Establishment of Time-Action Profiles for Regular and NPH Insulin Using Pharmacodynamic Modeling

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OBJECTIVE— To provide distinct definitions and quantify the establishment of onset, peak, and duration of action for insulins.

RESEARCH DESIGN AND METHODS— We administered single subcutaneous doses of 10 U regular insulin to 10 volunteer subjects and 25 U NPH insulin to 6 healthy male volunteer subjects on separate occasions. Each dose was given after an overnight fast during a glucose clamp to maintain an euglycemic state. We measured serum insulin concentrations and glucose infusion rates (GIR) from frequent blood sampling after each treatment. Serum insulin concentrations were related to GIR values at each collection time and a counter-clockwise hysteresis resulted. An effect compartment model was used to simultaneously describe the pharmacokinetics and pharmacodynamics of each insulin and to resolve the hysteresis.

RESULTS— From the resulting relationship, GIR could then be predicted, with onset and duration of action reflecting the time when effect compartment concentrations initially exceeded then declined below a 10% maximum possible effect (E_{max}) level. Ninety-five percent confidence intervals were constructed allowing a predictive range of values. For regular insulin, a mean onset of 0.75 h, peak of 2 h, and duration of 6 h was estimated. Mean values were also produced with NPH, with an onset of 3 h, peak of 6–7 h, and a duration of 13 h estimated.

CONCLUSIONS— This method estimates the onset, peak, and duration of insulin action. Although these estimates were from single doses, we believe they can provide good estimations of insulin activity.

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GIR, glucose infusion rate; RIA, radioimmunoassay; C_{max}, maximum serum insulin concentrations; t_{max}, time to C_{max}; AUC, area under the serum concentration versus time curve; R_{max}, maximum infusion rates of glucose infusion; TR_{max}, the time to R_{max}; G_{tot}, total glucose infused; E, measured effect; E_{max}, maximum effect possible; C_E, concentration of drug in the effect compartment; EC_{50}, drug concentration that producing an effect 50% of maximum; g, shape factor; CI, confidence interval.

Although regular and NPH insulins are recognized as short- and intermediate-acting insulins, respectively, definition of their time-activity profiles has been difficult. NPH insulin has been dosed according to maintenance of blood glucose levels over a 12- to 24-h period, and regular insulin dosed on an as-needed basis assuming an onset of 30–90 min after a subcutaneous injection (1). Although these are reasonable dosing methods, they rely on highly variable and somewhat subjective interpretations of time-activity profiles.

The time-activity profiles for regular and NPH insulin have been described previously. These include serum insulin concentration versus time profiles (2–10), blood glucose depression versus time profiles (4,9,11,13), and glucose requirement versus time profiles from a glucose clamp (2,5–8,12). Serious attempts are lacking from all but a few studies to define the onset and duration of action of these formulations in a consistent fashion. However, these assigned definitions have been somewhat arbitrary and vary widely (7,10,12,18–19). Universally accepted definitions have been elusive.

This study attempts to provide rigorous definitions of onset, duration, time to peak, and comparative intensity of two different types of insulin—regular and NPH. The definitions are based on simultaneous measurements of both serum insulin concentrations and glucose infusion rates by means of a glucose clamp technique. The value of this model is twofold: *1*) it depends on information gained from a method that has been used for several years in defining insulin time-action profiles, and *2*) it depends on both pharmacokinetic and pharmacodynamic measurements, not just one or the other.

RESEARCH DESIGN AND METHODS

— We compared two drugs: human regular insulin (Humulin R®, Lilly, Indianapolis, IN) and human

NPH insulin (Humulin N®, Lilly). Both mixtures were human insulin of recombinant origin and contained 100 U/ml, with 1 U equivalent to 38.1 μg (6.56 pmol) for regular insulin and 38.4 μg (6.61 pmol) for NPH insulin.

