

Inhalation of Insulin in Dogs

Assessment of Insulin Levels and Comparison to Subcutaneous Injection

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Pulmonary insulin delivery is being developed as a more acceptable alternative to conventional subcutaneous administration. In 15 healthy Beagle dogs (average weight 9.3 kg), we compared insulin distribution in arterial, deep venous, and hepatic portal circulation. Dogs received 0.36 units/kg s.c. regular human insulin ($n = 6$) or 1 mg (2.8 units/kg) or 2 mg (5.6 units/kg) dry-powder human inhaled insulin ($n = 3$ and 6, respectively). Postinhalation of inhaled insulin (1 or 2 mg), arterial insulin levels quickly rose to a maximum of 55 ± 6 or 92 ± 9 $\mu\text{U/ml}$, respectively, declining to typical fasting levels by 3 h. Portal levels were lower than arterial levels at both doses, while deep venous levels were intermediate to arterial and portal levels. In contrast, subcutaneous insulin was associated with a delayed and lower peak arterial concentration (55 ± 8 $\mu\text{U/ml}$ at 64 min), requiring 6 h to return to baseline. Peak portal levels for subcutaneous insulin were comparable to those for 1 mg and significantly less than those for 2 mg inhaled insulin, although portal area under the curve (AUC) was comparable for the subcutaneous and 2-mg groups. The highest insulin levels with subcutaneous administration were seen in the deep venous circulation. Interestingly, the amount of glucose required for maintaining euglycemia was highest with 2 mg inhaled insulin. We conclude that plasma insulin AUC for the arterial insulin level (muscle) and hepatic sinusoidal insulin level (liver) is comparable for 2 mg inhaled insulin and 0.36 units/kg subcutaneous insulin. In addition, arterial peak concentration following insulin inhalation is two times greater than subcutaneous injection; however, the insulin is present in the circulation for half the time. *Diabetes* 53:877–881, 2004

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AUC, area under the curve; FEIL, fractional extraction of insulin; IVC, inferior vena cava.

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Insulin replacement is essential in all patients with type 1 diabetes and in an increasing number of patients with type 2 diabetes. However, the initiation of insulin treatment is frequently delayed in the latter population and, in general, fraught with poor compliance in both populations, as subcutaneous administration is perceived as inconvenient and unacceptable by many patients and health care providers. For these reasons, considerable effort has gone into developing alternative, more acceptable forms of insulin delivery. Pulmonary insulin is an attractive option because it is minimally invasive. Preliminary results from controlled clinical studies indicate that inhaled human insulin is both as effective as subcutaneous regular human insulin and well tolerated (1–6) and that problems related to changes in pulmonary function and insulin antibodies are minimal (7).

This study was undertaken to elucidate other aspects of inhaled insulin not yet well understood; in particular, how the insulin concentrations in arterial, deep venous or portal circulation relate to one another after inhalation of insulin as opposed to after subcutaneous insulin injection. Unlike subcutaneous insulin, inhaled insulin is presumed to enter the body through pulmonary and deep bronchial veins after absorption into the alveolar capillary beds. Either way, insulin is thought to be drained into the left ventricle and, thus, directly into the arterial circulation, potentially generating higher initial arterial and portal insulin levels. This is of potential interest when assessing efficacy (portal) aspects of inhaled insulin. We therefore designed a study in dogs to measure plasma insulin in the femoral artery, portal vein, and the inferior vena cava (IVC) following administration of subcutaneous or inhaled insulin. We chose a dog model because multiple indwelling catheters in the vessels of interest can be used over a prolonged experimental period and the model is well characterized with respect to insulin-dependent glucose metabolism (8).

RESEARCH DESIGN AND METHODS

Fifteen healthy (seven male, eight female; overnight fasted) Beagle dogs (2–3 years old) were studied. The dogs' weights ranged from 8 to 10 kg and they were randomized to three exposure groups. Six dogs were assigned to subcutaneous administration of 0.36 units/kg regular human insulin, three dogs to inhalation of 1 mg dry-powder insulin, and six dogs to inhalation of 2 mg dry-powder insulin. All animals were killed at study end. The protocol was

reviewed and approved by the Lovelace Respiratory Research Institute (LRRI) Institutional Animal Care and Use Committee.

