Effect of Gender and Device Mouthpiece Shape on Bolus Insulin Aerosol Delivery Using the AER_x Pulmonary Delivery System

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Purpose. A study was designed to compare differences in insulin aerosol deposition profiles in healthy male and female subjects, as well as examine the effect of mouthpiece cross-sectional shape, volume, and taper on deposition profiles using a developmental AER_x pulmonary delivery system.

Methods. Six mouthpieces were screened in the laboratory, and three were selected for clinical investigation: a cylindrical mouthpiece with constant-cross-sectional area, an elliptical mouthpiece with constant-cross-sectional area, and a tapered elliptical mouthpiece with an exit cross-sectional area equal to one half the entrance cross-sectional area.

Results. There was no significant difference in the lung dose or in the deposition pattern between males and females ($p > 0.05$, by ANOVA). The cross-sectional shape of the mouthpiece had no significant effect on the clinical lung dose or the deposition pattern ($p > 0.05$, by ANOVA), although *in vitro* testing showed lower emitted dose values with the tapered elliptical mouthpiece (by ANOVA and Duncan's multiple range test, $\alpha = 0.05$). Using the tapered mouthpiece in the clinic resulted in significantly more deposition on the mouthpiece itself when compared to the nontapered mouthpieces.

Conclusion. Inhalation of insulin using the AER_x system was insensitive to differences in male and female respiratory tract geometry across all mouthpiece designs examined.

KEY WORDS: AERx; gender differences; mouthpiece design; pulmonary drug delivery; scintigraphy.

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ABBREVIATIONS: AER_x, Aradigm's pulmonary delivery system; ANOVA, analysis of variance; CC, cylindrical cross-section mouthpiece; ED, emitted dose; $FPF_{2.5}$, fine particle fraction smaller than 2.5 micrometers; FPD, fine particle dose; $FPF_{4.0}$, fine particle fraction smaller than 4.0 micrometers; GSD, geometric standard deviation; GCP, good clinical practices; HPLC, high-pressure liquid chromatography; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; ^{81m}Kr, krypton-81m; %LC, percent label claim; %LD, percent loaded dose; LE, long length, elliptical cross-section mouthpiece; LT, long length, tapered mouthpiece; MMAD, mass median aerodynamic diameter; MT, medium length, tapered mouthpiece; sC/P, central to peripheral lung deposition ratio; SE, short length, elliptical cross-section mouthpiece; ST, short length, tapered mouthpiece; ^{99m}Tc-DTPA, technetium-99m diethylene triaminepentaacetic acid; USP, United States Pharmacopeia.

INTRODUCTION

Understanding the factors that contribute to the efficiency and reproducibility of lung deposition is an important element in the development of pulmonary drug delivery systems for systemic effect or local action. Dose to the lung, and the distribution of the aerosol within the lung, are the most direct metrics for judging the effectiveness of pulmonary delivery. Lung dose efficiency is directly affected by the amount retained in the device and deposited in the oropharynx. Svartengren *et.al.* reported that different levels of oropharyngeal deposition of inhaled aerosols can occur in females compared to males (1). In that study, volunteers inhaled monodisperse 3.6-μm Teflon particles at a controlled flow rate of 22 L/min. Healthy females had a median mouth and throat deposition of 30%, whereas healthy males had a median mouth and throat deposition of 16%. This suggests the importance of including both genders in lung deposition studies. Additionally, delivery system mouthpiece shape and size may impact aerosol deposition in the device and oropharynx. For example, a tapered mouthpiece may result in more deposition in the mouthpiece and in the patient's oropharynx compared to a constant-cross-section mouthpiece due to differences in the aerodynamic flow profiles.

This paper reports the results of a gamma scintigraphy study using a developmental prototype of the handheld AER_x pulmonary delivery system (2) in a group of healthy volunteers to investigate the effects of different mouthpiece shapes on aerosol deposition in both males and females. The standard mouthpiece used with the AER_x system prior to this study was a mouthpiece with a cylindrical shape. The motivation behind the study was the selection of a more ergonomic and aesthetically pleasing mouthpiece that did not adversely impact drug delivery performance.

The screening experiments compared five elliptical mouthpieces (three tapered, two untapered) to the existing cylindrical design. Based on those data, one tapered design and one nontapered design were selected for use in the clinical study along with the original cylindrical design as a control.