Study design

Data were derived from two separate studies performed at the Lilly Laboratory for Clinical Research (Indianapolis, IN). The protocols for these studies were reviewed by Indiana University/Purdue University Institutional Review Board (Indianapolis, IN). Informed consent was obtained from all participants. Study subjects were healthy men 22–45 years of age and within 15% of their ideal body weight for their age, height, and frame size (the Metropolitan Life Health Insurance tables were used as a reference). Before study acceptance, all subjects were given a physical examination, including complete blood and urine chemistry evaluations, a chest X ray, an electrocardiogram, and a 2-h glucose tolerance test. All participants were in good health and met World Health Organization criteria for normal glucose tolerance, i.e., fasting glucose values <6.4 mM and blood glucose <7.8 mM 2 h after a 75-g glucose load (1). All of the participants completed their assigned treatments. The participants were similar in age (39 ± 8.4 years, mean ± SD), height (177 ± 9.8 cm), weight (71.7 ± 9.5 kg), and body mass index (23.0 ± 2.79 kg/m^2).

All treatments were given by subcutaneous injection. A single dose of 10 U was given to 10 volunteer subjects for the regular insulin treatment, and 6 volunteer subjects received 25 U for the NPH treatment. All doses were administered subcutaneously after an overnight fast. The subjects remained fasted and at bedrest during the entire data collection period. The treatments were given without blinding the volunteer subjects, the nurses, or the investigator.

The treatments were administered during glucose clamps conducted using the Biostator® (Diagnostics Division, Miles, Mishawaka, IN) and its built-in glucose clamp algorithm (20,21). The participants fasted overnight and remained fasting and at bedrest during the glucose clamps, which lasted up to 12 h following regular insulin and up to 24 h following NPH. The Biostator's glucose analyzer was calibrated to each participant's capillary whole blood glucose by adjusting the pump ratio to match capillary glucose concentrations. The glucose clamp was maintained 0.3 mM below the subject's fasting glucose level. The assigned insulin dose was administered once stabilization was achieved. Capillary whole blood glucose samples were assayed with a YSI 2300 STAT® glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). The glucose infusion rates and blood glucose values were recorded from the Biostator using a Compaq Deskpro® 386/20e (Compaq Computer Corporation, Houston, TX) personal computer.

Blood samples were collected after each dose for analysis of insulin. Samples were collected from an antecubital vein contralateral to that used for glucose and saline infusion of the glucose clamp. The infusion rate necessary to maintain the subject's blood glucose was continuously recorded by the Biostator. These glucose infusion rates (GIR) were then used as a glucodynamic measurement for insulin.

Sample analysis

Insulin radioimmunoassay (RIA) method. We used a commercially available RIA kit (Insulin Coat-ACount®) from Diagnostic (Los Angeles, CA) to measure the serum concentrations of insulin. We validated the RIA before the analysis of study samples, and all analyses were performed in accordance with the kit's instructions. Briefly, each incubation included buffer, serum or biosynthetic human insulin standard prepared in kit zero calibrator, ^{125}I-labeled porcine insulin, and a polyclonal guinea pig anti-porcine insulin antiserum. We incubated each binding reaction for 20–22 h at room temperature. Separation of bound- and free-labeled insulin was achieved by solid-phase antibody methodology using antibody-coated tubes. Assay data were analyzed by VAX computer using a weighted four-parameter logistic model algorithm. The insulin concentration of test samples was estimated from a standard curve of reference insulin prepared in the RIA kit's zero calibrator matrix. Each standard curve contained the following concentrations of human insulin: 0, 8.61, 17.2, 43, 86, 170, 430, 860, 1,720, 4,305, 8,610, and 17,220 pM.

The RIA's lower limit of detection was determined to be 8.61 pM. We assessed interassay precision and recovery by adding reference human insulin to serum pooled from fasted nondiabetic adults. Interday precision (% coefficient of variation) (n = 5) was 29.3% at 86 pM, 13.3% at 430 pM, and 13.3% at 4,305 pM. Recoveries for the serum control samples ranged from 92 to 114%. Interassay precision data for standard curve parameters ranged from 3.2 to 19.4%.

Pharmacokinetic and pharmacodynamic analyses

The serum insulin concentrations provided several summary pharmacokinetic parameters, including maximum serum insulin concentrations (C_{max}) (pM), time to C_{max} (t_{max}) (h), and the area under the serum concentration versus time curve (AUC) (pmol · h^{-1} · L^{-1}). Calculation of AUC was performed using the trapezoidal rule (14).

