Variability of the Metabolic Effect of Soluble Insulin and the Rapid-Acting Insulin Analog Insulin Aspart

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OBJECTIVE — To study the intra- and interindividual variability of the metabolic activity of soluble insulin and of the rapid-acting insulin analog insulin aspart after subcutaneous injection.

RESEARCH DESIGN AND METHODS — A total of nine healthy male volunteers received subcutaneous injections of soluble insulin (0.2 U/kg) in the abdominal region on each of the four study days. Another 10 volunteers received an injection of insulin aspart four times. Glucose infusion rates necessary to neutralize the blood glucose-lowering effect of the administered insulin were registered during euglycemic glucose clamps (blood glucose 5.0 mmol/l; basal intravenous insulin infusion 0.15 mU · kg⁻¹ · min⁻¹) over the subsequent 600 min. We investigated the variation in metabolic activity by calculating coefficients of variation (CVs).

RESULTS — In comparison to soluble insulin, subcutaneous injections of insulin aspart led to a more rapid onset of action and a shorter duration of action. Subcutaneous injection of the insulin preparations resulted in intraindividual CVs of the summary measures between 10 and 30% (soluble insulin vs. insulin aspart: maximal metabolic activity 15 ± 7 vs. 16 ± 10%, time to maximal metabolic activity 14 ± 10 vs. 11 ± 6%; NS between the preparations [means ± SD]). The decline to half-maximal activity after maximal activity showed a lower intraindividual CV with insulin aspart (19 ± 9 vs. 11 ± 5%; P = 0.018). The interindividual CVs were higher than the intraindividual CVs (26 vs. 23 vs. 19, and 26 vs. 17%). Generally, the pharmacodynamic variability was higher than the pharmacokinetic variability. For the pharmacokinetic measures, the intra- and interindividual variability in t_max was lower for insulin aspart than for soluble insulin.

CONCLUSIONS — The metabolic effect of soluble insulin shows an intraindividual variability of 10–20% in healthy volunteers, even under strictly controlled experimental conditions. The overall variability of action of insulin aspart was comparable to that of soluble insulin.

For clinical purposes, quantitative data about the intraindividual variability of the metabolic effect of subcutaneous injected insulin should be helpful. To quantify the intraindividual variability of the metabolic effect, the euglycemic clamp technique was used in two studies (8,9). In the study by Galloway et al. (8), the metabolic effect of long-acting zinc insulin was investigated. Ziel et al. (9) studied soluble insulin, but on two study days and with a study duration of 360 min only. Thus to date, knowledge about the variability of the metabolic effect of currently available short-acting insulin preparations is limited.

Subcutaneous injection of rapid-acting insulin analogs like insulin aspart and insulin lispro may lead to a reduced variability of insulin action due to the weaker self-association tendency of insulin monomers into hexamers (10). Insulin aspart was shown to have a more rapid onset of action and a shorter duration of action after subcutaneous injection than soluble insulin (11,12). In clinical experiments, the use of insulin lispro was reported to result in a reduced variability of the metabolic effect (13). Nevertheless, it has not been investigated under controlled experimental conditions, so far, if the variability of the metabolic effect of rapid-acting insulin analogs is lower than that of soluble insulin. We studied the variability of insulin action and of insulin absorption after injecting identical doses of soluble insulin or insulin aspart subcutaneously into the abdominal region of healthy volunteers on four study days.