Catheterization. Approximately 3 weeks before the study, indwelling Silastic catheters (0.040 ID; Helix Medical, Carpinteria, CA) were inserted under isoflurane anesthesia into the IVC, the left femoral artery, and the hepatic portal vein as previously described (8) for blood sampling. The catheters were filled with a heparin/saline solution and buried subcutaneously. They remained in their subcutaneous pocket between surgery and the experiment. On the day of the study, the catheters were removed from their pocket and flushed with saline. Silastic catheters were also inserted on the day of the study into cephalic or saphenous veins for infusion of somatostatin or glucose. Postsurgery, blood was taken for complete blood cell count. One dog was given 250 mg ampicillin because of an increased white blood cell count.

Drug administration. In all dogs, $0.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ somatostatin (Bachem California, Torrance, CA) was infused via a cephalic vein catheter starting ~4 min after administration of exogenous insulin (the time required to move the animal from the operating room to the experimental lab). Infusion of D-50 glucose into a second peripheral venous catheter was started at the same time as the start of somatostatin at a rate of $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

Dogs assigned to receive inhaled insulin were anesthetized to allow intubation and controlled endotracheal insulin administration. Dogs were given 0.2 ml of acepromazine followed 15 min later by 5% inhaled isoflurane until disappearance of palpebral or pedal reflexes. The maintenance dose of isoflurane was 2%. A modified P2.3 device developed by Inhale Therapeutics (currently Nektar Therapeutics, San Carlos, CA) was used to administer the inhaled insulin. Following induction of apnea by ventilating the dogs 10–15 times with the anesthesia bag, an ~800-ml pulse of air administered through the P2.3 device at ~15 l/min followed by a breath hold of about 2 s was used to expose the dogs to dry-powder insulin (the total lung capacity of an anesthetized dog weighing 10 kg is ~1.4 l and the inspiratory capacity is ~800 ml). Insulin was delivered from blister packs (Nektar Therapeutics) containing 1 mg dry-powder human insulin using the P2.3 device. The contents of two 1-mg blister packs was administered to dogs receiving the 2-mg dose in a single inhalation. Dogs receiving 1 mg insulin were administered only one blister. The duration of the induced apnea was <1 min so that a single controlled deep respiration followed by a breath hold could be achieved. Following insulin administration, the administration of isoflurane was discontinued and the dog was transferred to the experimental laboratory where it was placed in a sling. Dogs recovered consciousness within ~10 min. Animals were therefore studied in the conscious state and the duration of the anesthesia was less than 30 min in total.

Dogs assigned to receive subcutaneous insulin, 0.36 units/kg regular human insulin (Humulin; Eli Lilly, Indianapolis, IN), were anesthetized and treated as described above, including administration of the 800-ml pulse of air through the P2.3 device. However, insulin was administered by subcutaneous injection into the left flank area.

Blood sampling. Blood samples (4 ml from the femoral artery, 2 ml from the IVC and portal vein) were collected in K_2EDTA -containing tubes (Becton Dickinson, Franklin Lakes, NJ) preanesthesia (jugular vein), preinsulin administration (artery), and at 4 min coincident with the start of the somatostatin and glucose infusions. Samples were also taken at 9, 14, 34, 49, 64, 79, 94, 124, 154, 184, 214, 244, 304, and 364 min. Additional arterial blood samples were taken as required in order to maintain the glucose clamp. Glucose was immediately measured in duplicate using the arterial blood sample and a Bayer Glucometer Elite, and the glucose infusion rate was adjusted to maintain euglycemia. The remainder of the arterial sample and all venous samples were kept on ice until centrifuged.

Analytic methods. Plasma from arterial samples was separated into two equal aliquots, one for C-peptide analysis and one for insulin analysis. Samples for C-peptide measurement had 50 μl aprotinin (Pentex Bovine Aprotinin; Serologicals Proteins, Kankakee, IL) added per milliliter of plasma. The plasma samples taken from the venous (portal and IVC) and arterial sites were immediately frozen and stored at -20°C until assayed by a previously described method (9). It should be noted that human and canine insulin differ by one amino acid and that they cross-react completely in the insulin assay used. Measurement of canine C-peptide levels allowed an estimation of endogenous insulin secretion (10).

