Effects of Human Insulin on Insulin Binding Antibody Production in Nondiabetic Subjects

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OBJECTIVE — To test the hypothesis that human insulin may have a low immunogenicity and that short-term exposure may not cause endogenous insulin antibody production.

RESEARCH DESIGN AND METHODS — Randomized double-blind prospective study. Serum samples collected for insulin binding antibodies and measured by a sensitive immunochemical assay. Subjects were seven healthy nondiabetic patients who had never received exogenous insulin. Each subject received 6 separate monthly injections of human insulin. On four occasions, both regular and NPH insulin were administered. On the other two occasions, either NPH or regular insulin was administered alone.

RESULTS — Mean ± SE basal insulin antibody levels (1.2 ± 0.2 μg/L) increased to a maximal level of 4.5 ± 0.8 μg/L after four injections. Thereafter, antibody levels declined to an end-of-study value of 2.5 ± 0.3 μg/L. This represented a highly significant overall increase ($P < 0.001$). A control group of six insulin-dependent diabetic subjects treated with human insulin over the same period as the test subjects demonstrated no change in insulin antibody concentrations (2.8 ± 0.7–2.7 ± 0.6 μg/L).

CONCLUSIONS — These results suggest that human insulin preparations, when administered subcutaneously, may be more immunogenic than previously considered. The antigenic response was rapid, because only four subcutaneous injections were sufficient to produce insulin antibody levels in nondiabetic patients similar to those observed in insulin-dependent diabetic patients receiving chronic insulin replacement therapy.

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The most commonly used insulin regimen for insulin-treated patients is twice-daily short- and intermediate-acting insulin (1). The use of human insulin preparations manufactured by recombinant DNA technology or enzymatically modified pork insulin has become more widespread. Insulin-induced antibody formation occurs after beef and to a lesser extent pork and human insulin treatment (2). The clinical relevance of insulin-binding antibodies is controversial. It has been suggested that insulin antibodies prolong the action of subcutaneously injected insulin (3), cause nocturnal hypoglycemia (3), and affect daily insulin dose requirements (4). However, other studies have concluded that insulin antibodies are not important factors in the control of diabetes (5) and would be of no clinical relevance as more diabetic subjects are treated with human insulin (6). It is not known how quickly antibodies develop after exposure to a typical human insulin treatment regimen in nondiabetic subjects. This study investigated whether six subcutaneous injections of short- and intermediate-acting human insulin, given at monthly intervals, could alter insulin binding antibody levels in nondiabetic subjects.

RESEARCH DESIGN AND METHODS

— Seven healthy nondiabetic female subjects aged 24–30 yr (mean ± SE 26.9 ± 1.0 yr), with a mean body mass index of 22 ± 1 kg/m^2 were studied. Mean ± SE fasting blood glucose was 4.5 ± 0.1 mM and mean HbA_1 was 6.1 ± 0.6% (normal range 5–7.5%). None of the subjects were taking medication and had never received exogenous insulin. All gave informed written consent, and local ethical committee approval was obtained. Each subject received, in a randomized order, six separate monthly injections of either 20 U s.c. human regular and NPH insulin (4 occasions), 14 U s.c. human NPH (1 occasion), or 6 U s.c.

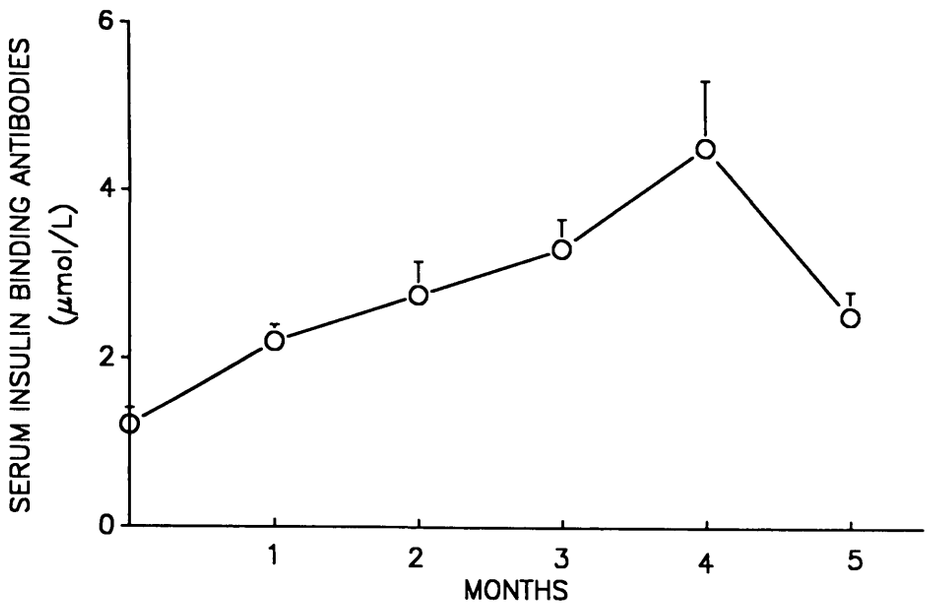

Figure 1—*Mean changes of serum insulin-binding antibody concentrations after monthly subcutaneous injections of human insulin in nondiabetic subjects.*

