Intra-individual Variability of the Metabolic Effect of Inhaled Insulin Together With an Absorption Enhancer

**OBJECTIVE** — To study the metabolic effect and the variability of the effect elicited by inhalation of 87.2 U insulin powder combined with an absorption enhancer. The metabolic effect was compared with that of 10.2 U regular insulin injected subcutaneously and of 5.5 U regular insulin given intravenously.

**RESEARCH DESIGN AND METHODS** — In this single-center open euglycemic glucose clamp study, 13 healthy male volunteers received 5 insulin administrations on separate study days: once as an intravenous dose, once as a subcutaneous injection, and 3 times by inhalation, in randomized order. Glucose infusion rates (GIRs) necessary to keep blood glucose concentrations constant at 5.0 mmol/l were determined over an 8-h period after administration.

**RESULTS** — After inhalation of the insulin powder aerosol, the onset of action was substantially more rapid than after subcutaneous insulin injection, and maximal action was reached earlier (86 ± 47 vs. 182 ± 53 min, P < 0.0001). The maximal glucose infusion rate after inhalation of insulin was comparable to that after subcutaneous insulin injection (9.2 ± 2.6 vs. 8.8 ± 2.8 mg · kg⁻¹ · min⁻¹, NS). The metabolic effect in the first 2 h after inhalation was significantly greater than that after subcutaneous insulin injection (amount of glucose infused: 0.88 ± 0.25 vs. 0.59 ± 0.20 g · kg⁻¹ · 120 min⁻¹, P < 0.0001). However, the total metabolic effect after inhalation and subcutaneous injection was comparable (2.50 ± 0.76 vs. 2.56 ± 0.69 g · kg⁻¹ · 480 min⁻¹, NS). The relative bioefficacy of inhaled insulin calculated in relation to the data from the subcutaneous insulin application was 12.0 ± 3.5% (absolute bioefficacy 10.1 ± 3.1%) but was highest in the first 2 h after application (18.5 ± 3.7%; absolute bioefficacy 8.2 ± 4.1%). The intraindividual variability of the metabolic response induced by insulin inhalation was 14 ± 9% for the maximal glucose infusion rate, 15 ± 10% for the time-to-maximal effect, and 16 ± 12% for the total amount of glucose infused.

**CONCLUSIONS** — This feasibility study shows that inhaled insulin with an absorption enhancer has a pronounced metabolic effect compared with the results of a previous study of inhaled insulin without an enhancer. The intraindividual variability of the metabolic effect was comparable with that of inhaled and subcutaneously injected insulin.

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Insulin molecules can be absorbed into the blood stream after inhalation from the alveoli (1). In a previous study, we showed that inhalation of an insulin powder aerosol containing solid insulin particles (99 U) led to a relative bioavailability of 7.8 ± 3.5% and a relative bioefficacy of 7.6 ± 2.9% (2). This bioefficacy was comparable with that achieved with nasal insulin administration using absorption enhancers but implies that a large amount of insulin has to be used with either administration form to achieve a sufficient metabolic effect (3–7). Nevertheless, the time-action profile seen with inhalation of insulin showed promising properties (i.e., a rapid onset of action and a relatively long duration of action).

Co-administration of nonionic surfactants increased insulin absorption after inhalation 3- to 4-fold in animal experiments (8). Thus, the use of an absorption enhancer may increase bioavailability and bioefficacy considerably and would probably also improve the time-action profile of inhaled insulin. This study in healthy subjects investigated the bioavailability, bioefficacy, and within-subject variability of response to inhaled biosynthetic insulin with an absorption enhancer (bile salt, an endogenous substance) in comparison with intravenous and subcutaneous administration of insulin.

**RESEARCH DESIGN AND METHODS**

Subjects
This single-center study was open and randomized and had a 5-period crossover design. A total of 13 healthy subjects (age 27 ± 3 years [range 23–34], BMI 22.7 ± 2.0 kg/m² [19.4–26.2], weight 73 ± 9 kg [55–85], normal lung function [forced expiratory volume (FEV1) 4.6 ± 0.5 liters (3.8–5.3)], 104 ± 10% of the predicted value, all insulin IgG antibody negative, all nonsmokers [urine cotinine negative]) were randomized. Written informed consent was given by the volunteers after a detailed explanation of the study procedures. The study was approved by the local ethics committee and was carried out according to the principles of the Declaration of Helsinki and of good clinical practice.