Estimation of liver sinusoidal insulin. Liver sinusoidal insulin exposure was estimated for the three groups assuming that 20% of blood flow to the liver is arterial in origin (11). This approach assumes that the portal, venous, and arterial blood mix at or near the beginning of the hepatic sinusoids, and that the calculated estimate represents the average insulin concentration at the start of the sinusoids. Obviously, the sinusoidal insulin value will drop as the blood travels through the liver because the latter organ destroys approximately half of the insulin that reaches it (12,13). Concurrent infusion of somatostatin has been shown to have little effect on the fractional extraction

of insulin (FEIL) by the liver. In an earlier study, the FEIL was 42% in the absence of somatostatin and 49% in its presence (14). Thus, the use of somatostatin in the present study, which was necessary to ensure that plasma insulin was exogenous in origin, should not have affected the pharmacokinetics of insulin in a significant manner.

Statistical methods. The level of significance was $P < 0.05$ (two-sided test). Statistical comparisons between groups were made using two-way analysis of variance, while intragroup differences from baseline were calculated using one-way analysis of variance (StatView, Calabasas, CA). The Scheffe procedure and Fisher's protected least-significant difference test for multiple comparisons were used post hoc when significant F ratios were obtained. Area under the curve (AUC) was calculated using the trapezoidal rule (WinNonlin 3.1; Pharsight, Mountain View, CA). All values are presented as means \pm SEM.

RESULTS

Arterial insulin. In response to inhaled insulin (1 or 2 mg), arterial insulin levels rapidly rose to a peak of $55 \pm 6 \mu\text{U/ml}$ at 14 min in the 1-mg group (two dogs peaked at 14 min and one at 19 min) and $92 \pm 9 \mu\text{U/ml}$ at 9 min in the 2-mg group (four dogs peaked at 9 min and two at 19 min) (Fig. 1). With both doses of inhaled insulin, plasma levels tended to remain elevated for a short period of time before returning to levels seen in overnight-fasted, conscious dogs by 3 h. In contrast, arterial plasma insulin after 0.36 units/kg subcutaneous administration rose more slowly, peaking at $55 \pm 8 \mu\text{U/ml}$ by 64 min before gradually returning to baseline by the end of the experiment. AUC was similar for subcutaneous insulin ($10,123 \pm 917 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$) and 2 mg inhaled insulin ($8,629 \pm 1,196 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$) but smaller for 1 mg inhaled insulin ($5,707 \pm 1,115 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$).

Portal insulin. Portal insulin levels rose to the greatest extent in the 2-mg inhaled-insulin group (to $74 \pm 11 \mu\text{U/ml}$ at 34 min). Peak levels were comparable between the subcutaneous insulin (64 min) and 1-mg inhaled-insulin (34 min) groups at 37 ± 6 and $43 \pm 9 \mu\text{U/ml}$, respectively (Fig. 1).

Hepatic-sinusoidal insulin. Hepatic-sinusoidal AUC was estimated by integrating measured arterial and portal plasma insulin levels. The AUC seen with 2 mg inhaled insulin ($7,256 \pm 1,054 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$) was indistinguishable from that observed in the subcutaneous insulin group ($7,224 \pm 723 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$) and significantly larger than that seen with the 1-mg inhaled-insulin group ($4,496 \pm 805 \mu\text{U} \cdot \text{ml}^{-1} \cdot 364 \text{ min}^{-1}$; $P < 0.05$) (Fig. 2).

Deep venous insulin. In response to inhaled insulin, whether 1 or 2 mg, deep venous insulin levels rose rapidly to a peak of $51 \pm 9 \mu\text{U/ml}$ in the 1-mg group and $80 \pm 16 \mu\text{U/ml}$ in the 2-mg group at 34 and 19 min, respectively (Fig. 1). With both doses of inhaled insulin, plasma levels tended to remain elevated for a short period of time before returning to baseline by 3 h. In contrast, insulin levels after 0.36 units/kg s.c. administration rose more slowly, peaking at $78 \pm 10 \mu\text{U/ml}$ at 64 min before gradually returning to baseline toward the end of the experiment.

