

# The Effect of Insulin Antibodies on the Metabolic Action of Inhaled and Subcutaneous Insulin

A prospective randomized pharmacodynamic study

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**OBJECTIVE** — To assess the impact of the development of high- or low-affinity insulin antibodies (IABs) on postprandial glucose tolerance, duration of insulin action, and clinical safety in patients with type 1 diabetes receiving inhaled insulin (Exubera).

**RESEARCH DESIGN AND METHODS** — This study consisted of a prospective, randomized, open-label, parallel-group trial in which 47 patients with type 1 diabetes received NPH insulin twice daily plus either premeal inhaled insulin (INH group;  $n = 24$ ) or premeal subcutaneous regular insulin (SC group;  $n = 23$ ) for 24 weeks. Meal challenge and euglycemic clamp studies were performed on consecutive days at baseline, week 12, and week 24. Adverse events were monitored.

**RESULTS** — For the INH and SC groups, mean ( $\pm$ SD) IAB levels were  $3.5 \pm 3.9$  and  $2.6 \pm 4.1$   $\mu$ U/ml at baseline, respectively, compared with  $101.4 \pm 140.4$  and  $4.3 \pm 9.4$   $\mu$ U/ml at week 24. At week 24, the changes from baseline were similar for the INH and SC groups for maximal plasma glucose concentration ( $C_{max}$ ) (adjusted ratio for treatment group difference 0.99 [90% CI 0.95–1.03]), area under the plasma glucose concentration time curve (adjusted ratio for treatment group difference 0.98 [0.88–1.08]), and duration of insulin action (adjusted treatment group difference 29 min [–49 to 108]). No adverse events were attributed to IABs.

**CONCLUSIONS** — In patients with type 1 diabetes treated with inhaled insulin, development of high- or low-affinity IABs did not impair postprandial glucose tolerance, alter the time-action profile of insulin, or impact tolerability. No clinical relevance of IABs was identified in this study.

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**B**efore the introduction of highly purified porcine and recombinant human insulins into clinical practice, the use of less purified animal-derived insulin in patients with diabetes was associated with a near 100% prevalence of

detectable insulin antibodies (IABs) (1). Although mean antibody levels are now lower in patients treated with subcutaneous human insulin, ~50% of such patients have measurable IABs (2). Since the initial description of IABs in the 1950s,

the effect of antibody development, from low levels at baseline to levels commonly observed during clinical therapy, on key glycemic parameters has not been tested in a prospectively designed randomized controlled study.

Multiple cross-sectional studies over the years have investigated whether IABs can cause alterations in insulin pharmacokinetics or pharmacodynamics. Although some studies have shown associations between IABs and delayed rises in free insulin concentrations (3,4) or prolonged half-life of free insulin (3,5) after insulin dosing, the clinical impact of these observations remains unclear (6). Using a standardized meal challenge test, one study showed a relationship between high-affinity IAB binding and impaired postprandial glucose tolerance (7), although such a relationship has not been consistently reported (8,9). Significantly, although some studies have reported a deleterious effect of IABs on long-term glycemic control (10–12), most have not (8,13–18). Notably, the syndrome of immunologic insulin resistance has been reported in patients with high levels of maximum insulin binding (5).

Furthermore, reports of prolongation of insulin half-life have not been associated with an increased incidence of clinical hypoglycemia (14), although rare cases of unusual hypoglycemic syndromes associated with insulin therapy-related antibodies have been described (19–21). In addition, hypoglycemia thought to be due primarily to high levels of low-affinity binding has been described in the insulin autoimmune syndrome (Hirata's Syndrome) (22) and in rare cases of monoclonal gammopathy (23–25)

Inhaled insulin offers a noninvasive method of insulin delivery via the pulmonary mucosa. Exubera (Pfizer, New York, NY/sanofi-aventis, Bridgewater, NJ in collaboration with Nektar Therapeutics, San Carlos, CA) is a novel aerosol delivery system that enables delivery of inhaled, rap-