human regular insulin (1 occasion). Each injection was given by the same investigator with the same type of insulin syringe (0.5-ml syringe, Plastipak, Becton Dickinson, London) at 0900 with the ambient temperature of the room kept constant (21.3 ± 0.4°C). The insulin was injected at 45° into the anterior abdominal wall 8 cm lateral to the umbilicus. The insulin preparations used were manufactured by either recombinant DNA technology (Humulin M3, Humulin S, and Humulin I, Lilly, Indianapolis, IN) or enzymatic modification of pork insulin (Actraphane, human mixtard, Novo Nordisk, Bagsvaerd, Denmark). To prevent hypoglycemia after each injection, subjects were maintained normoglycemic with a 10% glucose infusion for 6 h, and then consumed a carbohydrate-enriched diet for another 6 h.

IgG insulin binding capacity was measured by a sensitive immunochemical assay (7,8) with a lower limit of detection (0.1 μg/L), interassay coefficients of variation (c.v.) of 11% at 0.9, 8.4% at 1.7, and 8.8% at 6.3 μg/L. Data are expressed as means ± SE and analyzed with standard parametric one-way analysis of variance with a repeated-measures design.

RESULTS — Baseline insulin antibody concentrations were all within the normal reference range of our assay for nondiabetic subjects (0.1–2.0 μg/L). One month after the first injection, insulin antibody concentrations increased by nearly twofold (1.2 ± 0.2–2.2 ± 0.2 μg/L). Insulin antibody concentrations increased after successive injections, reaching a maximal level of 4.5 ± 0.8 μg/L after the fourth injection (Fig. 1). Thereafter, antibody levels fell to 2.5 ± 0.3 μg/L at the end of the study. The overall increase of insulin antibody concentration was highly significant ($P = 0.001$; analysis of variance). A control group of six insulin-dependent diabetes mellitus (IDDM) subjects attending our laboratory in a separate metabolic study were examined for monthly insulin antibody levels over a similar time span as the test subjects. Mean age was 30 ± 6 yr, HbA_1 was 9.0 ± 0.6% (reference range 5–7.5%), and mean duration of diabetes was 11 ± 2 yr. All had been treated with monocomponent pork and then human insulin since diagnosis. Initial insulin antibody levels were 2.8 ± 0.7 μg/L and those obtained at the end of the study were 2.7 ± 0.6 μg/L. This represented an insignificant mean change of only ~4%. Serial antibody levels, in this group, remained stable over the 6-mo period with a mean C.V. of 7.5 ± 2%.

CONCLUSIONS — This study demonstrates that only two injections (s.c.) of human insulin are sufficient to cause a doubling in insulin-binding antibodies. The peak insulin antibody levels of 4.5 ± 0.8 μg/L obtained from the subjects in this study are similar to antibody levels obtained from our assay for well-controlled IDDM patients receiving human or monocomponent pork insulin. The insulin antibody concentrations obtained in this study would not be expected to cause problems in diabetic metabolic control because another study has shown virtually no effect on insulin sensitivity when insulin-binding antibodies are <25 μg/L (8). There are some problems in the measurement of insulin-binding antibodies because the assays are not standardized (9). Therefore, in this study, antibody levels were expressed as both absolute concentrations and percentage increase. Thus, the exact numerical value may be less important than the overall relative rise. In this study, each subject's peak antibody level was increased three- to fivefold compared with basal. This large relative rise, coupled with the precision of our assay in the low range, emphasizes how quickly injections (s.c.) of human insulin produces a significant increase in insulin-antibody concentrations.

The exact cause for the stimulus of antibody production after human in-

sulin administration is not known. Insulin is stored in the β-cells as hexameric crystals. After release into the portal vein, it dissociates into dimers and then monomers. However, after injection (s.c.) of available human insulin preparations, polymerization may occur (10). A polymer is more immunogenic than a monomer, and hence may be the stimulus for antibody production (11). However, we could not find any evidence that a particular insulin preparation was any more immunogenic than another.

We cannot comment on any effects seasonal variation may have on insulin IgG binding, but other monthly antibody data from our laboratory obtained from IDDM subjects over a 6-mo period showed a mean change of only ~4%. Thus, we assume that the antibody rise that occurred in this study was caused by the human insulin injections (s.c.).

In conclusion, we have shown that human insulin, when administered subcutaneously, may be more immunogenic than previously considered. The antigenic response was immediate in nondiabetic subjects, because four injections (s.c.) produced antibody levels comparable to those observed in IDDM patients receiving chronic insulin replacement therapy.

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