RESEARCH DESIGN AND METHODS

Subjects
A total of 20 healthy male volunteers (age 26 ± 1 [range 23–28] years; BMI 22.8 ± 1.9 [18.9–26.0] kg/m²) participated in this double-blind study. After receiving detailed oral and written information, all volunteers undersigned an informed consent. The study was performed according to the principles of Good Clinical Practice and the declaration of Helsinki and was approved by the local ethical committee.
Study protocol During the morning of each of the four study days, the volunteers were connected to a Biostator after having fasted for at least 12 h. Blood glucose was kept constant at a target level of 5.0 mmol/l by means of the euglycemic glucose clamp technique (14). A low-dose basal intravenous insulin infusion of 0.15 mU · kg⁻¹ · min⁻¹ was maintained continuously throughout the whole experiment in order to establish comparable baseline insulin levels on all study days. Baseline glucose requirements were registered for 90 min, before insulin was injected subcutaneously by means of a syringe (0.3-ml Microfine IV+, Becton Dickinson, Heidelberg, Germany; needle length 13 mm) into the abdominal region. Glucose infusion rates (GIR) necessary to keep blood glucose constant were registered during the subsequent 600 min. The four study days were separated by at least 7 days. The volunteers were instructed to keep their body weight constant and to abstain from strenuous physical exercise during the study period.

Identical insulin doses of 0.2 U/kg body weight (14.4 ± 1.6 [range 12–17] U) were injected each time with the same injection technique in the umbilical region by the same investigator (L.H.). The needle was inserted at a 45° angle into a lifted skinfold in order to ensure subcutaneous injection. Nine volunteers received subcutaneous injection of soluble insulin on each of the four study days (Actrapid HM, U100, Novo Nordisk, Bagsvaerd, Denmark), whereas 10 other volunteers received injections of insulin aspart (B28Asp; U100, Novo Nordisk). The 10th volunteer of the soluble insulin group developed an acute otitis media before the first study day, which had to be treated with antibiotics and led to this subject dropping out of the study. The two groups of volunteers did not differ with respect to age and BMI.

Blood samples for estimation of plasma glucose, serum insulin, and serum C-peptide concentrations were collected in 30-min intervals before the injection, and thereafter in intervals of 10 min (0–60 min after injection), 15 min (60–120 min), and 30 min (120–600 min), respectively. Serum insulin and serum C-peptide concentrations were estimated by commercial radioimmunoassays (Pharmacia Insulin RIA 100, Pharmacia Uppsala, Sweden, intra-assay coefficient of variation [CV] 4.9% and interassay CV 4.9%) and an enzyme-linked immunosorbent assay (ELISA) (DAKO C-peptide, DAKO Diagnostics, Cambridgeshire, U.K.; intra-assay CV 6.4% and interassay CV 8.7%) by Medi-Lab, Copenhagen, Denmark. The Pharmacia Insulin RIA 100 does not measure the concentration of insulin aspart proportionally to human insulin concentrations. Therefore, the following corrections formula was used to calculate the correct insulin aspart concentrations in the blood:

\[
\text{Insulin aspart}_{\text{corrected}} = F \times \frac{1,503 \times \text{insulin aspart} \, \text{(fraction)}}{1,398 - \text{insulin aspart} \, \text{(fraction)}}
\]

with \( F \) denoting the dilution factor.

Statistical methods
A lognormal function was fitted to each of the individual time-action profiles registered (15). This function allowed calculation of the following pharmacodynamic summary measures: maximal glucose infusion rate (GIR_max), time to GIR_max (t_max), time to half-maximal GIR values before and after GIR_max (early t₅₀% and late t₅₀%), and the area under the curve (AUC) of GIR time profiles. The coefficient of variation (CV = SD / x) was calculated for the summary measures in order to describe the intraindividual and interindividual variability of insulin action. Intraintdividual CVs were calculated from the results obtained with each volunteer on the four study days. Interindividual CVs were calculated from the individual mean values of all volunteers with an identical insulin preparation. An unpaired, two-sided t test was used for statistical comparison of the intraintdividual CV of the summary measures obtained with the two insulin preparations. A P value <0.05 was used as significance level. To describe the individual variability of the summary measures graphically, the individual means and their SDs were presented (16).

Variability of insulin absorption was studied by fitting a polynomial function to the individual serum insulin concentration profiles. The following pharmacokinetic summary measures were estimated graphically: maximal serum insulin concentration (C_max), time to C_max (t_max). The AUCs were calculated for different time periods under the intraintdividual insulin profiles. The variability of these pharmacokinetic summary measures was studied as described for the pharmacodynamic parameters.