C-peptide. Arterial C-peptide levels averaged 0.1–1.5 ng/ml throughout the study (Fig. 3), indicating that 75–90% inhibition of basal insulin release was achieved (10) and that after the first 20 min postdosing, ~1 $\mu\text{U/ml}$ of arterial insulin and 3 $\mu\text{U/ml}$ of portal insulin could be attributed to endogenous insulin secretion.

Glucose infusion. As shown in Fig. 3, normal glucose levels were maintained throughout the study, without significant intra- or intergroup differences. There were,

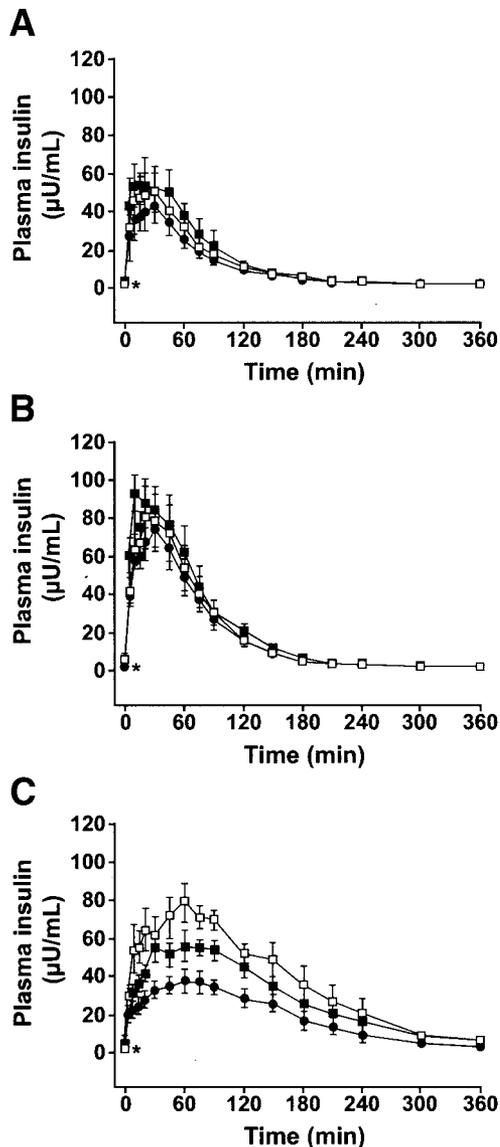


FIG. 1. Arterial, portal and deep venous plasma insulin after inhalation of 1 mg (A) or 2 mg (B) of human insulin or after subcutaneous injection of 0.36 units/kg of regular human insulin (C). All values are presented as means \pm SE. ■, arterial; ●, portal; □, deep venous. *Estimated values.

however, significant differences in the amounts of intravenous glucose required to ensure euglycemia. Glucose infusion rates were highest with 2 mg inhaled insulin peaking at $19.8 \pm 2.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 94 min compared with 1 mg inhaled insulin and subcutaneous insulin ($12.0 \pm 4.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [79 min] and $13.2 \pm 3.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [124 min], respectively). Altogether, $3.1 \pm 0.5 \text{ g/kg}$ glucose was required for the 2-mg group, while less was needed to maintain euglycemia in the subcutaneous groups ($2.6 \pm 0.5 \text{ g/kg}$; NS) and the 1-mg inhaled-insulin group ($1.4 \pm 0.6 \text{ g/kg}$; $P < 0.05$) (Fig. 2).

DISCUSSION

In summary, the time course of plasma insulin was clearly different following the two routes of administration. At both doses used in this study, inhaled insulin peaked almost immediately postadministration, reaching a plateau

