Adverse Effects of Insulin Antibodies on Postprandial Plasma Glucose and Insulin Profiles in Diabetic Patients Without Immune Insulin Resistance

Implications for Intensive Insulin Regimens

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SUMMARY

To assess the possible influence of moderate titer insulin antibodies on diabetic glycemic control, we examined insulin-antibody equilibrium binding characteristics, postprandial glucose tolerance, and plasma free-insulin profiles after subcutaneous injection of both porcine and human insulin (0.15 U/kg) in 12 patients with insulin-dependent diabetes mellitus under conditions simulating intensive insulin therapy. The patients' antibodies bound porcine and human insulin indistinguishably, and their plasma glucose and free-insulin profiles after ingestion of a standard meal were similar with both insulins. Initial increases in plasma free-insulin levels after injection of both insulins were negatively correlated with both insulin-antibody binding ($r = -.55$, $P < .006$) and postprandial hyperglycemia (peak level $r = -.56$, $P < .006$); the latter was positively correlated with insulin-antibody binding ($r = .48$, $P < .02$). The effects of insulin antibodies on postprandial plasma free-insulin and glucose levels could be accounted for substantially by the association constant of the high-affinity insulin-antibody binding sites (K_1); patients in the highest quartile for K_1 had significantly slower initial increments in plasma free insulin (0.31 ± 0.04 vs. 0.46 ± 0.06 μU/min, $P < .05$) and greater postprandial hyperglycemia (peak value 237 ± 10 vs. 166 ± 12 mg/dl, $P < .001$) than patients in the lowest quartile. We conclude that moderate insulin-antibody titers commonly found in insulin-treated patients can slow the early increase in plasma free insulin after subcutaneous injection and that this impairs postprandial glucose tolerance; such an effect may limit the effectiveness of intensive insulin therapy.

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Although it has been known for ~30 yr that most diabetic patients treated with insulin develop insulin antibodies (1), the influence of these antibodies on glycemic control remains controversial (2,3). Occasionally, high titers of insulin antibodies may cause insulin resistance (4), but, with a few exceptions (5,6), there has been little evidence that moderate insulin-antibody titers below levels associated with insulin resistance impair glycemic control (7–20). It has even been suggested that insulin antibodies might be advantageous in acting as a buffer or reservoir to prevent rapid fluctuations in insulin availability (20–22).

Nevertheless, several recent studies have demonstrated that commonly observed insulin-antibody titers prolong the half-life (23–25), enlarge the distribution space (23,24), and increase the metabolic clearance rate (23–25) of intravenously infused insulin. Moreover, it has also been shown that such antibody titers can adversely alter the pharmacokinetics of subcutaneously injected regular insulin (24,26–28); however, the consequences of these alterations on glycemic control have not been evaluated.

Because the success of intensive insulin regimens depends largely on use of regular insulin to mimic the normal rapid pancreatic β-cell response to meals (29,30), and because insulin antibodies may delay increases in plasma free insulin after subcutaneous injection (24,27,28), we examined the effect of insulin antibodies and their equilibrium binding characteristics on plasma free-insulin profiles and postprandial plasma glucose responses in patients with insulin-dependent diabetes mellitus (IDDM) under conditions simulating intensive insulin therapy.

MATERIALS AND METHODS

Subjects. Informed written consent was obtained from 12 C-peptide–negative patients with IDDM (3 men, 9 women)

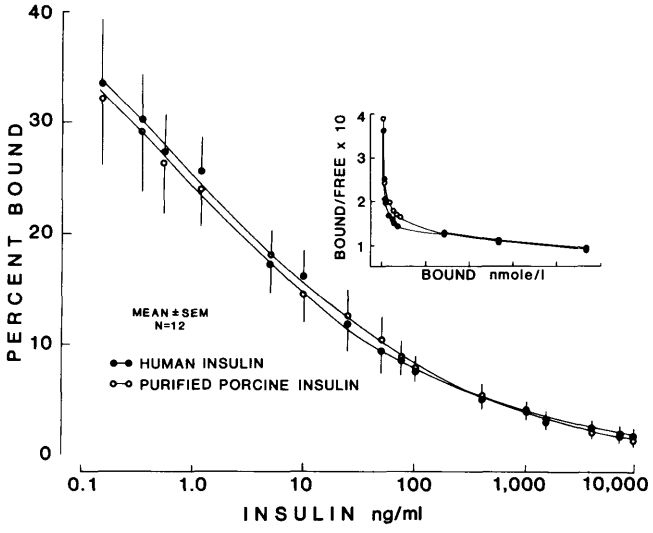

FIG. 1. Displacement curve and Scatchard plot (*insert*) comparing antibody binding of purified porcine and human insulin in sera from 12 patients with insulin-dependent diabetes mellitus.