In addition to pharmacokinetic parameters, summary pharmacodynamic parameters were also generated. The maximum infusion rates of glucose infusion (R_{max}) (mmol/min), the time to R_{max} (TR_{max}) (h), and the total glucose infused for the duration of the study (G_{tot}) (mmol) were derived from the glucose infusion data.

An effect compartment model was used to interrelate the serum insulin

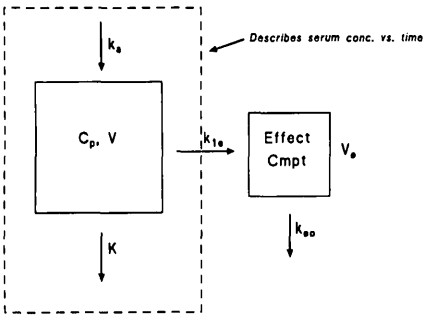

Figure 1—*The effect compartment model.*

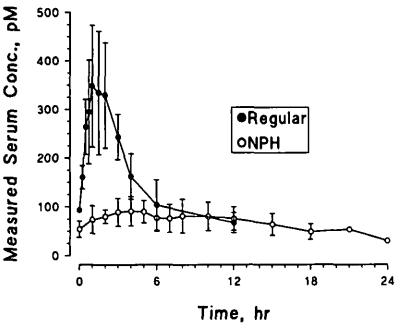

Figure 2—*Mean measured serum insulin concentrations after a single 10-U dose of regular (soluble) insulin and a 25-U dose of NPH (isophane) insulin. Bars represent SD. All doses were subcutaneous.*

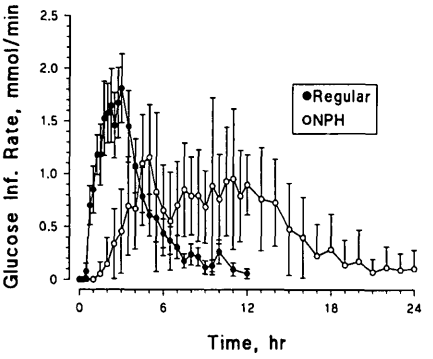

Figure 3—*Mean glucose infusion rates (GIR) needed to maintain euglycemia after a single 10-U dose of regular (soluble) insulin and a 25-U dose of NPH (isophane) insulin. Bars represent SD.*

concentrations with the induced glucose infusions (15). Our laboratory has previously used a simplified effect compartment model to successfully describe insulin pharmacodynamics from an intravenous admimstration (16), but we have never described subcutaneous administrations.

A one-compartment model, used to describe insulin serum concentration versus time data, was expanded with an effect compartment to simultaneously describe the GIR versus time data (Fig. 1). The computer program ADAPT II (17) installed on a VAX 8800 was used to perform this task. In this model, k_a is the absorption rate constant and K the elimination rate constant describing the measurable serum concentrations of drug, k_{1e} is the transfer rate constant of drug to the effect compartment, k_{eo} the rate constant associated with the elimination of effect, V the apparent volume of the distribution of the serum compartment, and V_e the volume of distribution of the effect compartment. The units of all rate constants are h^{-1}; the volume terms (V, V_e) are in units of L.

The effect was described by the Hill equation (14,15):

$$E = \frac{E_{max} \cdot C_E^{\gamma}}{EC_{50}^{\gamma} + C_E^{\gamma}}$$

where the measured effect (E), the maximum effect possible (E_{max}), the concentration of drug in the effect compartment (C_E), the concentration of drug that produces an effect that is 50% of maximum (EC_{50}), and the shape factor (γ) that describes the slope of the sigmoidal curve defining the effect relationship. For this study, the measured effect was GIR.

