma glucose level that is observed clinically could occur by an increase in R_d, a decrease in rate of appearance (R_a), or both. Other possible explanations are discussed in a recent review (6).

Stimulation of glucose uptake by muscle could be the result of a "push" or "pull" mechanism. If the mechanism only involves the movement of glucose into the cell, then glucose might be expected to be pushed into both glycogen and the glycolytic pathway, the latter increasing oxidation and lactate release. Because hind-limb lactate output did not occur in the inhalation group, even when muscle glucose uptake was six- to sevenfold greater than basal (between 65 and 155 min), a pull mechanism seems more likely.

Possible mechanisms of increased nonhepatic glucose disposal associated with insulin inhalation have been postulated, including nitric oxide– (NO), carbon dioxide–, or oxygen-mediated events, possibly via carotid body sensing (8). Another potential mediator of this effect is insulin inhibition of ACE in the lung (15–23). ACE is a membrane-bound glycoprotein found mostly in the lung but also in non–pulmonary vascular beds. It is inhibited by insulin (24–27), and several large clinical studies have demonstrated that ACE inhibition protects at-risk hypertensive patients from developing type 2 diabetes (28,29). ACE converts angiotensin I into angiotensin II (ATII) and inactivates bradykinin. Bradykinin and ATII effects are mediated through the bradykinin receptor subtype 2 (BK_2) and ATII receptor subtype 1 (AT_1), respectively. Binding of bradykinin to BK_2 directly increases NO levels, enhances insulin signaling and sensitivity (via insulin receptor substrate-1 [IRS-1] phosphorylation and phosphoinositide 3-kinase [PI3K] activity), increases GLUT4 translocation in skeletal muscle, and decreases vascular resistance, thereby increasing insulin-dependent glucose uptake in muscle and fat (20,21,23). ATII reduces skeletal muscle glucose uptake by inhibiting insulin signaling, decreasing GLUT4 biosynthesis, and reducing capillary flow (30). When BK_2 is selectively blocked by HOE-140 (bradykinin B2-receptor antagonist), there is a decrease in muscle GLUT4 translocation and glucose uptake (31). On the other hand, antagonism of AT_1 results in increased insulin sensitivity while infusion of ATII induces insulin resistance (30). Thus, inhibition of ACE by insulin in the lung may be the means by which increased glucose disposal occurs following insulin inhalation. Furthermore, ACE inhibition would be expected to decrease MAP. Unfortunately, this measurement was only available in a limited number of the studies, and the high standard error prevents more definitive conclusions. Clearly, further studies are required to verify any of the hypothetical mechanisms discussed above.

One potential concern raised by the observed increase in glucose disposal following insulin inhalation in dogs and the decreased fasting plasma glucose levels seen in patients with diabetes is the possibility of increased postprandial hypoglycemic risk. Interestingly, however, the number of overall hypoglycemic events was lower in patients treated with inhaled than in those treated with subcutaneous insulin (2,3,5), although severe hypoglycemic rates were either not different (2,5) or slightly higher (3). In addition, increased inhalation-associated insulin action can easily be offset by adjusting the insulin dose to avoid hypoglycemia.

In summary, this study demonstrates that the entry of inhaled insulin (Exubera) into the lung results in a significant augmentation of muscle but not hepatic glucose uptake compared with intravenous delivery of insulin.

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