Journal of Pharmacokinetics and Pharmacodynamics, Vol. 30, No. 3, June 2003 (© 2003)

Pharmacokinetic and Pharmacodynamic Properties of Insulin Aspart and Human Insulin

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Received January 20, 2003—Final May 8, 2003

The preferred approach to determine the pharmacokinetic (PK) and pharmacodynamic (PD) properties of insulin analogues is the euglycemic glucose clamp. Currently, non-compartmental data analytical approaches are used to analyze data. The purpose of the present study is to propose a novel compartmental-model for analysis of data from glucose clamp studies. Data used in this trial only involved 18 of the 20 originally treated subjects. Data was obtained from a crossover trial where 18 healthy subjects each received a single subcutaneous (sc) dose of 1.2 nmol/kg (body weight) insulin aspart (IAsp) or 1.2 nmol/kg human insulin (HI) during a euglycemic glucose clamp after overnight fast. Serum insulin and glucose concentrations were measured and the glucose infusion rate (GIR) was adjusted after dosing, to maintain blood glucose near basal levels. Individual model parameters were estimated for IAsp, HI, and the corresponding glucose and GIR data. We found statistically significant differences between most of the HI and IAsp pharmacokinetic parameters, including the sigmoidicity of the time course of absorption (1.5 for HI vs. 2.1 for IAsp (unit less), P=0.0005, Wilcoxon Signed-rank test), elimination rate constant (0.010 min^{-1} for HI vs. 0.016 min^{-1} for IAsp (P=0.002)). The PD model parameters were mostly not different, except for the rate of insulin action (0.012 min^{-1} for HI vs. 0.017 min^{-1} for IAsp (P=0.03)). The model may provide a framework to account for different PK properties when estimating the PD properties of insulin and insulin analogues in glucose clamp experiments.

KEY WORDS: pharmacokinetic; pharmacodynamic; insulin aspart; modeling.

INTRODUCTION

Administration of human insulin (HI) to patients with diabetes is studied intensely with the purpose to find new treatments that better mimic normal pancreatic insulin release. The search has led to the development of

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1567-567X/03/0600-0221/0

short acting insulin analogues with focus on faster absorption compared to HI (1). For type 1 diabetic patients the slow absorption of HI may result in early postprandial hyperglycemia and late postprandial hypoglycaemia (2,3). The absorption of HI is delayed due to a tendency to self-associate into dimeric, tetrameric and hexameric units (4). In the insulin analogue insulin aspart (IAsp), the amino acid proline in position B28 has been replaced by aspartic acid. The effect of this substitution is a reduction in the tendency of the insulin molecule to self-associate. Clinical studies have shown that the onset of the hypoglycemic effect for IAsp is faster than for regular HI due to a faster absorption. Thus IAsp injected immediately before a meal provides better control of postprandial blood glucose than HI (5–7). The euglycemic glucose clamp study is the most common used methodology (8–12) to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) of new insulin analogues. The description of the PK and PD properties of HI and insulin analogues is most often done in terms of non compartmental methods where parameters such as maximal serum insulin concentration (C_{max}), time of maximum serum insulin concentration (t_{max}), maximal glucose infusion rate (GIR_{max}), time to GIR_{max} (t_{max}) and area under the GIR curve. In only a few publications investigators have attempted to use compartmental models for the analysis of euglycemic glucose clamp data that follow a subcutaneous (sc) dose of insulin (11). There are several compelling reasons to apply a PK-PD, model-dependent, analysis approach to these data. First, one may clearly describe and separate the pharmacokinetics of the insulin analogue from its effects on a biological endpoint, e.g., insulin action on glucose disappearance. Using the current methods of analyzing data from clamp experiments, a possible difference in PD properties of a given analogue may be difficult to estimate if the analogue has both different PK and PD compared with that of insulin. Another reason to apply model dependent data analysis is the possibility to extend the results to different experimental protocols and perform model-based simulations under different conditions. In the present study, we propose such an integrated model and we use the identified model parameter estimates to compare the PK and the PD of IAsp and HI.

The model consists of a PK component describing the sc insulin PK as proposed by Berger (13) and of a PD component, following in structure the minimal model proposed by Bergman (14).

RESEARCH DESIGN

The healthy subject data came from a previous published study (15) where 20 healthy subjects were studied. The mean age was 31.1 years (range: 19.0–40.0 years). Mean height was 180.26 cm (range: 172.0–195.0 cm), mean

weight was 76.4 kg (range: 59.6–96.8 kg), and mean BMI was 23.6 kg/m^2 (range: 20.0–27.0 kg/m^2). Two subjects dropped out of the study, thus only data from the 18 subjects that completed the entire study and only data where IAsp and HI were given sc in the abdomen were analyzed. The present experimental protocol and the study design are summarized as such: following an overnight fast, each subject received a single dose (1.2 nmol/kg BW) of either IAsp (NovoLog®, Novo Nordisk, Bagsvaerd, Denmark) or HI (Novolin® R, Novo Nordisk, Bagsvaerd, Denmark) by sc injection into the abdominal region. A glucose infusion at variable rate (GIR) was used to maintain the desired blood glucose level and was started immediately after the injection of the test preparations. Blood samples were collected regularly from 90 min prior to dosing and continued for 10 hr post dose. A GIR at a variable rate was used to maintain the desired blood glucose level, immediately after the administration of either IAsp or HI.