Study protocol
After an overnight fast, the subjects came to the clinic and were connected to a Biostator (Life Science Instruments, Elkhardt, IN) targeting blood glucose 5.0 mmol/l, continuous
Table 1—Pharmacodynamic and pharmacokinetic summary measures after inhalation of 87.2 U insulin with an absorption enhancer, subcutaneous injection of 10.2 U regular insulin, and intravenous administration of 5.5 U on different study days in 13 healthy volunteers (baseline values subtracted)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Inhaled insulin</th>
<th>Subcutaneous injection</th>
<th>Intravenous injection</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose requirements (mg·kg⁻¹·min⁻¹)</td>
<td>2.1 ± 0.8</td>
<td>2.2 ± 1.4</td>
<td>1.8 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Time to early half-maximal effect (min)</td>
<td>14 ± 5</td>
<td>43 ± 12</td>
<td>6 ± 4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Maximal metabolic effect (mg·kg⁻¹·min⁻¹)</td>
<td>9.2 ± 2.6</td>
<td>8.8 ± 2.8</td>
<td>8.2 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>GIR t_max (min)</td>
<td>86 ± 47</td>
<td>182 ± 53</td>
<td>44 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to late half-maximal effect (min)</td>
<td>277 ± 68</td>
<td>352 ± 61</td>
<td>138 ± 56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic effect after 8 h (mg·kg⁻¹·min⁻¹, without baseline subtraction)</td>
<td>4.2 ± 1.8</td>
<td>4.1 ± 1.9</td>
<td>3.3 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>AUC in the first 120 min after insulin administration (g·kg⁻¹·120 min⁻¹)</td>
<td>0.88 ± 0.25</td>
<td>0.59 ± 0.20</td>
<td>0.74 ± 0.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC in the 480 min after insulin administration (g·kg⁻¹·480 min⁻¹)</td>
<td>2.50 ± 0.76</td>
<td>2.56 ± 0.69</td>
<td>1.48 ± 0.62</td>
<td>NS</td>
</tr>
<tr>
<td>Basal serum insulin (pmol/l)</td>
<td>62 ± 17</td>
<td>61 ± 16</td>
<td>66 ± 26</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal serum insulin (pmol/l)</td>
<td>606 ± 199</td>
<td>179 ± 45</td>
<td>3409 ± 697</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to maximal concentration (min)</td>
<td>9 ± 3</td>
<td>105 ± 59</td>
<td>4 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum insulin after 8 h (pmol/l, without baseline subtraction)</td>
<td>87 ± 23</td>
<td>77 ± 26</td>
<td>62 ± 22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AUC in the first 120 min after insulin administration (nmol·l⁻¹·120 min⁻¹)</td>
<td>29.4 ± 9.0</td>
<td>15.5 ± 5.3</td>
<td>29.5 ± 4.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC in the 480 min after insulin administration (nmol·l⁻¹·480 min⁻¹)</td>
<td>52.5 ± 17.3</td>
<td>44.3 ± 9.5</td>
<td>34.8 ± 10.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated.
resulted in a more rapid increase to higher maximal values than subcutaneous insulin administration (Fig. 1B, Table 1). The serum insulin profiles for the 2 modes of administration were comparable after 90 min, with a slow decline thereafter. After 8 h, serum insulin levels were still a little higher than baseline levels. The AUC of the serum insulin profiles for the first 120 min after administration and also the total AUC was greater with inhaled insulin than with subcutaneous insulin injection.

Absolute bioavailability of insulin after inhalation was estimated to be 10.2 ± 5.0% and relative bioavailability 14.5 ± 4.4% (intraindividual CV 19 ± 6%). During the first 2 h after inhalation, absolute bioavailability was estimated to be 6.4 ± 2.3% and relative bioavailability to be 25.1 ± 10.7%, reflecting the different time-action profiles obtained with the 2 methods of administration (intraindividual CV 17 ± 6%). After insulin inhalation, the intraindividual variability was 19 ± 7% for the maximal serum insulin concentrations, 25 ± 12% for the time to maximal levels, and 19 ± 6% for the total area under the serum insulin levels.