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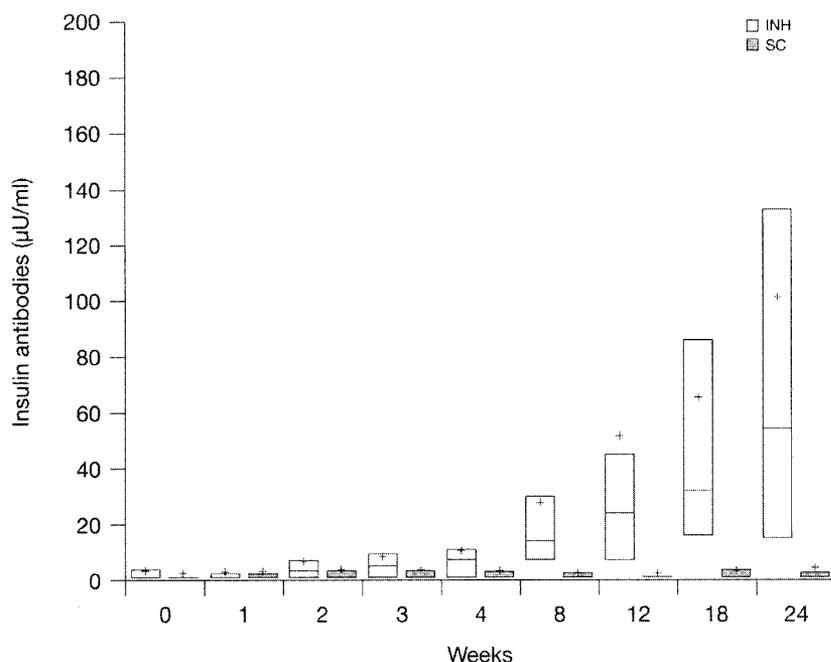
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**Abbreviations:** AUC, area under the curve; GIR, glucose infusion rate; IAB, insulin antibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**Figure 1**—IAB response curve in the INH and SC groups throughout the study. The box plots represent the median (center horizontal line), mean (indicated by +), and the 25th and 75th percentiles (bottom and top edges of the box). Three subjects receiving inhaled insulin had IAB levels >240 µU/ml during the study. The maximum antibody level achieved with inhaled insulin therapy was 641 µU/ml compared with 41 µU/ml in patients treated with subcutaneous insulin, both of which occurred at week 24.

id-acting, dry-powder human insulin. The results of several clinical trials have demonstrated that inhaled insulin is effective, well tolerated, and well accepted in people with either type 1 or type 2 diabetes (26,27). However, in studies with Exubera and with other inhaled insulin preparations, patients receiving inhaled insulin, particularly those with type 1 diabetes, have developed greater serum IAB concentrations than patients receiving comparator therapies (28). These antibodies have been shown to be primarily IgG, as is the case with antibodies associated with subcutaneous insulin (28).

This prospective, randomized study investigated the relationship between the development of high- and low-affinity antibody binding and postprandial glucose tolerance, duration of insulin action, and safety in patients with type 1 diabetes treated with inhaled insulin.

**RESEARCH DESIGN AND METHODS**

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, 1989. The protocol was approved by the local institutional review board, and all patients provided written informed consent.

This 24-week, prospective, randomized, open-label, parallel-group trial consisted of a screening visit, a 4-week lead-in dose titration period in which patients received subcutaneous insulin, and a 24-week randomized treatment phase during which the patients underwent three inpatient evaluations. Men and women aged 18–50 years with type 1 diabetes, HbA<sub>1c</sub> (A1C) levels 5.0–9.0%, baseline IAB levels ≤20 µU/ml, and fasting C-peptide levels ≤0.3 pmol/ml were eligible for inclusion. Smokers and patients with lung disease were excluded. (Additional inclusion/exclusion criteria are presented in 26.)

Eligible patients were randomized by a computer-generated randomization scheme to receive outpatient therapy with either premeal inhaled insulin (INH group) or premeal subcutaneous regular human insulin (SC group), along with NPH insulin twice daily, for a 24-week treatment period.

**Inpatient evaluations**

A 2-day inpatient evaluation was performed at weeks 0, 12, and 24. Each evaluation consisted of a standardized meal challenge on day 1 followed by a euglycemic

mic glucose clamp on day 2. Following randomization, but before the baseline inpatient evaluation, dose-finding studies were performed to confirm an appropriate study drug dose of either inhaled or subcutaneous regular insulin for all three inpatient evaluations. In this way, the study drug dose was optimized for the individual patient for the standard meal at baseline, and this dose remained fixed for both components of each inpatient evaluation. For patients randomized to receive the INH regimen, the optimized dose of inhaled insulin was administered within 5 min of the standardized meal, consistent with its anticipated onset of action. For patients randomized to receive the premeal SC regular insulin regimen, the optimized subcutaneous regular insulin dose was administered 15 min before the meal, consistent with European labeling recommendations for the subcutaneous insulin employed. The study dose of subcutaneous insulin for the inpatient meal challenge and euglycemic clamp studies was administered by study staff.

In the meal challenge studies, patients were admitted for evaluation at 6:00 A.M. (± 15 min), after fasting for at least 8 h. A continuous regular insulin infusion at a rate of 0.2 mU · kg<sup>-1</sup> · min<sup>-1</sup> was maintained throughout the meal challenge study. Glucose was infused at a variable rate by a biostator (glucose-controlled insulin infusion system; MTB Medizintechnik, Ulm, Germany), if necessary, to keep plasma glucose at a stable concentration of 7.2 mmol/l. At noon (12:00 P.M.), the glucose infusion was stopped and patients received their individualized study drug doses before the meal challenge. The test meal (a 450-kcal solid meal containing 13.2 g protein, 10.9 g lipids, and 73.2 g carbohydrates) was to be consumed within 15 min. Blood glucose measurements were performed immediately before study drug dosing and at protocol-specified time points during the subsequent 6 h.