RESULTS — The mean serum insulin concentration versus time profiles from each administration are shown in Fig. 2. Mean glucose infusion rates are provided in Fig. 3. Summary pharmacokinetic and pharmacodynamic parameters for each insulin formulation are provided in Table 1. TR_{max} for both regular and NPH insulin occurs at a later time than t_{max}, which shows a delay between maximum serum concentrations and maximum pharmacodynamic effects. This is more obvious for regular insulin but occurs with both regular and NPH insulins. This delay is even more evident when serum concentrations are plotted against the GIR values for each time serum insulin concentrations are collected (Fig. 4). Concentration versus response typically results in a sigmoid-shaped curve. However, because of the delay present between serum insulin concentrations and effect, we found a counter-clockwise hysteresis loop. Thus, for any one serum insulin concentration two different effects can be induced depending on the time after administration. The size of the hysteresis loop depends on the time difference between the serum concentrations and GIR. As evidence of this, the hysteresis loop in Fig. 4 is greater for regular insulin than for NPH.

Table 1—*Summary of pharmacokinetic and pharmacodynamic parameters*

	Insulin	
Parameter	Regular	NPH
C_{max} (pM)	308 ± 133	127 ± 58.1
t_{max} (h)	1.7 ± 0.67	5.7 ± 3.5
AUC ($pmol \cdot h^{-1} \cdot L^{-1}$)	1,212 ± 206	1,240 ± 442
R_{max} (mmol/min)	2.18 ± 1.00	1.36 ± 0.380
TR_{max} (h)	3.0 ± 1.5	5.9 ± 2.4
G_{tot} (mmol)	451 ± 166	661 ± 342

Data are means ± SD.

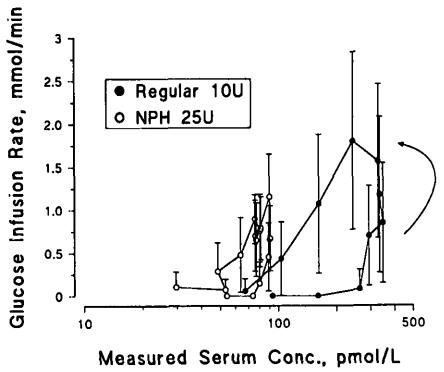

Figure 4—*Relationship of measured serum concentrations to the GIR at each time when serum concentrations were measured. A time-dependent hysteresis results, with the arrow indicating the progression of data with time for both regular and NPH insulin. Bars represent SD.*

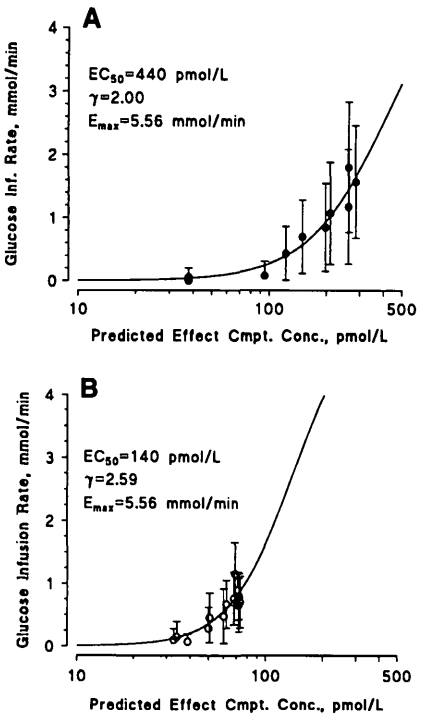

Figure 5—*Relationship between measured GIR and predicted effect compartment concentrations, showing the closure of the hysteresis loop for both regular (A) and NPH (B) insulin.*

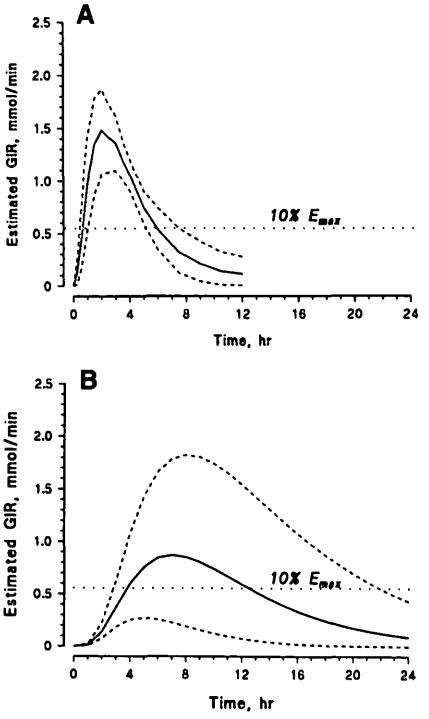

Figure 6—*Estimated GIR from the effect compartment concentrations, showing the 10% E_{max} above which an observed clinical effect is produced. The solid line represents the mean GIR and the dashed lines on represent the 95% CI for both regular (A) and NPH (B) insulin.*