The continuous baseline intravenous insulin infusion and the administered insulin resulted in mean serum C-peptide levels of 0.6 nmol/l throughout the experiments with all forms of administration.

Tolerability of insulin inhalation

The insulin inhalation was generally well tolerated by the volunteers (i.e., no adverse event pattern that could be related to this form of administration was observed).

**CONCLUSIONS** — Inhalation is a novel administration form for insulin that is under clinical development. Important questions with this kind of administration are as follows: 1) Does it induce a sufficient metabolic effect relatively rapidly after administration, allowing sufficient prandial insulin substitution? 2) Is the variability of the induced metabolic effect comparable to that after subcutaneous insulin injection?

Our feasibility study demonstrated for the first time in humans that the addition of an absorption enhancer leads to a considerably greater metabolic effect than that seen with inhalation of pure insulin, which was previously studied (2). The observed relative bioefficacy was 7.6% for the 360-min duration of the study with inhalation of pure insulin, compared with 12.0% in this study (duration 480 min). To show the differences in glucose consumption in the 2 studies, mean GIRs and serum insulin concentrations registered with inhalation of pure insulin and insulin with an absorption enhancer are given in Fig. 2A and B. The difference in metabolic action is especially pronounced in the first 2 h after inhalation, which is the critical time period in which to limit postprandial glycemic excursions. In other words, the total metabolic activity induced might be comparable with different insulin administration forms, but the time when this activity occurs is important.

Appropriately designed meal-related studies with diabetic patients are necessary to examine whether the greater metabolic effect soon after administration gives a better postprandial metabolic control than subcutaneous injection of regular insulin (or rapid-acting insulin analogs) (9–11).

The onset of action seen with insulin plus the absorption enhancer after inhalation of the insulin powder aerosol was similar to or even more rapid than that seen with rapid-acting insulin analogs given by subcutaneous injection, but the
Variability of inhaled insulin

In other studies with inhaled insulin, without an absorption enhancer, a relative bioavailability of ~10% was reported (15-17). In these studies, bioefficacy was estimated as a decline in blood glucose (i.e., stimulation of counterregulatory hormones might have occurred, allowing no precise description of the pharmacodynamic properties of the insulin preparations used) (18). Thus, the results of such studies are difficult to compare with our data.

Inhalation of insulin was well tolerated in our study. The enhancer used is an endogenous substance (bile salt). However, use of an absorption enhancer gives rise to safety concerns. There are no data on the long-term effects of inhaling considerable amounts of bile salt (and insulin) over extended periods of time. If this substance has any negative effects, they are currently unknown and it remains to be clarified whether the benefits outweigh the risks of using an additional substance.

Insulin inhalation led to a good reproducibility of the metabolic effect induced, at least under our controlled experimental conditions. The within-subject variability after inhalation was about the same for the total amount of glucose infused (CV 16%) as for the total AUC under the serum insulin profile (19%). Quantitative information about the intraindividual variability of the metabolic effects induced by subcutaneous injection of regular insulin is limited (19,20). In a study that we performed under similar conditions in healthy subjects, the within-subject variability was evaluated after subcutaneous administration of regular insulin or of the rapid-acting insulin analog insulin aspart in identical doses on 4 study days (21). The intraindividual CVs obtained in that study were comparable to those seen with inhaled insulin.

In summary, inhalation of an insulin powder aerosol with an absorption enhancer led to a pronounced metabolic effect soon after administration. The variability in the induced metabolic effect was comparable to that of regular insulin after subcutaneous administration. This feasibility study indicates that the powder aerosol used might be beneficial for prandial insulin substitution in patients with type 2 diabetes who are reluctant to take injections and, therefore, use oral drugs even if insulin therapy is indicated.

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References

Figure 2 — Mean glucose infusion rates (A) and serum insulin concentrations (B) registered in healthy subjects after inhalation of pure insulin (99 U, ---) or insulin with an absorption enhancer (bile salt, 87.2 U, —) in this and a previous study (2).