At 8:00 A.M. on day 2 of each inpatient evaluation, after an overnight fast, a glucose clamp study was begun, employing a constant insulin infusion of 0.2 mU · kg<sup>-1</sup> · min<sup>-1</sup> and blood glucose clamp level at 7.2 mmol/l. Blood glucose measurements were performed continuously by the biostator and confirmed by reference measurements in at least 30-min intervals with a laboratory device (Super GL; Ruhrtal Labortechnik, Mönnesee-Delecke,

Germany). Study medication was administered at 10:00 A.M., after which the blood glucose clamp level was maintained for 12 h with a variable infusion of glucose.

### Outpatient management

Two dosage strengths of inhaled insulin (1 and 3 mg) were used to administer the aerosolized inhaled insulin powder by means of a dry-powder inhaler (Nektar Therapeutics, San Carlos, CA). Doses of inhaled insulin were administered immediately before meals. Doses of NPH insulin in both treatment groups were administered before breakfast and at bedtime. Home glucose monitoring measurements were used to adjust insulin dose to achieve bedtime and premeal glucose targets of 5.5–7.7 and 4.4–6.6 mmol/l, respectively. In both the INH and SC insulin groups, recommended doses were set weekly based on the previous week's mean glucose monitoring results.

### IAB measurements

Blood samples were collected at weeks –4, –3, 0, 1, 2, 3, 4, 8, 12, 18, and 24 for routine measurement of IABs using a radioligand binding assay based on a modification of previously described methodology (28). Bound and unbound insulin was removed using acid treatment followed by charcoal extraction. After centrifugation to remove the charcoal-bound insulin, insulin-depleted samples were incubated with radiolabeled human recombinant insulin (Aventis Pharma Deutschland, Frankfurt, Germany) at a final concentration of  $1.4 \times 10^{-10}$  mol/l for 24 h at 4°C. IAB complexes were precipitated with polyethylene glycol (15.3% [wt/vol]), and the precipitates were quickly washed with buffer (11% polyethylene glycol) and allowed to dry. Scintillant was added and the amount of radioactivity measured using a Microbeta Trilux counter (Perkin Elmer). To measure nonspecific binding, high concentrations of unlabeled insulin were added to radiolabeled insulin and insulin-extracted samples. Percent-specific IAB binding for each sample was calculated by subtracting the mean nonspecific binding counts from the total binding counts for each sample and dividing by the total counts of insulin added. Specific insulin-binding capacity (microunits of insulin bound per milliliter of sample) was calculated by multiplying the percent binding by the amount of insulin added ( $\mu$ U) and

dividing by the volume of serum tested. The lower limit of quantitation for this assay was 2.1  $\mu$ U/ml.

Assays to measure IAB insulin-binding activity at a range of affinities were also employed. These were performed once for each inhaled insulin-treated study patient, using end-of-study or last-visit serum specimens. For measurement of high-affinity binding, serum was incubated with insulin at  $1.4 \times 10^{-10}$  mol/l, conditions similar to those employed for the routine IAB measurement described above. At higher concentrations of insulin, lower-affinity antibodies will bind to insulin at equilibrium. Thus, to measure low-affinity IABs, test serum was incubated with  $10^{-8}$  mol/l insulin. The  $10^{-8}$ -mol/l concentration (1,508  $\mu$ U insulin/ml) was selected since it is within the range of total (bound and free) insulin concentrations observed in patients with high levels of IABs (29,30). This assay measures binding capacities of both low- and high-affinity IABs. The lower limit of quantitation for the high-affinity assay was 4.3  $\mu$ U/ml and for the low-affinity assay was 471  $\mu$ U/ml.

### Safety

General safety assessments performed during the study included physical examination, vital signs, laboratory safety tests, and the recording of observed and volunteered adverse events.

A hypoglycemic event was defined as any symptom characteristic of hypoglycemia with prompt resolution after carbohydrate intake, any blood glucose measurement of  $\leq 59$  mg/dl (3.3 mmol/l) associated with hypoglycemic symptoms, or any blood glucose measurement  $< 49$  mg/dl (2.7 mmol/l). A severe hypoglycemic event was defined as a hypoglycemic event during which the patient was unable to treat himself or herself, had at least one neurological symptom, and had either a blood glucose reading  $< 49$  mg/dl (2.7 mmol/l) or resolution of the patient's symptoms occurred with glucose or glucagon administration.

### Statistical analyses

The change from baseline in peak plasma glucose concentration ( $C_{\max}$ ) and the area under the plasma glucose concentration time curve ( $AUC_{0-120}$ ) were calculated based on data from the meal challenge tests. The study sample size ( $n = 40$ ) provided a power of 80% to detect a treat-

ment group difference of 38 mg/dl (2.1 mmol/l) in peak postprandial glucose concentration.