MATERIALS AND METHODS

Rationale for Mouthpiece Designs

Four new mouthpiece designs, short tapered (ST), long tapered (LT), short elliptical (SE), and long elliptical (LE), were generated for the initial *in vitro* screening experiments. Table I presents a summary of the mouthpiece designs that were considered along with the original cylindrical constant (CC) cross-section design. The medium tapered (MT) design, eventually selected for use in the clinical study, was added to the screening study after the initial experiment because the ST design was considered unacceptable based on the results of the initial experiment.

Two-dimensional attributes were considered crucial when designing the mouthpieces: the internal volume of the mouthpiece, which correlates with the residence time for evaporation of aerosol droplets; and the overall length of the mouthpiece, which impacts the overall size of the device. Additionally the outer diameter was constrained by the ability of

Table I. Summary of Mouthpiece Designs for Initial *in Vitro* Screening

	Cylindrical	Long ellipse	Short ellipse	Long taper	Medium taper[a]	Short taper
Abbreviation	CC	LE	SE	LT	MT	ST
Overall length (cm)	6.4	6.6	5.7	8.9	7.5	6.4
Internal volume (ml)	50	50	43	59	50	40
Inlet area (sq. cm)	7.9	7.5	7.5	7.5	7.5	7.5
Exit area (sq. cm)	7.9	7.5	7.5	3.7	3.7	3.7
Major-minor ratio at inlet	1.0	1.2	1.2	1.2	1.2	1.2
Major-minor ratio at exit	1.0	1.2	1.2	1.9	1.9	1.9
Length prior to taper (cm)	NA	NA	NA	6.4	5.0	3.9
Angle of taper[b] (deg)	NA	NA	NA	30	30	30

[a] Not evaluated in initial mouthpiece screening experiments.
[b] Measured at the point on the mouthpiece where the taper begins.

patients to place the mouthpiece comfortably in their mouth. The dimensional values for design CC were considered to be the baseline for this evaluation. As can be seen in Table I, design LE retains the same internal volume and roughly the same length as design CC. Design SE investigates the effect of length on evaporation in order to explore a reduction in the size of the device. Design LT has the same length and internal volume prior to tapering as design CC has overall, whereas design ST retains the same overall length as design CC. Design MT provides a shorter alternative to LT, with a larger volume for aerosol evaporation than design ST. The tapered design may be more ergonomically desirable, but the increased air-stream linear velocity at the exit could result in increased throat deposition and reduced system performance.

In Vitro Evaluation of Aerosol Properties

Aliquots (50 μl) of a liquid formulation of insulin were individually sealed into proprietary unit-dosage forms for use in the developmental AER_x device (3). The AER_x device initiates aerosol delivery at a preprogrammed inspiratory flow rate and inspired volume and delivers the aerosol dose over a fixed period of time (1.1 s in this study). The emitted dose (ED), expressed as a percent of loaded dose (%LD), was measured by collecting the aerosol deposited on a glass-fiber filter attached to the mouthpiece of the device. The mouthpiece deposition was measured by collecting the aerosol deposited on the mouthpiece. Throughout this paper, the emitted dose is defined as the amount of insulin or radiolabel in the aerosol that exits the mouthpiece. The loaded dose is defined as the total amount of insulin or radiolabel sealed into the dosage form.

In the mouthpiece screening experiments the particle size distribution was measured using a laser-diffraction particle-sizing system (Sympatec, HELOS/BF System, Zellerfeld, Germany) rather than by cascade impaction (CI) because of concern in the screening experiments that the effects of mouthpiece geometry on particle size could be obscured by continued evaporation in the CI apparatus itself. Previous experience with AER_x has shown post-mouthpiece evaporation in the CI to be an important consideration, when evaluating the extent of aerosol evaporation. The measurement taken from the HELOS system was the fraction of the volume of particles smaller than 2.5 μm. This fraction is referred to in this paper as the fine particle fraction-2.5 ($FPF_{2.5}$). The $FPF_{2.5}$ is similar to the fine particle fraction-4.0 ($FPF_{4.0}$), the fraction of particles smaller than 4.0 μm, which are expected to deposit in the peripheral lungs, as determined by cascade impaction at 60 LPM. As the aerosol particles evaporate during transit through the AER_x device, the particle size distribution shifts toward a relatively monodisperse distribution centered around 2.5 μm. Therefore, the $FPF_{2.5}$, with a smaller cutoff closer to the final expected median particle size, was used rather than $FPF_{4.0}$ for mouthpiece screening to better detect subtle changes in the extent of evaporation. The $FPF_{2.5}$ value for each experimental run was calculated by measuring 20 instantaneous aerosol particle size distributions (evenly spaced in time throughout the 1.1 s bolus delivery process), calculating the instantaneous $FPF_{2.5}$ value for each time point, and then time-averaging these values using the instantaneous optical concentration as a weighting factor. It is assumed that the instantaneous optical concentration is representative of the volume of particles present at a given time point, which allows for a more accurate means of averaging the instantaneous $FPF_{2.5}$ values. After selecting mouthpieces to be used in the clinical study, subsequent *in vitro* clinical-system performance testing used an Anderson Cascade Impactor fitted with a USP inlet port (4) to measure the particle size distribution.