aged 29 ± 2 yr (mean ± SE). C-peptide negativity was defined as a <0.2-ng/ml plasma increment after 1 ng i.v. glucagon (31); the sensitivity of the C-peptide assay is 0.06 ng/ml. The subjects were nonobese (105 ± 3% ideal body wt; Metropolitan Life Insurance Tables, 1959) and had a duration of diabetes of 11 ± 2 yr and a glycosylated hemoglobin of 9.1 ± 0.4% (upper limit of normal 7.5%) (32).

Protocol. Subjects were studied twice; on each occasion they were withdrawn from their long-acting insulin for 48 h (lente) or 72 h (ultralente) before study and were managed solely by multiple subcutaneous injections of regular insulin. Between 1600 and 1900 h the evening before study they were admitted to the Mayo Clinic General Clinical Research Center, connected to a closed-loop insulin infusion device (Biostator) as previously described (26), and maintained euglycemic overnight. The next morning between 0700 and 0800 h the insulin infusion from the Biostator was fixed constant at the rate that had been given during the preceding 2 h, and this was maintained throughout the study. After an equilibration period of 1–1.5 h, regular insulin (0.15 U/kg s.c.) was injected 2 cm to the left or right of the umbilicus. To compare the effects of antibody recognition of porcine insulin and human insulin, on one occasion purified pork insulin and on the other occasion biosynthetic human insulin (both Lilly, Indianapolis, IN) were administered; the order of the injections was random. One-half hour after injection of insulin, a standard meal (10 kcal/kg) consisting of 50% carbohydrate, 35% fat, and 15% protein was given, and patients were asked to eat this meal within 10–15 min.

Analyses. Arterialized venous blood samples (26) were obtained before injection of insulin and at 10 to 30-min intervals thereafter for measurement of plasma glucose (YSI glucose analyzer, Yellow Springs, OH) and plasma free-insulin concentrations (33). Insulin-antibody binding in the absence (B_0) and presence of competing unlabeled porcine and human insulin was determined by a modification of the method of Goldman et al. (17), as previously described (24), at a final plasma dilution of 1:10 both with ^{125}I-labeled (A-14) human insulin and ^{125}I-labeled (A-14) porcine insulin as tracers (courtesy of Dr. R. Chance, Lilly). Scatchard analysis for determination of equilibrium binding characteristics of the insulin-antibody–binding sites was performed with a nonlinear least-squares regression computer program and a two-site model (34). Unless indicated otherwise, data are given as means ± SE. Differences between porcine and human insulin were evaluated by paired Student's *t* tests corrected, when appropriate, for repeated measurements (35). Correlations were performed with simple least squares; multiple linear regression was used to assess correlation of individual equilibrium binding kinetics with plasma glucose and insulin responses (36).

RESULTS

Insulin-antibody binding. Insulin-antibody binding of ^{125}I-labeled porcine and ^{125}I-labeled human insulin (B_0) were not significantly different and were highly correlated ($r = .84$, $P < .001$) (Fig. 1). Scatchard analysis indicated the presence of two classes of antibody-binding sites consistent with numerous previous reports (1,2,8,15–18,20,24; Fig. 1, *insert*); the capacity and association constants of these binding sites (Table 1) did not differ significantly for porcine and human insulin and were similar to values previously found by other investigators in patients not considered to be insulin resistant (1,2,8,16–18,20,24).

Plasma glucose and free-insulin profiles. Basal intravenous insulin infusion rates and both baseline plasma glucose and plasma free-insulin concentrations were comparable before administration of each insulin (18 ± 1 vs. 18 ± 1 mU/min purified porcine and human insulin, respectively) (Fig. 2). Postprandial plasma glucose responses were virtually identical after injection of both insulins. Plasma free-insulin concentrations were not significantly different at any time after injection of the insulins, although plasma free-insulin levels with human insulin were somewhat lower than those with purified porcine insulin 150 min after meal ingestion.