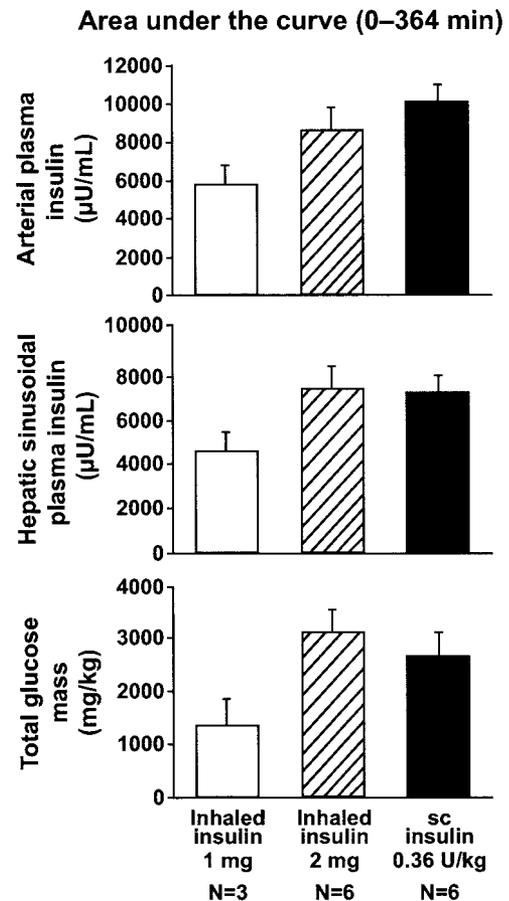


FIG. 2. AUC (0–364 min) after inhalation of 1 or 2 mg of human insulin or after subcutaneous administration of 0.36 units/kg regular human insulin. All values are presented as means \pm SE.

within the first few minutes before quickly returning to baseline by ~ 3 h. These results conform well to the pharmacokinetics of inhaled insulin observed in humans. In contrast, subcutaneous administration of regular human insulin showed a delayed rise in plasma insulin.

The inhibition of endogenous insulin secretion began between 5 min (the start of somatostatin infusion) and 10 min (the first point at which the plasma C-peptide level was reduced). By 20 min the plasma C-peptide concentration reached a level of 0.1–0.15 ng/ml in each group, a reduction of $\sim 75\%$ from the level normally seen in an overnight-fasted dog (0.6 ng/ml). The 0 time C-peptide levels were lower in these dogs than would normally be expected, probably as a result of the anesthesia required for insulin inhalation in the dog. Nevertheless, the amount of insulin in the circulation arising from endogenous secretion over the first 20 min of the study probably ranged from the basal value of $\sim 6 \mu\text{U/ml}$ to a value of $\sim 1 \mu\text{U/ml}$, averaging perhaps $3 \mu\text{U/ml}$. Since the peak arterial plasma insulin level in the 1 mg inhalation group was $56 \mu\text{U/ml}$, $<6\%$ of the arterial insulin could be attributed to endogenous release in that group at that time. In the 2-mg group it would be $<4\%$; in the subcutaneous group, $\sim 8\%$. After the first 20 min, the amount of arterial insulin derived from pancreatic secretion was between 1 and $2 \mu\text{U/ml}$ in all groups. Essentially, therefore, the plasma insulin profiles represent exogenously administered insulin. On that

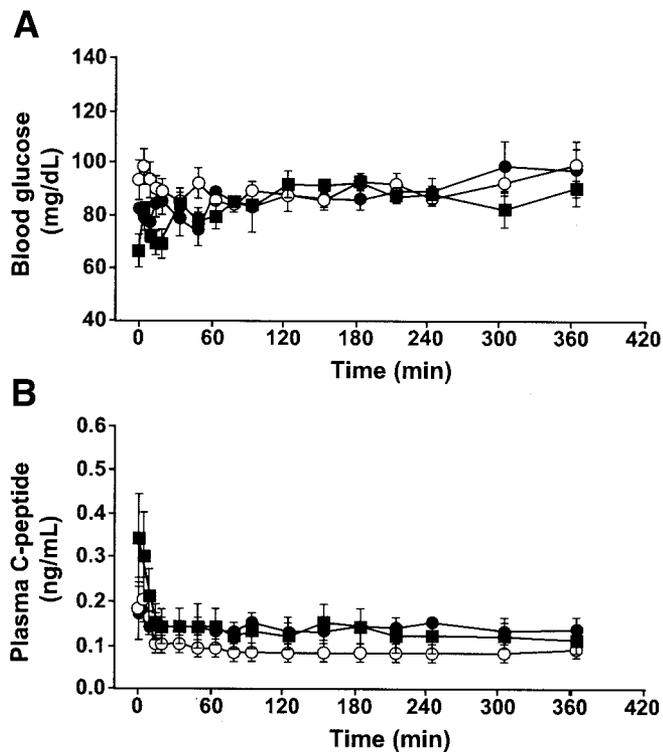


FIG. 3. Arterial blood glucose (A) and arterial C-peptide (B) levels after inhalation of 1 or 2 mg of human insulin or subcutaneous administration of 0.36 units/kg of regular human insulin. All values are presented as means \pm SE. ●, 1 mg inhaled insulin; ■, 2 mg inhaled insulin; ○, 0.36 units/kg s.c. insulin.

basis, we can conclude that inhaled insulin is completely cleared from the dogs within \sim 4 h, while $>$ 6 h are required with subcutaneous insulin. In fact, by extrapolating the insulin curves, a 7- to 8-h clearance time for subcutaneous insulin would be predicted.