Initial mouthpiece screening experiments, utilizing the HELOS system to measure particle size distributions, were performed at a flow rate of 70 L/min, at the high end of the preset flow-rate actuation-window of the developmental AER_x device (45–70 LPM). However, these experiments were conducted using a laboratory-scale version of the AER_x system. The functionality of the laboratory-scale system was the same as the developmental AER_x, however the ability to monitor the aerosolization process was greatly enhanced (3). The subsequent *in vitro* clinical-system performance tests were performed at a flow rate of 55 L/min, near the middle of the flow-rate actuation-window of the device, using the developmental AER_x device. The amount of insulin was quantified by reversed-phase HPLC using a Merck Hibar LiChrosorb (Merck and Co., Whitehouse Station, NV, USA) RP-18 analytical column (4.0 mm i.d. × 250 mm, 5-μm particle size) with an acetonitrile mobile phase and a run time of 35 min. The mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle fraction ($FPF_{4.0}$ – the fraction of aerosol particles smaller than 4.0 μm), and the fine particle dose (FPD = $FPF_{4.0}$*ED – the percent of the loaded dose expected to be deposited in the alveoli and avail-

able for transport to the blood stream) are reported. The MMAD and GSD were calculated by fitting the cascade impaction stage data to a log normal distribution using a residual sum of squares minimization algorithm.

Preparation and *in Vitro* Evaluation of Radiolabeled Aerosol

As in the clinical study, insulin formulation was mixed with ^{99m}Tc-DTPA (^{99m}technetium-diethylene triaminepentaacetic acid)/0.9% w/v saline to make a solution containing approximately 2.5 mg insulin and 0.93 MBq ^{99m}Tc-DTPA per 50 μl of solution. Aliquots (50 μl) of this solution were sealed into unit-dosage forms and analyzed for emitted dose, and particle size distribution using cascade impaction. The radioactivity was quantified by gamma counting (Wallac Wizard 1470) against a linear standard curve, with each measurement corrected for radioisotope decay and background radiation. The insulin was quantified by reversed-phase HPLC as described above.

Human Clinical Study Protocol

Fifteen healthy volunteers (6 males and 9 females, ages 20–47 years, nonsmoking for the preceding 12 months) completed the study. Each volunteer was dosed once with each of the three mouthpieces using the developmental AER_x system. Treatment order was randomly assigned. Each dose was separated by at least 48 h. On each day of dosing, AER_x dosage forms were prepared containing approximately 2.5 mg of insulin and 5.3 MBq ^{99m}Tc-DTPA in 50 μl of solution. Each dosage form was imaged prior to administration to accurately determine mass balance. After inhalation and a 5-s breath-hold, the volunteer exhaled into a low resistance air filter. Within two minutes of dosing, a lateral head and neck image of 60-s duration was taken, to measure the deposition in the oropharyngeal region. All images were gathered using a GE Maxi Camera 400 A, with low energy general purpose collimator, and commercially available nuclear medicine software (MAPS10,000 Web Link Medical Ltd. Marlow, Buckinghamshire, UK) for image processing. The volunteer was then provided with approximately 50 ml of water to rinse out their mouth, which they transferred to an appropriately labeled container. They were then given a slice of bread and a drink of water to encourage removal of the radioactivity from the oropharyngeal region into the stomach. Thereafter, acquisition of anterior and posterior thorax/abdomen views of 120 s and a further lateral image of 60 s were acquired, resulting in a total imaging time of approximately 6 min. In addition to the volunteer, images were taken of all components of the device, dosage form, exhalation filter and mouth wash to perform a mass balance based on radioactivity count. Each measurement was corrected for radioisotope decay, background radiation, and tissue attenuation where appropriate. The mass balance calculations were performed as a means of assessing the appropriateness of the derived, volunteer-specific tissue attenuation factors (5). Before the first day of dosing, an outline of each volunteer's lungs was imaged using ^{81m}Kr gas. Tissue attenuation correction factors were derived for the lungs, stomach/abdomen and oropharyngeal region, following transmission imaging of each subject. Intrapulmonary (regional) lung deposition was quantified from the measured radioactivity in the central (C) and peripheral (P) regions of the lung (6), normalized with that measured from ^{81m}Kr ventilation images to yield a specific regional deposition parameter (sC/P). A value of 1.00 indicates a homogeneous deposition equivalent to that of ^{81m}Kr gas, whereas higher values of sC/P indicate more central deposition. The study was conducted following the tenets of the Declaration of Helsinki and was approved by the Simbec Independent Ethics Committee. Informed consent was obtained from the subjects participating in the study, and the study was conducted per ICH guidelines and GCP.