The euglycemic clamp studies were used to determine the following end points: glucose infusion rate (GIR),  $GIR_{\max}$  (maximum GIR),  $GIR AUC_{0-120}$  (the total amount of glucose infused for the interval 0–120 min),  $t_{50\text{-early}}$  (the time before  $t_{\max}$  when the smoothed curve is at 50% of  $GIR_{\max}$ ),  $t_{50\text{-late}}$  (the time after  $t_{\max}$  when the smoothed curve is at 50% of  $GIR_{\max}$ ), and the duration of insulin action ( $t_{50\text{-late}} - t_{50\text{-early}}$ ).

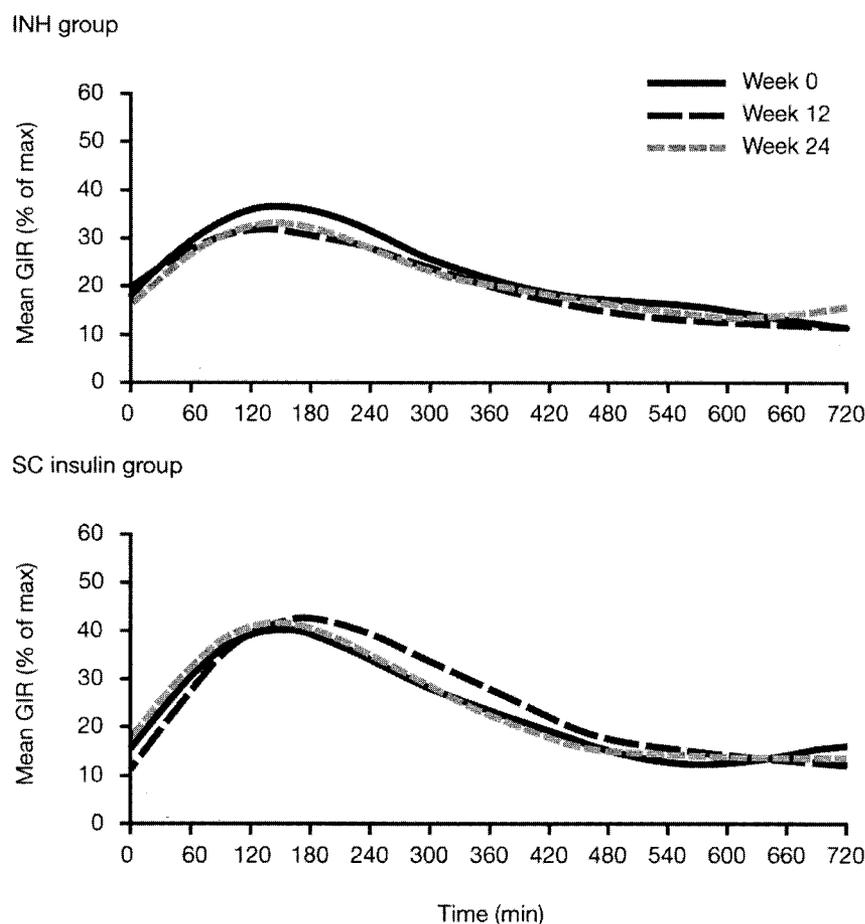
AUC end points were mathematically derived using the trapezoidal rule, and  $GIR_{\max}$  values were taken from the maximum of the smoothed polynomial GIR curve. The SAS PROC MIXED procedure for repeated data analysis (SAS Institute, Cary, NC) was performed to estimate the longitudinal effects of the two treatments on the pharmacodynamic parameters described above. Treatment group comparisons were performed using logarithmic transformations of meal challenge  $C_{\max}$  and  $AUC_{0-120}$ , as well as euglycemic clamp  $GIR AUC_{0-120}$ . Week 24 versus baseline ratios were calculated for all parameters. INH-to-SC insulin treatment group ratios and associated 90% CIs were calculated using an adjusted model that incorporated treatment, study week, and baseline measurement as terms in the analysis. Similar analyses (without log transformations) were performed for  $t_{50\text{-late}} - t_{50\text{-early}}$ . For both treatment groups, IAB levels, A1C, and hypoglycemic events were summarized over the course of the study.

## RESULTS

### Demographics and participant flow

A total of 74 patients were screened for the study, and 47 patients were randomized. One patient from each treatment group withdrew consent after randomization but before treatment; a total of 23 patients in the INH and 22 patients in the SC insulin group received study treatment. One patient in the INH and four patients in the SC insulin group discontinued treatment (all withdrew study consent). Thus, 22 patients in the INH and 18 patients in the SC insulin group completed the study. The treatment groups were similar at screening with respect to demographic and clinical characteristics (mean age 37.6 vs. 35.9 years, mean BMI





**Figure 2**—Mean GIR normalized to  $GIR_{max}$  by patient at each clamp time point (minutes).

24.8 vs. 24.7 kg/m<sup>2</sup>, percentage of male subjects 74 vs. 55%, duration of diabetes 16.6 vs. 18.0 years, baseline A1C levels 7.0 vs. 7.4%, and baseline IAB levels 3.5 vs. 2.6  $\mu$ U/ml) for the INH and SC insulin groups, respectively.