Relationships between plasma free insulin, postprandial glucose responses, and insulin-antibody binding. There was a significant negative correlation between the rate of increase in plasma free insulin over the 90-min interval after injection of insulin and the postprandial plasma glucose response when the latter was assessed both in terms of peak plasma glucose concentration ($r = -.56$, $P < .006$) and plasma glucose area under the curve ($r = -.43$, $P < .04$)

TABLE 1
Insulin-antibody binding characteristics

	Human insulin	Purified porcine insulin
B_0* (%)	34.8 ± 7.4	38.7 ± 8.0
High-affinity binding sites		
Capacity (nM)	6.9 ± 2.1	6.6 ± 1.6
Association constant (10^9 M)	208 ± 70	202 ± 71
Low-affinity binding sites		
Capacity (nM)	219 ± 44	231 ± 39
Association constant (10^6 M)	2.3 ± 0.1	2.3 ± 0.1

*Binding of ^{125}I-labeled insulin (0.16 ng/ml) in the absence of added unlabeled insulin.

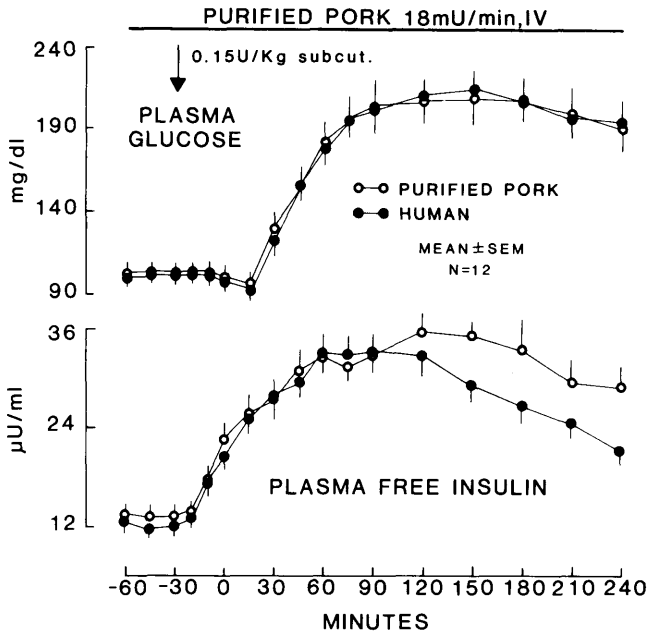

FIG. 2. Comparison of postprandial plasma glucose and free-insulin concentrations after subcutaneous injection of purified porcine and human insulin 30 min before meal ingestion (0 min) in patients with insulin-dependent diabetes mellitus.

(Fig. 3). Thus, the slower the initial increase in plasma free-insulin concentration, the poorer was postprandial glucose tolerance. There was no significant correlation found between the total increase in plasma free insulin (area under the curve) and postprandial glucose tolerance, indicating the relative importance of the early increases in plasma insulin (20,30,37,38).

The initial rate of increase in plasma free insulin was negatively correlated with insulin-antibody binding (B_0) (Fig. 4, *right panel*; $r = -.55$, $P < .006$); the latter was positively correlated with postprandial plasma glucose responses (Fig. 4, *left* and *middle panels*, both $P < .02$). Thus, the greater the insulin-antibody binding (B_0), the slower the initial rate of increase in plasma free insulin and the poorer the postprandial glucose tolerance.

Role of high- and low-affinity insulin-antibody binding sites. Using multiple linear regression, insulin-antibody binding (B_0) was found to be significantly correlated with only the capacity ($r = .45$, $P < .04$) and the association constant ($r = .52$, $P < .01$) of the high-affinity binding sites (Fig. 5). The initial rate of increase in plasma free insulin ($r = -.46$, $P < .04$), the peak postprandial plasma glucose level ($r = .52$, $P < .01$), and the area under the curve for the postprandial plasma glucose responses ($r = .53$, $P < .01$) were all found to be significantly correlated with only association constants of the high-affinity insulin-antibody binding sites.

DISCUSSION

Our studies reaffirm the importance of early increases in plasma insulin in limiting postprandial hyperglycemia (29,30,37,38) and demonstrate that insulin antibodies, even when present in amounts less than those associated with insulin resistance, can reduce the increase in plasma free insulin after subcutaneous insulin injection.

Francis et al. (28) recently found similar effects of moderate insulin-antibody titers on plasma free-insulin kinetics. However, their studies used doses of regular insulin somewhat larger than those generally used clinically (~20 U) and were conducted after withdrawal of insulin when subjects were markedly hyperglycemic (~300 mg/dl). Thus, the relevance of their observations on changes in plasma glucose concentrations are difficult to assess. The results of our studies, which were performed under conditions simulating intensive insulin therapy, clearly indicate that antibody-induced alterations in insulin kinetics can adversely affect postprandial glucose tolerance.