RESULTS

In Vitro Aerosol Characterization: Mouthpiece Screening Experiments

Results from emitted dose testing are presented in Table II, A. Although there appeared to be differences in the mean values for emitted dose with each mouthpiece, none were statistically different from design CC ($p > 0.05$, by ANOVA). It is interesting to note, however, that design ST was the only mouthpiece tested for which the mean emitted dose was lower than that for the control. Although this decrease is not statistically significant in this data set at the 95% confidence level, it may indicate a change in system performance when considered with the data described below.

Similar to the emitted dose measurements, neither the LE, SE nor LT designs showed statistically significant differences in mouthpiece deposition relative to the control ($p > 0.05$, by ANOVA). However, design ST did show a statistically significant (by ANOVA and Duncan's multiple range test, $\alpha = 0.05$) increase in mouthpiece deposition relative to all other mouthpieces. Design ST had the lowest internal volume (Table I), and as such, the degree of evaporation may be lower than that for the other designs. Combined with the fact that the mouthpiece is tapered, it is plausible that the combination of larger particles encountering a tapered geometry resulted in additional mouthpiece deposition.

Particle Size Distribution

Measurements of the $FPF_{2.5}$ were not made during the same experimental runs as the emitted dose and mouthpiece deposition measurements, due to the physical incompatibility of the filter collection and HELOS systems. However, these measurements were made under the same experimental op-

Table II. Average Emitted Dose and Mouthpiece Deposition of Candidate Mouthpieces

	A					B	
	CC	LE	SE	LT	ST	LT	MT
Avg. emitted dose (%LD[a]) (n = 5)	70.7	71.3	71.6	74.3	68.5	63.4	64.6
SD	3.7	4.1	0.8	2.6	1.6	3.4	2.9
Avg. mouthpiece (%LD) (n = 5)	9.6	6.8	7.8	6.7	14.4	ND	ND
SD	1.9	4.0	3.9	3.4	0.7	ND	ND

[a] %LD, the percent loaded dose, is the percent of the drug that was loaded in the dosage form. The difference between the amount listed in the table and 100% was retained in the device.

erating conditions. The results for $FPF_{2.5}$ are presented in Table III.

The $FPF_{2.5}$ values for all mouthpieces were statistically similar to the control ($p > 0.05$, by ANOVA). However, this could be confounded by the relatively high level of variability in the data, as the nominal trends that were observed appear consistent with fundamental principles of aerosol science. For example, the $FPF_{2.5}$ values for the CC and LE designs were nearly identical, and these are designs that are nearly identical in terms of length and internal volume. Furthermore, the SE design showed a lower $FPF_{2.5}$ value than the LE design, as would be expected of a mouthpiece with shorter length and consequently shorter residence time for evaporation. The LT design showed the highest $FPF_{2.5}$ value, as would be expected of the mouthpiece with the largest internal volume, and the ST design showed by far the lowest $FPF_{2.5}$ value, as would be expected of the mouthpiece with the smallest internal volume. The fact that the mean $FPF_{2.5}$ value for the ST design was so low further validates the hypothesis that the ST design showed nominally lower emitted dose because incomplete evaporation resulted in deposition on the tapered surface.