#### IAB levels

In the INH group, IAB levels, as measured in the routine radioligand binding assay, gradually increased from baseline (mean  $3.5 \pm 3.9$ , median 1.1  $\mu$ U/ml), reaching peak levels at week 24 (mean  $101.4 \pm 140.4$ , median 54.0  $\mu$ U/ml). The maximum IAB level observed with inhaled insulin therapy in this study was 641  $\mu$ U/ml. In contrast, for the SC insulin group, IAB levels at baseline (mean  $2.6 \pm 4.1$ , median 1.1  $\mu$ U/ml) were stable until week 18, after which they gradually increased to a mean of  $4.3 \pm 9.4$  and median of 1.1  $\mu$ U/ml at week 24 (Fig. 1).

Eleven of 23 end-of-study samples from inhaled insulin-treated patients had low-affinity binding (incubation at  $10^{-8}$

mol/l insulin concentration) below the limit of quantitation for the assay. Measurable levels of low-affinity IABs at end of study ranged from 677 to 3,360  $\mu$ U/ml. The corresponding levels of high-affinity insulin binding (incubation at  $1.4 \times 10^{-10}$  mol/l insulin concentration) in the same samples ranged from 41 to 387  $\mu$ U/ml. For each sample, binding capacity at  $10^{-8}$  mol/l insulin was greater than that at  $1.4 \times 10^{-10}$  mol/l insulin concentration.

#### Glucose tolerance

In patients treated with inhaled insulin for 24 weeks, the adjusted week 24 versus baseline ratios for postprandial glucose ( $C_{max}$  or  $AUC_{0-120}$ ) were similar to those in patients treated with subcutaneous insulin. For inhaled and subcutaneous insulin, respectively, these ratios were  $C_{max}$  0.98 vs. 0.99, adjusted ratio for treatment group difference 0.99 (90% CI 0.95–1.03) and  $AUC_{0-120}$  0.95 vs. 0.97, adjusted ratio for treatment group difference

0.98 (0.88–1.08) (Table 1). In patients treated with inhaled insulin, neither high- or low-affinity IAB binding was correlated with postprandial glucose  $AUC_{0-120}$  (Fig. 3A).

#### Duration of insulin action

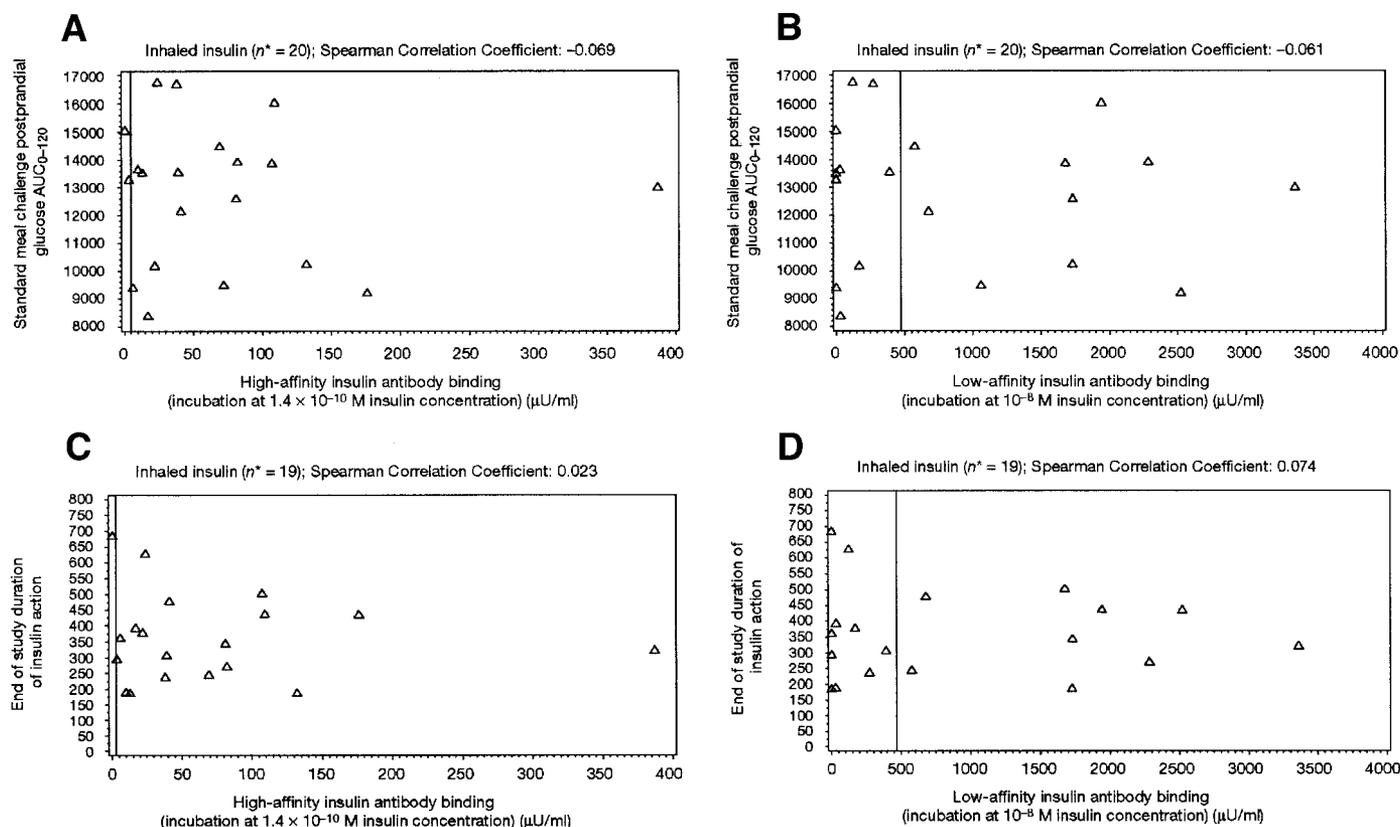
Mean GIR normalized to  $GIR_{max}$  by patient at baseline, week 12, and week 24 are shown in Fig. 2. The adjusted change from baseline to week 24 in the duration of insulin action for patients treated with inhaled insulin was  $-3$  min and for patients treated with subcutaneous insulin was  $-32$  min; treatment group difference 29 (90% CI  $-49$  to 108). Treatment group differences in  $AUC-GIR_{1-120}$  and  $GIR_{max}$  (Table 1) were not statistically significant. Scatterplots (Fig. 3C and D) demonstrate no correlation between the level of high- or low-affinity IABs and the duration of insulin action in patients treated with inhaled insulin.