The failure of most previous studies to find an effect of insulin antibodies on glycemic control could be explained by several factors (7–20). Most studies examined the relationship between insulin-antibody titer and insulin dose in patients who were poorly controlled and/or those who were being treated predominantly with intermediate-acting insulin preparations (e.g., lente/NPH). Insulin doses may not necessarily reflect actual insulin requirements, as certainly would be the case in patients who were in poor metabolic control. Furthermore, the impact of insulin antibodies on insulin pharmacokinetics would not be expected to be as great in patients treated with intermediate-acting insulins as in patients being treated predominantly with short-acting insulin such as those on intensive insulin regimens. Finally, in patients with poor metabolic control, other factors (e.g., non-

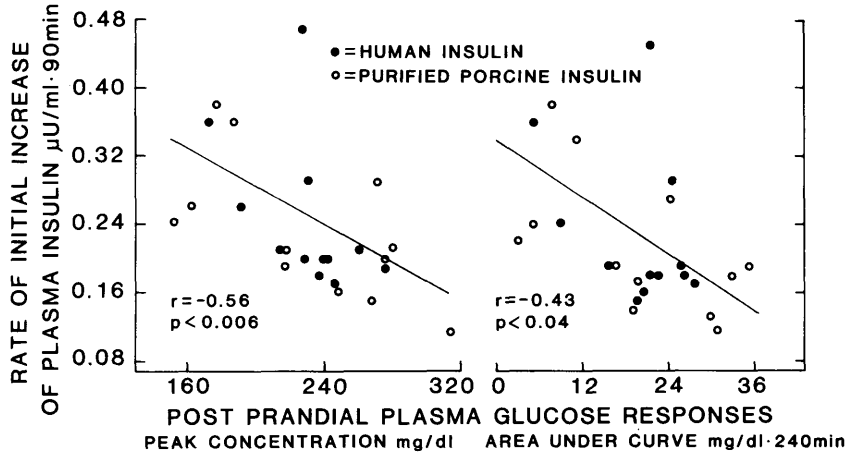

FIG. 3. Correlation between initial increase in plasma free insulin after subcutaneous injection of purified porcine and human insulin with postprandial glucose tolerance in patients with insulin-dependent diabetes mellitus.

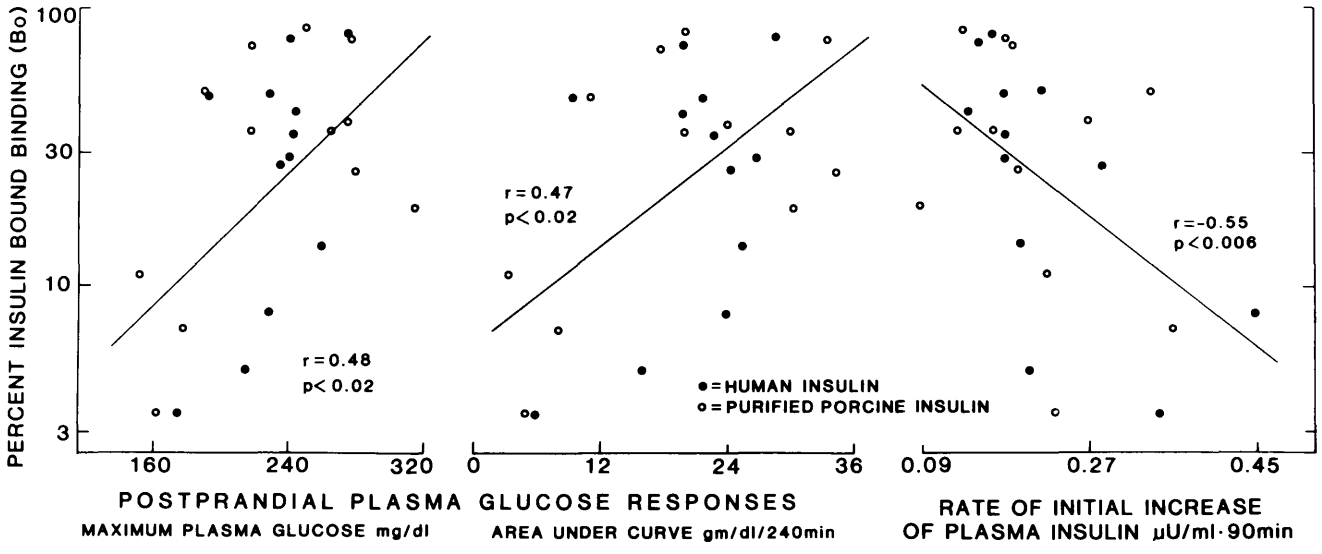

FIG. 4. Correlation between insulin-antibody binding, postprandial glucose tolerance, and initial increase in plasma free insulin in patients with insulin-dependent diabetes mellitus.

compliance with diet or inappropriate insulin dose) may overshadow the effect of insulin antibodies. Our results are particularly relevant to patients in whom achievement of near normoglycemia is being attempted with intensive insulin therapy.