Mouthpiece Selection

Based on these initial data, a decision was made to evaluate one of the straight designs (LE or SE) and one of the tapered designs (LT or ST) in the human study along with the original CC design. Of the two straight mouthpieces, the LE design was more desirable because it showed slightly better evaporative characteristics than the SE design, and the overall length of the LE design was still within the size specification constraints for the AER_x device. Of the two tapered mouthpieces, the performance with the LT design was superior to that with the ST design, but the overall length of the LT design exceeded the constraints for the AER_x device. Therefore, a compromise design was proposed—the medium-length tapered mouthpiece, or the MT design. This design was similar to the LT and ST designs, except that it had an overall length of 7.5 cm, a length prior to taper of 5.0 cm, and a total internal volume of 50 ml. Confirmatory testing prior to the next phase of experiments confirmed that the MT design produced aerosol performance results equivalent to the LT design (Table II, B) in a head-to-head comparison. These data are different from those in the initial experiment (Table II, A) because they were conducted using a different laboratory-scale AERx with a nonoptimized set of motor parameters to control the aerosolization. This resulted in an extrusion pressure that was too low, which in turn resulted in a slightly lower aerosolization efficiency. However, the data indicate consistent performance between the LT and MT mouthpiece designs. This conclusion is supported by the experimental data in Table IV, discussed below. The original laboratory scale AER_x was used to generate the particle size distribution data in Table III.

Table III. Average $FPF_{2.5}$[a] for Candidate Mouthpieces

	CC	LE	SE	LT	ST	MT
$FPF_{2.5}$[a] (n = 3)	0.414	0.398	0.369	0.440	0.300	0.341
SD	0.078	0.128	0.082	0.071	0.064	0.029

[a] $FPF_{2.5}$, fraction of the aerosol that is smaller than 2.5 μm.

Table IV. *In Vitro* Aerosol Characterization for Clinical Development AER_x Device

	CC	LE	MT
Avg. emitted dose (%LD[a]) (n = 10)	70.1	69.9	66.5
SD	1.0	1.6	2.3
%RSD	1.5	2.3	3.4
Mouthpiece deposition (%LD) (n = 2)	N/A	4.1, 3.9	6.6, 6.0
CI inlet port deposition (%LD) (n = 2)	0.8, 1.4	1.1, 0.7	0.6, 0.9
MMAD (μm)	2.2	2.3	2.3
GSD	1.35	1.36	1.34
$FPF_{4.0}$[b]	0.93	0.91	0.92
FPD[c] (%LD)	65.3	63.9	61.5

[a] %LD, the percent loaded dose, is the percent of the drug that was loaded in the dosage form.

[b] Fine particle fraction-4.0 ($FPF_{4.0}$) is the fraction of aerosol droplets in the emitted dose (ED) with aerodynamic diameters <4.0 μm.

[c] Fine particle dose (FPD) is the product of the $FPF_{4.0}$ and the ED, expressed as a percent of loaded dose.

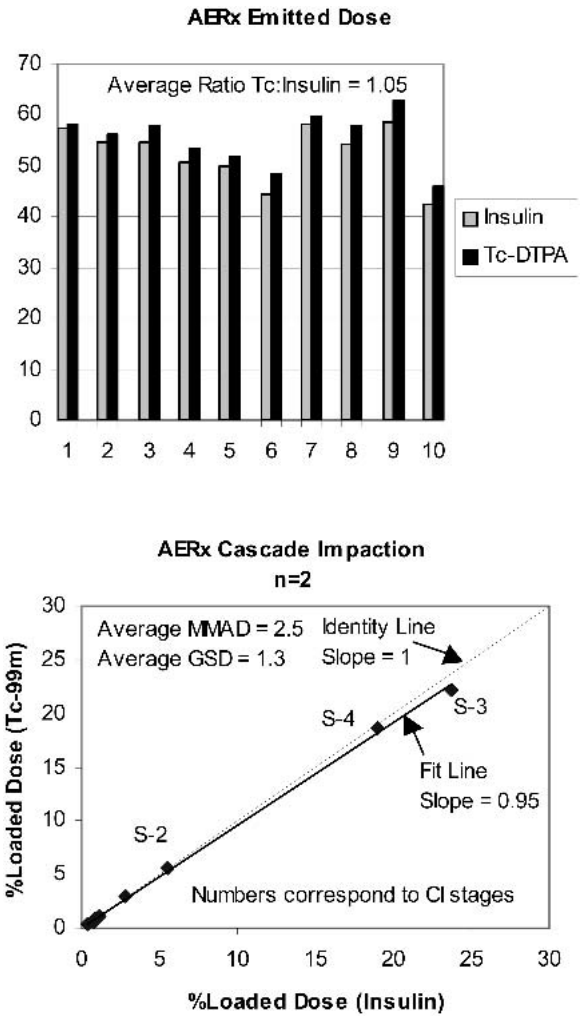

Fig. 1. Distribution of the insulin and radiolabel in developmental AER_x aerosol testing.