#### Outpatient efficacy and safety

A1C was stably maintained in both treatment groups throughout the study (Table 1). For fasting plasma glucose, after 24 weeks of therapy, patients receiving inhaled insulin experienced a greater reduction from baseline ( $-23$  mg/dl) than patients receiving subcutaneous insulin ( $+2.7$  mg/dl). In patients receiving inhaled insulin therapy, fasting plasma glucose did not correlate with high- or low-affinity IAB levels (Fig. 4C and D).

None of the patients in either the INH or SC insulin groups discontinued the study because of adverse events or laboratory abnormalities. In the INH arm there were 985 hypoglycemic events over 126.6 subject-months of exposure, yielding an event rate of 7.8 events per subject-month compared with 1,041 hypoglycemic events over 111 subject-months of exposure and an event rate of 9.4 events per subject-month in patients receiving subcutaneous insulin.

Three patients treated with inhaled insulin had four severe hypoglycemic events compared with two patients receiving subcutaneous insulin, who each experienced one severe hypoglycemic event. Two of four severe hypoglycemic events in inhaled insulin-treated patients occurred within 3 days of initiation of inhaled insulin therapy. Three of four severe hypoglycemic events in inhaled insulin-treated patients were reported as serious adverse events because they were judged by the investigator to be important



**Figure 3**—Scatterplots of postprandial glucose  $AUC_{0-120}$  (A and B) and duration of insulin action ( $t_{50-late} - t_{50-early}$ ) (C and D) in inhaled insulin–treated patients according to high- and low-affinity antibody binding, respectively, at week 24.  $n^*$  is the number of subjects with end-of-study results. Vertical lines represent lower limit of quantitation (LLoQ) of antibody assays.

medical events. The severe hypoglycemic events that occurred in subcutaneous insulin–treated patients were not reported as serious adverse events. No patients discontinued the study because of hypoglycemia, and there were no nonhypoglycemia-related serious adverse events reported in this study. Furthermore, all cases of severe hypoglycemia were successfully treated without hospitalization, and there were no reported clinical sequelae.

In patients treated with inhaled insulin, the level of either high- or low-affinity IABs did not influence the total number of outpatient hypoglycemic events (Fig. 4A and B), and IAB levels did not identify those at risk for severe hypoglycemia. Furthermore, no adverse events were attributed to the increased IAB levels in the INH group.

**CONCLUSIONS**— This study investigated the pharmacodynamic impact of the development of IABs with inhaled insulin therapy in patients with low IAB levels at baseline.