In this study the adverse effects of insulin antibodies were correlated with the association constant of the high-affinity class of insulin-binding sites but not the number of high- and low-affinity binding sites or the association constant of the low-affinity binding sites. Figure 5 shows the plasma free-insulin and glucose profiles of patients in the highest and lowest quartile for high-affinity binding-site association constants. Patients with the highest association constants had much slower initial increments in plasma free insulin (0.31 ± 0.04 vs. 0.46 ± 0.06 $\mu U \cdot ml^{-1} \cdot min^{-1}$, $P < .05$) and greater postprandial hyperglycemia (peak values 237 ± 10 vs. 166 ± 12 mg/dl, $P < .001$). We interpret this finding to indicate that the number of insulin binding sites is not ordinarily rate limiting for binding of insulin and that, at physiologic plasma insulin concentration, the association constant of the high-affinity insulin binding sites is the major determinant of the amount of insulin bound.

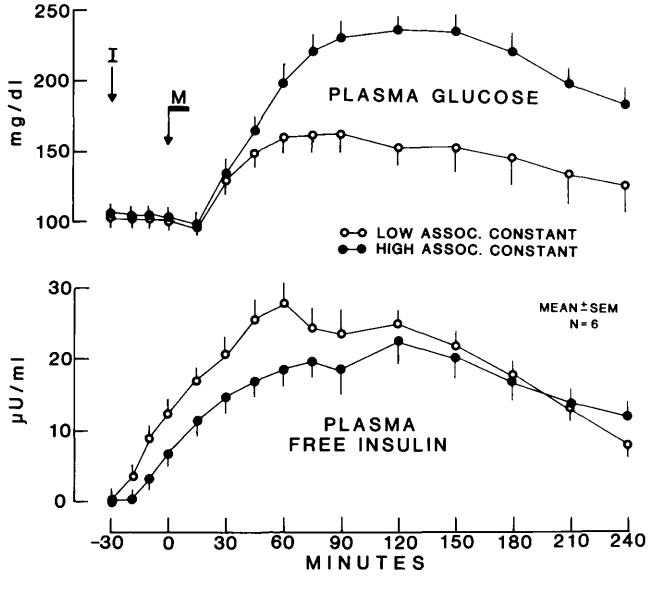

FIG. 5. Postprandial plasma glucose and free-insulin profiles of patients with insulin-dependent diabetes in highest and lowest quartile for association constants of high-affinity insulin-antibody binding sites.

Except for somewhat lower plasma free-insulin levels observed several hours after injection of human insulin, consistent with some reports of a shorter duration of action of subcutaneously injected human insulin (39,40), we found no difference in plasma free-insulin and glucose profiles when purified porcine or human insulin was injected. Similar results have generally been found (41,42) and are readily explained by the fact that these insulins have virtually identical intrinsic activity (41,42) and by the fact that the patients studied have had antibodies that bound porcine and human insulin indistinguishably (43) as in this study.

Note, however, that these results do not necessarily indicate that there would be no difference in plasma insulin kinetics among patients who are being treated with different species of insulin or those who have been switched from nonhuman to human insulin. Treatment with less immunogenic insulins should result in lower insulin-antibody titers (19,44) and plasma insulin kinetics would be expected to be less adversely affected.

The clinical implications of our findings pertain mainly to patients on intensive insulin regimens whose goal is achievement of near normoglycemia without undue risk of hypoglycemia. As suggested by the studies of Gray et al. (23) and Bolli et al. (26), increasing insulin doses in an attempt to overcome postprandial hyperglycemia resulting from the diminution of early increases in plasma free insulin due to insulin antibodies could cause late hyperinsulinemia and hypoglycemia because insulin antibodies also prolong the duration of action of insulin (23–28). Thus, for some patients on intensive insulin regimens, the effects of insulin antibodies

on insulin kinetics may hinder the achievement of near normoglycemia.

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