Table V. *In Vivo* Aerosol Deposition Comparing Males and Females

		Mouthpiece[a]	Oropharynx (pre-wash)	Oropharynx, stomach, & mouth wash	Lungs	Exhaled	sC/P
Females (all designs)	Avg.	7.6	13.3	19.6	80.2	0.2	1.28
	SD	2.6	4.3	5.8	5.8	0.1	0.48
	%RSD	34.6	32.2	29.6	7.2	69.9	37.6
Males (all designs)	Avg.	7.1	17.5	21.8	78.1	0.1	1.47
	SD	3.1	9.5	10.1	10.1	0.1	0.51
	%RSD	42.9	54.1	46.4	12.9	89.4	34.7
Females (LE only)	Avg.	5.9	13.9	21.1	78.8	0.2	1.29
	SD	1.7	4.0	5.6	5.6	0.1	0.37
	%RSD	29.0	28.9	26.5	7.1	73.2	28.8
Males (LE only)	Avg.	5.7	21.9	25.4	74.4	0.2	1.51
	SD	1.7	13.9	14.5	14.5	0.1	0.50
	%RSD	29.1	63.5	57.2	19.5	51.0	33.1

[a] Expressed as a percent of loaded dose (%LD); all other values expressed as a percent of emitted dose (%ED).

Clinical System Performance Testing

In Vitro Radiolabeled Aerosol Characterization

The results from the *in vitro* radiolabeled formulation characterization studies are shown in Fig. 1. These results demonstrated that the radiolabel and the insulin were distributed equivalently in the aerosol (conducted with design CC). Only one mouthpiece was used, as the type of mouthpiece does not affect whether the radio-tracer and drug are equivalently distributed in the aerosol.

In Vitro System Performance Characterization

The aerosol was also characterized without the radiolabel using the developmental prototype handheld AER_x system used to conduct the clinical study. The results for the three mouthpieces used in the clinical study are shown in Table IV. The discrepancy between the emitted dose levels in Fig. 1 and Table IV is the result of improvements to the system performance prior to the clinical study. The emitted dose using design MT was significantly lower (by ANOVA and Duncan's multiple range test, $\alpha = 0.05$) than the other two mouthpieces. The lower emitted dose corresponds with a small apparent increase in mouthpiece deposition for the tapered mouthpiece, although deposition was only measured on two mouthpieces (Table IV). All other *in vitro* results are statistically similar ($p > 0.05$, by ANOVA) for all three mouthpieces.

In Vivo Study Results

Fifteen healthy volunteers completed the study. No adverse events directly attributable to the insulin formulation or to the delivery method were observed during the study.

Complete accountability of the radiolabel was demonstrated in the *in vivo* scintigraphy study. The average mass balance was 112% (SD = 7%) of the loaded activity. Indicating that the attenuation factors derived for each volunteer were reasonable. The distribution of aerosol in the lungs is characterized by the ratio of aerosol in the central lungs to that in the peripheral lungs (sC/P) (6). The scintigraphy data for all mouthpiece designs are summarized in Table V. ANOVA was performed with treatment as the main effect to determine differences between the mouthpiece configurations. ANOVA indicated that there were no statistically significant differences ($p > 0.05$) between males and females with respect to oropharyngeal deposition, lung deposition, aerosol distribution within the lungs (sC/P), or mouthpiece deposition across all three mouthpieces. There was similarly no statistically significant difference between males and females for the LE design alone (the mouthpiece selected as a result of this study) for all regions of aerosol deposition as shown in Table V. However, the data are somewhat confounded by the high variability seen in one volunteer's lung dose as shown in Fig. 2, subject number 12. Though there is no reason to exclude the subject 12 data from the analysis, the variability in lung dose for that subject is clearly much larger than for the others.