Blood samples for the determination of HI or IAsp, and C-peptide were collected every 30 min from 90 min prior to dosing, every 10 min the first hour post dose and then every 30 min throughout the 10 hr study.

METHODS

Pharmacokinetic Model for HI and IAsp

The PK model was based on a model for sc insulin absorption previously described by Berger (13). The model assumes that insulin follows a dose dependent absorption profile and is eliminated from a central compartment following first-order kinetics. The model as described by Berger (13) includes two parameters, slope and intercept (a and b) that describe the time required for 50% of the insulin dose to be absorbed (T_{50}); however, we found that the intercept parameter b was unidentifiable from HI or IAsp data, probably because the model contained too many parameters, thus we excluded it from our version of the model. Another possibility would have been to use the model $T_{50} = b$ as the former model will have limited applicability outside the normalized dose given in the present study. With this change, all parameters were found to be identifiable.

Equation (1) shows the mathematical representation of the PK model that describes exogenous insulin amounts:

$$\frac{dA(t)}{dt} = \frac{st^{s-1} \cdot T_{50}^{s}}{(T_{50}^{s} + t^{s})^{2}} \cdot dose - k \cdot A(t) \tag{1}$$

where $A(t)$ (pmol) is the amount of exogenous insulin in serum, s (unit less) describes the observed sigmoidicity in the time course of absorption, T_{50} =

$a \cdot dose$, where a (min/pmol) characterizes the dose dependency of the absorption time, and k (min^{-1}) is the first-order elimination constant.

Serum C-peptide concentration can be used to infer endogenous insulin secretion, since it is secreted equimolarly with insulin and its extraction by the liver is negligible. Various models for endogenous insulin production/C-peptide secretion rate were investigated for their potential use (16–18); however, none of the currently available models was able to describe the observed (almost constant) serum C-peptide concentrations (see Fig. 1). Therefore, we concluded that the influence of exogenously administered HI or IAsp on the secretory process was negligible, and a simple constant basal insulin secretion level was assumed. This assumption may introduce bias in the model estimates, as the C-peptide levels do change during the clamp experiment, however the impact on the model estimates is regarded as non significant, as there is no systematic difference between IAsp and HI data. Equation (2) describes the model for the observed total (endogenous and exogenous) serum insulin concentration:

$$I(t) = \frac{A(t) + I_b \cdot V_I}{V_I} \tag{2}$$

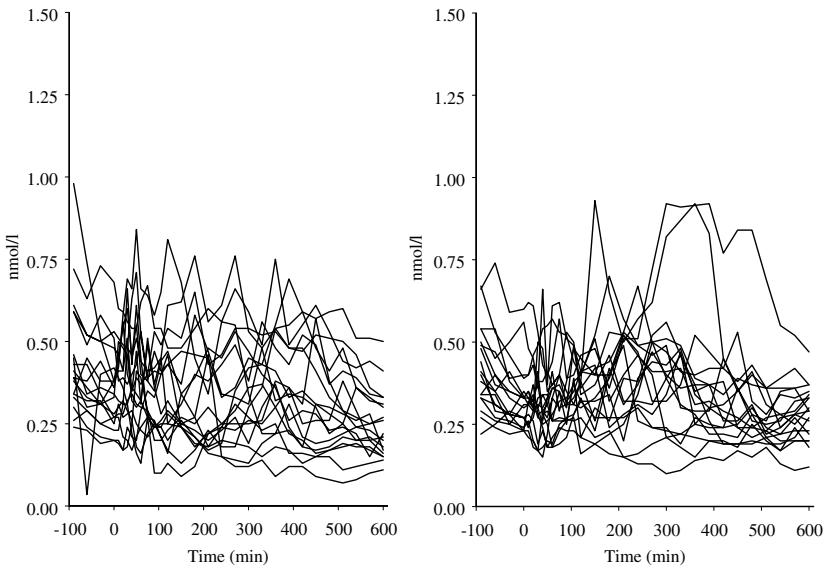

Fig. 1. Serum C-peptide concentrations after dosing with IAsp (left) and HI (right) for all subjects.

where $I(t)$ (pmol/L) is the total serum insulin concentration, I_b (pmol/L) is the basal endogenous insulin concentration assumed to be constant, and V_I (L) is the distribution volume for insulin. Equation (2) can be fitted to the insulin time course measured after either IAsp or HI administration, and determines the PK model. Unknown parameters for each subject are: s, a, k, Ib, and V_I. IAsp and HI clearance (CL) was calculated from the product of V_I and k.