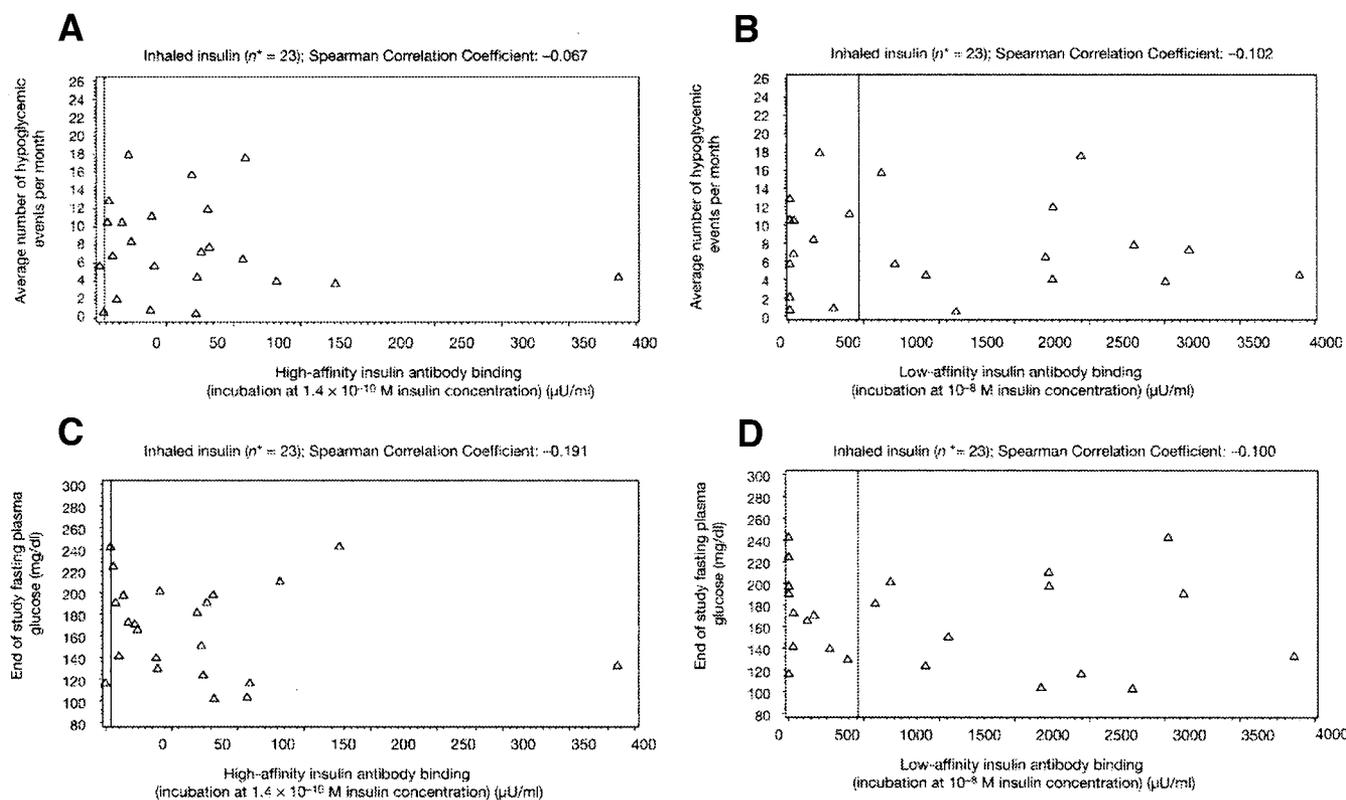
Both high- and low-affinity antibodies were measured in this study. Binding capacities at  $10^{-8}$  mol/l insulin (low- and high-affinity antibodies) were only measured in samples with detectable binding capacities at  $1.4 \times 10^{-10}$  mol/l insulin. Binding capacities for samples measured at  $10^{-8}$  mol/l insulin were greater than those observed at  $1.4 \times 10^{-10}$  mol/l. Therefore, the low-affinity antibodies had a greater binding capacity than the high-affinity IABs. Binding capacities of subject samples at the two insulin concentrations appeared to correlate, i.e., samples with highest binding capacities at the  $1.4 \times 10^{-10}$  mol/l insulin also had highest binding capacities at  $10^{-8}$  mol/l. We have also measured the binding capacities in these samples at  $10^{-9}$  and  $10^{-7}$  mol/l insulin concentrations (data not shown), and binding capacities at these incubations also correlated with high-affinity binding. These observations suggest that the immune response to inhaled insulin consists of a heterogeneous population of antibodies representing a range of binding affinities and binding capacities, as has been

described for subcutaneous insulin–associated antibodies (32,33). No correlations were observed between the binding capacity at any of these insulin concentrations and pharmacodynamic parameters.

Pharmacokinetic studies have shown associations between IAB levels or antibody binding affinity and pharmacokinetic responses, including delayed elevations in free insulin levels (3,4) and increases in the apparent half-life of free insulin (3,5). The present study was designed to assess the clinical relevance of the previous pharmacokinetic observations to the scenario in which type 1 diabetic patients would initiate inhaled insulin therapy by examining the relationship between IAB responses and postprandial glucose tolerance and/or duration of insulin action.