A statisical analysis of the radioactivity deposited in the mouthpieces indicates that the deposition, expressed as a percent of the loaded dose, in mouthpiece design MT is statisti-

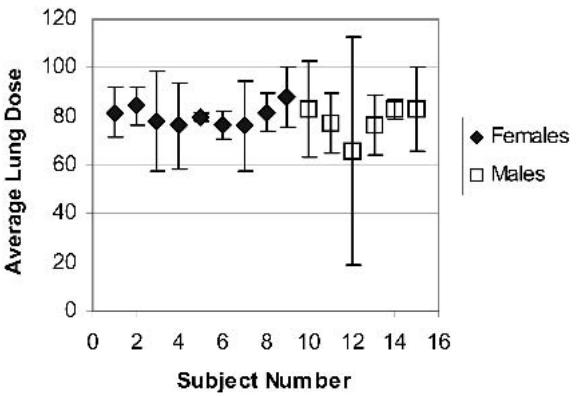

Fig. 2. Intrasubject lung dose variability. Error bars represent 95% confidence intervals. Subjects were randomly assigned to treatment order groups and are segregated here by gender for illustration purposes.

Table VI. *In Vivo* Aerosol Deposition Comparing Three Mouthpieces

		Mouthpiece[a]	Oropharynx (Pre-Wash)	Oropharynx, Stomach, and Mouth Wash	Lungs	Exhaled	sC/P
CC	Avg.	6.2	13.4	18.6	81.2	0.2	1.27
	SD	2.2	5.1	5.8	5.8	0.2	0.43
	%RSD	35.1	37.9	31.4	7.1	94.6	34.0
LE	Avg.	5.8	17.1	22.8	77.0	0.2	1.38
	SD	1.6	9.7	9.9	9.9	0.1	0.42
	%RSD	28.0	56.9	43.4	12.9	62.4	30.8
MT	Avg.	10.2	14.4	20.0	79.9	0.2	1.43
	SD	2.1	5.3	7.0	7.0	0.1	0.63
	%RSD	20.1	37.1	35.0	8.7	75.6	43.9

[a] Expressed as a percent of loaded dose (%LD); all other values expressed as a percent of emitted dose (%ED).

cally higher (by ANOVA and Duncan's multiple range test, $\alpha = 0.05$) when compared to CC, and LE mouthpieces (Table VI). Deposition in the lung, oropharynx and stomach, and exhalation filter expressed as a percent of emitted dose, was not statistically different for any of the three mouthpieces ($p > 0.05$, by ANOVA) (Table VI). The regional deposition, expressed as sC/P, was also not statistically different for any of the three mouthpieces ($p > 0.05$, by ANOVA) (Table VI).

There was no statistical difference in oropharyngeal deposition, lung deposition, or regional distribution between the three mouthpieces when the data were calculated as a percent of loaded activity ($p > 0.05$, by ANOVA).

DISCUSSION AND CONCLUSIONS

No statistically significant differences in the deposition of the radio-labeled insulin were observed between the nine women and six men in this study. The data showed that the aerosols delivered by the AER_x system were robust to potential differences between male and female respiratory tract geometry. There were also no statistically significant differences in the respiratory tract deposition patterns across the three mouthpieces examined *in vivo*. Although the MT mouthpiece had half the cross sectional area at the exit of the LE mouthpiece, resulting in twice the air stream velocity exiting the mouthpiece, there is neither a change in deposition in the oropharynx (pre-wash), nor for the combined oropharynx, stomach and mouthwash analysis. These data are consistent with 2-μm diameter particle data from a recent study by Lin *et.al.*, which reports the effects of mouthpiece diameter and flow rate on deposition of particles between 2 and 8 μm in a cast of a human airway (7). Regardless of mouthpiece configuration, the *in vitro* data show that the AER_x system tightly regulates the size of the aerosol so that more than 90% of the emitted dose is made up of droplets less than 4.0 μm that are suitable for deposition in the deep lung. This tight regulation is also demonstrated by the *in vivo* data. The AER_x system also has a patient-guided breath control system to encourage the patient breathing at an optimal flow rate during the inhalation. These features minimize the effect of differences in anatomy, and inter-patient technique variation on aerosol delivery.