The sample size assessment was based on the intrasubject variation comparison. With 20 subjects and four replications per subject, a true ratio of SDs of <0.5 and >2.0 would be discovered with 96% probability at a 5% significance level.

RESULTS
Blood glucose
Blood glucose concentrations were kept constant throughout the glucose clamp experiments at identical levels (soluble insulin vs. insulin aspart 5.0 ± 0.3 vs. 5.0 ± 0.2 mmol/l).

Time-action profile
Baseline glucose infusion rates were 1.0 ± 0.5 mg · kg⁻¹ · min⁻¹ with soluble insulin and 0.9 ± 0.5 mg · kg⁻¹ · min⁻¹ with insulin aspart (NS). As shown previously, the time-action profile of soluble insulin is characterized by a later onset of action, a later maximal effect, and later decline back to baseline values in comparison to the metabolic effects registered after subcutaneous injection of insulin aspart (P < 0.01; Fig. 1A, Table 1). The maximal metabolic effect registered was not different after subcutaneous injection of soluble insulin and insulin aspart. Injection of insulin aspart resulted in a higher AUC under the GIR profiles in the first 2 h after injection (P < 0.001). The overall metabolic effect over 10 h was comparable (Table 1).

The intraindividual variability of the summary measures of soluble insulin and insulin aspart were not significantly different (Table 1), only the decline to half-maximal activity after maximal activity showed a lower intraindividual variability with insulin aspart in comparison to soluble insulin. The interindividual CVs were 5–20% higher than the intraindividual ones (Table 1). The intra- and interindividual variability of some summary measures are given in Fig. 2A–E in a special presentation form. Summary measures do not provide information about the variability of insulin action over time after insulin injection. Calculation of the mean intraintdividual CV for each of the two insulin preparations showed that the CV was ~25% for both preparations between 60 and 360 min after injection (Fig. 2F).

Serum insulin profiles
Baseline serum insulin levels were 61 ± 11 pmol/l with soluble insulin and 55 ± 8 pmol/l with insulin aspart (NS). Serum insulin concentrations after subcutaneous injection of insulin aspart increased more rapidly and to higher concentrations in comparison with those obtained with solu-
Variability of insulin action

Figure 1—Glucose infusion rates (A) and serum insulin concentrations (B) after subcutaneous injection of 0.2 U/kg body weight of the rapid-acting insulin analog, insulin aspart, or of soluble insulin in healthy male volunteers on four study days (mean ± SEM).

Table 1—Intra- and interindividual variability of the metabolic effect and the serum insulin concentrations after subcutaneous injection of soluble insulin or insulin aspart

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soluble insulin (n = 9)</th>
<th>Insulin aspart (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Intraindividual CV (%)</td>
</tr>
<tr>
<td>Pharmacodynamic summary measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIR&lt;sub&gt;max&lt;/sub&gt; (mg·kg&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>9.5 ± 2.3</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>156 ± 29</td>
<td>14 ± 10</td>
</tr>
<tr>
<td>Early t&lt;sub&gt;50%&lt;/sub&gt; (min)</td>
<td>61 ± 12</td>
<td>16 ± 10</td>
</tr>
<tr>
<td>Late t&lt;sub&gt;50%&lt;/sub&gt; (min)</td>
<td>387 ± 68</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>AUC 0–120 min (mg·kg&lt;sup&gt;-1&lt;/sup&gt;·[120 min]&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>533 ± 216</td>
<td>27 ± 22</td>
</tr>
<tr>
<td>0–600 min (mg·kg&lt;sup&gt;-1&lt;/sup&gt;·[600 min]&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3,011 ± 548</td>
<td>13 ± 3</td>
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<tr>
<td>Pharmacokinetic summary measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (pmol/l)</td>
<td>195 ± 41</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>129 ± 36</td>
<td>24 ± 10</td>
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<tr>
<td>AUC 0–120 min (nmol·l&lt;sup&gt;-1&lt;/sup&gt;·[120 min]&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>16.1 ± 2.9</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>0–600 min (nmol·l&lt;sup&gt;-1&lt;/sup&gt;·[600 min]&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>52.9 ± 5.9</td>
<td>14 ± 10</td>
</tr>
</tbody>
</table>