The effect compartment model closed the hysteresis loop for both regular and NPH insulin (Fig. 5). The relationship between predicted-effect compartment concentrations and the measured GIR is a typical concentration versus response relationship. Other studies performed in our clinic have suggested E_{max} is attained between 4.45 and 6.67 mmol/min (D.C.H., J.R.W., unpublished observations). We assigned an E_{max} of 5.56 mmol/min to estimate the relationships for both regular and NPH insulins. A value corresponding to 10% E_{max} was used as a limitation beyond which a clinical effect was recognized (15).

From the predicted effect compartment concentrations and the sigmoidal relationship between the effect compartment concentrations and GIR, a time-action curve was constructed for the GIR values. The GIR associated with the 10% E_{max} was also included in this model, which is shown in Fig. 6. An onset of activity was assigned once the 10% E_{max} was exceeded and ceased once the curve fell below 10% E_{max}. In addition to the mean curves, the 95% confidence intervals (CI) are included for each of the simulations. Table 2 lists the 95% CI ranges for onset, peak, and duration of activity for both regular and NPH insulin. For regular insulin, the mean predicted onset occurred 0.75 h after administration, with a peak activity at 2 h and a duration of 6 h. For NPH, the mean onset was predicted at 3.5 h, with a peak predicted at 6–7 h after administration, and a duration of 13 h.

The variability of these predictions is high for NPH. This is attributable to, in part, the fewer subjects contributing to the study results. Another factor adding to this variability is the inherently variable absorption associated with NPH insulin (22,23). As indicated in Fig. 6, the variability is so great that the lower 95% CI lies below the minimum GIR over the entire 24-h period, suggesting that a 25-U dose may not induce any clinically relevant glucose demands in some individuals. Thus, the 95% CI stated in Table 2 for the onset and duration of NPH insulin is incomplete, with no definable end of onset or beginning of duration noted.

Despite the differences noted in drug delivery between regular and NPH insulins (10,24–26), one would expect the relationship developed between the predicted effect compartment concentrations and GIR to be similar. However, the sigmoidal curves have very different parameter values (EC_{50},γ) to describe the curve. Two factors influence this observation: *1*) the distribution time needed to move insulin from the pharmacokinetic compartment to the effect compartment, and 2) the rate of drug delivery (absorption) from the injection site. The distribution time is independent of the administration route and remains the same regardless of the route or

Table 2—*Predicted onset, peak, and duration of activity*

Insulin	Onset (h)	Peak (h)	Duration (h)
Regular	0.5–1.0	1.75–3.0	5–7.5
NPH	2.75–none*	5–8.5	none*–22

*The 95% CI, encompass the 10% E_{max} barrier, suggesting no effect may occur in some individuals after a 25-U dose.

rate of drug delivery. However, the rate of delivery will affect both the predicted concentrations in the effect compartment and the relative effect produced by those drug levels. Therefore, differences should be expected in these data. Indeed, intravenous insulin results in even greater differences of γ-values when compared with these data (16).

CONCLUSIONS— We realize there are some limitations to our estimations. Our clinical evaluations were performed in healthy volunteer subjects and not insulin-dependent patients. In addition, our data were limited by single doses of regular and NPH insulins. However, we selected doses that were commonly used for both insulin formulations. Despite these limitations, the model produced estimates that agree with more arbitrary determinations reported in the literature for both insulin formulations. We do not expect these estimates would have varied significantly had we performed the study in diabetic patients free from significant insulin antibody titers.

In conclusion, we have provided a method to estimate the onset, peak, and duration of action of insulin from two commonly used formulations. This method is not limited by the route of administration or type of insulin (i.e., lente, ultralente, etc.). However, this method does rely on complete collection of data; a glucose clamp should be conducted until the effects of the administered insulin have subsided.

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