The 2-mg dose of inhaled insulin and 0.36 units/kg s.c. insulin produced almost perfectly matched AUCs in the liver sinusoidal blood, while the arterial AUCs for insulin were slightly greater in the 2-mg inhaled-insulin group compared with the subcutaneous insulin group. The arterial insulin levels in both inhaled insulin groups were only slightly (and not significantly) higher than the corresponding portal or deep venous plasma insulin levels. In humans, a 6-mg dose of inhaled insulin, which may be typically given before a large meal, generates an average peak concentration of 69 μ U/ml in peripheral venous blood (T. Heise, personal communication). Because in the dog the arterial insulin concentration was only 15% greater than the venous level, it seems likely that the peak arterial concentrations in humans are unlikely to exceed 80 μ U/ml after inhalation of a 6-mg dose, which roughly corresponds to 18 units of subcutaneous insulin based on pharmacodynamic data (15). By comparison, insulin analogs administered by subcutaneous injection are associated with peripheral venous concentrations of 147–160 μ U/ml at a dose of 0.3 units/kg (16,17). This may correspond with arterial concentrations of 100–120 μ U/ml, given that we observed 30% lower arterial levels, as compared with central venous levels in the present study after subcutaneous injection.

However, a surprising finding in this study was that the

total glucose required to maintain euglycemia was \sim 20% greater in the 2-mg inhaled-insulin group compared with the subcutaneous insulin group, despite equivalent insulin levels at the liver and slightly lower insulin levels in the periphery. It should be noted that in the first 20 min or so of the 2-mg inhalation, the plasma glucose level was modestly lower than in the subcutaneous group. Had we infused a little more glucose early on in the inhalation group, the mass of glucose infused over the entire experimental period in that group would have been even greater than we observed, thus further supporting our conclusion. Whether this increased glucose requirement is due to the difference in insulin kinetics or an unknown factor associated with the route of administration remains to be clarified in a future study.

Variability of postadministration insulin levels was very similar whether comparing intra- or intergroup differences. Thus, this study confirms data obtained in clinical trials where the coefficient of variation for inhaled insulin was between 22 and 26% based on the AUC measured from 0 to 8 or 10 h (17–20). These figures are comparable to those seen following subcutaneous insulin injection in humans (17–21).

Relative bioavailability of inhaled insulin compared with subcutaneous insulin was \sim 7% in this study, which is consistent with clinical results obtained with this system in humans (10–11%) (20) and other dry-powder or liquid insulin inhalers (8–16%) (17,18,20–26). Given that the AUCs for arterial insulin were not significantly different in the two groups, this value was calculated by comparing the amount of insulin administered via subcutaneous injection (0.36 units/kg) or oral inhalation (5.6 units/kg). Our standard tests showed that 62% of the insulin in the blister was aerosolized, and we assumed that only 50% of that reached the deep lung. Thus, if one calculates bioavailability relative to the depot site (subcutaneous versus deep lung), the absorption from the lung is about one-fifth that from the subcutaneous site. Based on pharmacokinetic data, therefore, the dog inhalation model proved to be comparable to human studies, despite differences in drug administration, e.g., endotracheal “forced” inhalation in dogs versus voluntary inhalation in humans.

In summary, inhalation of insulin produced hormone levels in the arterial, portal, and venous circulation that were slightly but not significantly different. However, despite similar overall exposure of the tissues to the hormone (i.e., AUC), with the two routes of administration more glucose was needed with inhaled insulin compared with subcutaneous insulin to maintain euglycemia. This raises the possibility that inhaled insulin has a higher bioefficacy than subcutaneous insulin. However, because this study was neither designed nor powered appropriately to address this particular question, such a conclusion requires further study.

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