The summary measures were estimated after subtraction of baseline glucose infusion rates and serum insulin concentrations. *P < 0.05 vs. soluble insulin; †P < 0.001 vs. soluble insulin.
preparations studied revealed that the variability of action of insulin aspart was comparable to that of soluble insulin for most summary measures.

There are two possible explanations for the variability of insulin action: 1) pharmacokinetic variability (i.e., same doses of insulin lead to different plasma insulin concentrations), and 2) pharmacodynamic variability (i.e., similar plasma insulin concentrations induce different metabolic effects). Concerning the variability of insulin absorption (the pharmacokinetic part), a number of studies have been performed using radioactively labeled soluble insulin (1–7). With this semiquantitative method, an intrapatient CV of 15% and an interindividual CV of 30% has been estimated for the time to decline to half-maximal radioactivity over the insulin depot in the skin (T50%) for soluble insulin (7). Direct measurement of the serum insulin concentrations after subcutaneous insulin injection revealed a considerably higher intrapatient variability of Cmax and tmax of 64 and 107% (soluble insulin), 28 and 34% (lente insulin), and 44 and 68% (NPH-insulin) (17). Insulin absorption from the subcutaneous depot might also vary from injection site to injection site caused by the anatomical properties (e.g., local blood flow) and/or the local degradation of insulin (6,18,19). Injection of human insulin into the abdominal region resulted in a lower variability than that observed after injection of the respective porcine insulin preparation in the deltoid region (8).

The variability of insulin action (the pharmacodynamic part) was studied quan-
tatively with the euglycemic glucose clamp technique in two studies only (8,9). Galloway et al. (8) found intra- and interindividual CVs between 35 and 55% for the calculated summary measures for slow-acting insulin preparations employing an unbalanced study design with eight healthy volunteers. Ziel et al. (9) studied the variability of insulin absorption and insulin action with soluble insulin employing a similar study protocol as in our study, but with two study days and a study duration of 360 min only. In this study, the intraindividual CV for the total AUC below the insulin profile was 11%, which was lower than the CV for the AUC below the time-action profile (23%). Also in our study, the variability of the pharmacokinetic summary measures was lower than that of the pharmacodynamic parameters.

It is reasonable to assume, that in daily life the variability of insulin action in diabetic patients is even higher than that observed in healthy volunteers studied under controlled experimental conditions. Factors like insulin antibodies, changes in metabolic control, or previous hypoglycemic episodes with their impact on the actual insulin sensitivity were ruled out by our study design. The high variability of the metabolic response to application of identical insulin doses is a serious problem for insulin-treated diabetic patients and hamper the achievement of a good metabolic control without suffering from hypoglycemic events. The poor reproducibility of the metabolic effects of subcutaneous insulin therapy might be a source of frustration and uncertainty in many patients. Therefore, development of insulin preparations with a lower variability of action remains an important task for the future. From a clinical study with insulin lispro it was anticipated that the more rapid absorption of the insulin analog might result in a lower variability of insulin absorption and probably also insulin action in comparison to soluble insulin (13). Nevertheless, we did not observe a lower variability of insulin action under controlled experimental conditions, with the exception of the decline of the metabolic effect induced.

In summary, under experimental conditions in healthy volunteers, subcutaneous injection of soluble insulin resulted in an intra- and interindividual variability in insulin action of ~30%. The variability of action of insulin aspart was equivalent to that of soluble insulin.

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References