Both the *in vitro* and *in vivo* data demonstrate that the dose emitted from the AER_x system is very reproducible, resulting in well controlled lung doses. The reproducibility of the AER_x system is well established (8–10). Aerosol deposition predictability is based on reproducible control of particle size and inspiratory flow rate in the laboratory and in the human trials, as well as minimization of the major cause of variability of lung dose–oropharyngeal deposition. The deposition in the mouth and oropharynx was higher than expected (typically around 5–7% for AER_x) (8). This fortuitous result allowed better evaluation and comparison of the different mouthpiece designs, however, it may have contributed to a lack of agreement between *in vitro* $FPF_{4.0}$ and *in vivo* lung dose. The developmental AER_x data show that the fine particle fraction obtained from *in vitro* measurements for this system (Table IV) do not agree as well as expected (8) with the *in vivo* lung dose, expressed as a percent of emitted dose (Table VI). These results may be due to the use of a developmental AER_x device in which the aerosol evaporation conditions were not fully optimized. The use of an Anderson cascade impactor to measure the particle size distribution may have contributed to a lower *in vitro* MMAD and a higher $FPF_{4.0}$ due to continued evaporation after exiting the mouthpiece, but before depositing on the collection plates. *In vivo*, the high humidity of the respiratory tract may not allow further evaporation after exit from the mouthpiece, resulting in a higher than expected oropharyngeal deposition based on cascade impaction measurements.

Though none of the mouthpieces showed significant differences in lung dose, there was higher mouthpiece deposition on the MT design *in vivo*, as well as indications of reduced emitted dose and increased mouthpiece deposition with the MT design *in vitro*. This increased mouthpiece deposition may be due to the narrowing of the MT mouthpiece and concomittant acceleration of the air flow prior to exit from the mouthpiece. Based on these data, as well as feedback from clinical study volunteers, the LE design was selected for use in the AER_x system.

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REFERENCES

1. M. Svartengren, M. Anderson, G. Bylin, K. Philipson, and P. Camner. Mouth and throat deposition of 3.6 μm radiolabeled particles in asthmatics. *J. Aerosol Med.* **4**:313–321 (1991).
2. J. Schuster, S. Farr, D. Cipolla, T. Wilbanks, J. Rosell, P. Lloyd, and I. Gonda. Design and performance validation of a highly efficient and reproducible compact aerosol delivery system: AER_x®. In R. N. Dalby, P. R. Byron, and S. J. Farr (eds.), *Respiratory Drug Delivery VI,* Interpharm Press, Buffalo Grove, IL, 1998, pp. 83–90.
3. J. Schuster, R. Rubsamen, P. Lloyd, and J. Lloyd. The AER_x® aerosol delivery system. *Pharm. Res.* **14**:354 (1997).
4. United States Pharmacopeia, 27th Revision, Chapter <601>, Aerosols United States Pharmacopeial Convention, Inc. Rockville, Md. 2004.
5. P. Colthorpe, G. Taylor, and S. J. Farr. A comparison of two non-invasive methods for quantifying aerosol deposition in the lungs of rabbits. *J. Aerosol Med.* **10**:255 (1997).
6. J. E. Agnew, R. M. Bateman, D. Pavia, and S. W. Clarke. Radionuclide demonstration of ventilatory abnormalities in mild asthma. *Clin. Sci.* **66**:525–531 (1984).
7. T. C. Lin, P. N. Breysse, B. L. Laube, and D. L. Swift. Mouthpiece diameter affects deposition efficiency in cast models of the human oral airways. *J. Aerosol Med.* **14**:335–341 (2001).
8. S. J. Farr, S. J. Warren, P. Lloyd, J. K. Okikawa, J. A. Schuster, A. M. Rowe, R. M. Rubsamen, and G. Taylor. Comparison of in vitro and in vivo efficiencies of a novel unit-dose liquid aerosol generator and a pressurized metered dose inhaler. *Int. J. Pharm.* **198**:63–70 (2000).
9. S. J. Farr, J. A. Schuster, P. Lloyd, J. L. Lloyd, J. K. Okikawa, and R. M. Rubsamen. AER_x® development of a novel liquid aerosol delivery system: Concept to clinic. In P. R. Byron, R. N. Dalby, and S. J. Farr (eds.), *Respiratory Drug Delivery V*, Interpharm Press, Buffalo Grove, IL, 1996, pp. 175–185.
10. D. Cipolla, B. Boyd, R. Evans, S. Warren, G. Taylor, and S. Farr. Bolus administration of INS365: Studying the feasibility of delivering high dose drugs using the AER_x® pulmonary delivery system. In R. N. Dalby, P. R. Byron, S. J. Farr, and J. Peart (eds.), *Respiratory Drug Delivery VII,* Interpharm Press, Buffalo Grove, IL, 2000, pp. 231–240.