**Glucose tolerance**

Van Haefen, Heiling, and Gerich (7) found a difference of  $\sim 100$  mg/dl in peak postprandial glucose concentrations under conditions simulating intensive insu-



**Figure 4**—Scatterplots of average number of outpatient hypoglycemic events (A and B) and fasting plasma glucose (C and D) in inhaled insulin-treated patients according to high- and low-affinity antibody binding, respectively, at week 24.  $n^*$  is the number of subjects with end-of-study results. Vertical lines represent lower limit of quantitation (LLoQ) of antibody assays.

lin therapy when 12 patients with type 1 diabetes were stratified into quartiles based on association constants for high-affinity IABs, and patients in the highest stratum were compared with those in the lowest.

The sample size in the present study was sufficient to detect a difference between treatment groups of 38 mg/dl. A potential difference between the present study and Van Haeften et al. (7) is the range of insulin antibody levels in patients studied. In Van Haeften et al. (7), mean human IAB binding was 34.8% ( $\pm$ SD 7.4%,  $n = 12$ ). Using a similar semiquantitative assay in a pooled analysis of type 1 diabetic patients receiving inhaled insulin therapy, the mean rose to 31% binding (SD 20.3%,  $n = 363$ ) after 3–6 months of inhaled insulin exposure (28). However, quantitative comparisons between different assays should be interpreted with caution due to differences in assay formats and in concentrations of radiolabeled insulin used.

Another potential difference between Van Haeften, Heiling, and Gerich and the present study is the degree to which the

test doses of insulin were optimized for the test meal and for the individual patient. The mean postprandial glucose excursions observed in the present study were low, indicating that an optimal dose of study drug had been identified for most patients and that tight postprandial glycemic control was maintained throughout the study. Under these conditions, which controlled for nonantibody variables, neither high- nor low-affinity IAB binding was predictive of postprandial glucose levels.

In a study of 35 patients with immunologic insulin resistance syndrome, Davidson and DeBra (5) found a mean maximal 125I-insulin binding capacity of 191 mU/ml (range 13–1,080). Maximal insulin binding capacity was not measured in the present study, but it is likely that the antibody levels observed were lower than those seen in Davidson and DeBra's study. Therefore, the present study cannot rule out the possibility of immunologic insulin resistance syndrome in patients who achieve higher IAB responses than those evaluated. In theory, chronically worsened postprandial glu-

ose tolerance could translate into worsened overall glucose control as measured by A1C. The absence of an IAB effect on postprandial glycemic control observed in the present study, however, is consistent with the findings of other studies (8,13–18) that have shown no effect of IAB levels on long-term metabolic control in patients treated with subcutaneous insulin and is also consistent with the results of previous studies with inhaled insulin, in which IABs were not shown to have an impact on glycemic control as measured by A1C (28).

#### Duration of insulin action

It has also been suggested that IABs, especially of low-affinity binding, may prolong the duration of insulin action and therefore predispose to delayed hypoglycemia. Furthermore, the mean half-life of infused radiolabeled beef insulin was markedly longer in patients with immunologic insulin resistance and high maximum insulin binding capacity (5). A connection to clinical hypoglycemia, however, has only been documented in a small number of case reports (19–21), in

which distinctly unusual hypoglycemia syndromes were described.

In the present study, the changes from baseline in duration of insulin action were comparable between the two treatment groups (Table 1). Furthermore, in patients treated with inhaled insulin, changes in duration of insulin action were not observed to correlate with high- or low-affinity IAB binding (Fig. 3), and no correlation was found between IABs and outpatient hypoglycemic event rates (Fig. 4A and B). Although effects on duration of insulin action cannot be ruled out for patients who attain IAB levels higher than those observed in the study, our findings are consistent with the lack of relationship between IABs and severe hypoglycemia reported by Wredling (14).

In the present study, there was also no correlation between IAB level and fasting blood glucose level, suggesting that the improvement in fasting blood glucose observed in this and other studies (26, 27,34) is not due to an effect of IABs on the duration of insulin action. The lack of relationship between subcutaneous insulin-associated antibodies and fasting glucose has been previously described (9).

### Safety

In the present study, no adverse events were attributed to the increased IAB levels in the INH group, and both treatments were well tolerated.

The overall hypoglycemic event rate was lower in the inhaled insulin-treated patients compared with those treated with subcutaneous insulin, although two additional severe hypoglycemic events observed in the INH arm relative to the SC arm occurred early after inhaled insulin regimen initiation and probably reflect a period of early dose adjustment. The small numbers of events do not allow conclusions to be drawn regarding the incidence of severe hypoglycemia in patients treated with inhaled versus subcutaneous regular insulin. A previous study (35) showed that multiple doses of insulin glargine did not result in cumulative prolongation of insulin action. The lack of an increase in the duration of insulin action after 24 weeks of dosing with inhaled insulin in the present study likewise argues against the possibility of inhaled insulin dose accumulation.

In conclusion, in patients with type 1 diabetes treated with inhaled insulin, IAB levels were greater than in patients treated

with subcutaneous insulin, but the observed elevation in IABs was not associated with impaired glycemic control, increased duration of insulin action, or increased hypoglycemic event rates. No adverse clinical effects of IABs were identified.

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