Pharmacodynamic Model for Glucose Disappearance

The minimal model of glucose disappearance as originally proposed by Bergman (14) was used to determine IAsp and HI PD properties. The model's uniquely identifiable parameters are usually estimated using data from a standard or otherwise modified intravenous glucose tolerance test (IVGTT). The system of algebraic-differential equations shown below describes the minimal model, which is extended to accommodate the presence of a variable glucose infusion:

$$\frac{dx(t)}{dt} = p_2 \cdot S_I[I(t) - I_b] - p_2 \cdot x(t) \qquad x(0) = 0$$

$$\frac{dq(t)}{dt} = D(t) + S_G \cdot G_b \cdot V_G - S_G \cdot q(t) - x(t) \cdot q(t) \quad q(0) = G_b \cdot V_G \qquad (3)$$

$$g(t) = \frac{q(t)}{V_G}$$

where $x(t)$ (min^{-1}) is insulin action, p_2 (min^{-1}) describes the rate of insulin action, $q(t)$ (mmol) is glucose mass, $D(t)$ (mmol min^{-1}) is the glucose dose, i.e., the variable GIR in our case, G_b (mmol/L) is basal blood glucose concentration, S_G (min^{-1}) describes glucose effectiveness, which measures glucose effect *per se* at basal insulin level to stimulate glucose disposal and to inhibit endogenous production, S_I ($\text{min}^{-1}\,\text{pmol}^{-1}\,\text{L}$) is the insulin sensitivity parameter, which measures the ability of insulin to enhance glucose disposal, and V_G (L) is the glucose distribution volume. The main components of the combined PK and PD model are summarized in Fig. 2.

Parameter Estimation

The combined model given by Eqs. (1)–(3) was fitted simultaneously to individual subject HI and glucose data, and to IAsp and glucose data. The appropriate, individualized GIR was used in all cases. This resulted in individual PK and PD estimates for both IAsp and HI. For the estimation of the PK and the PD model parameters, non-linear extended least squares

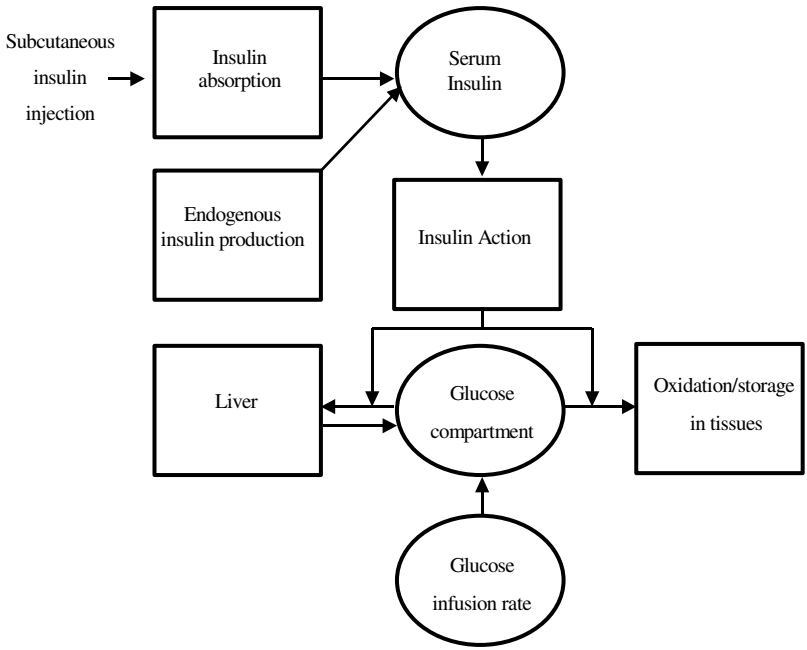

Fig. 2. Principal model components for the combined PK and PD model. Arrows indicate interactions as detailed by Eqs. (1)–(3).

regression analysis, as implemented in the SAAM II software system (University of Washington and SAAM Institute, Seattle, WA) (19), was used. Measurement errors were assumed Gaussian, zero mean and with a constant fractional standard deviation that was different for HI, IAsp and glucose, and was estimated from data, as is customary.

PK parameters were also estimated separately, and fixed when estimating the PD parameters, in order to determine the effect of likely PD model misspecification on the PK parameters.

Statistics

Parameter estimates were compared by the Wilcoxon Signed Rank test. All tests were made as within-subject comparisons at the 5% significance level. Statistical analyses were made using S-Plus 2000, professional release (MathSoft, Seattle, WA). Values are means ± SE unless otherwise specified.

RESULTS

The serum insulin profiles for IAsp and HI were markedly different, as were the GIR profiles shown in Fig. 3. However, the PK model we adopted

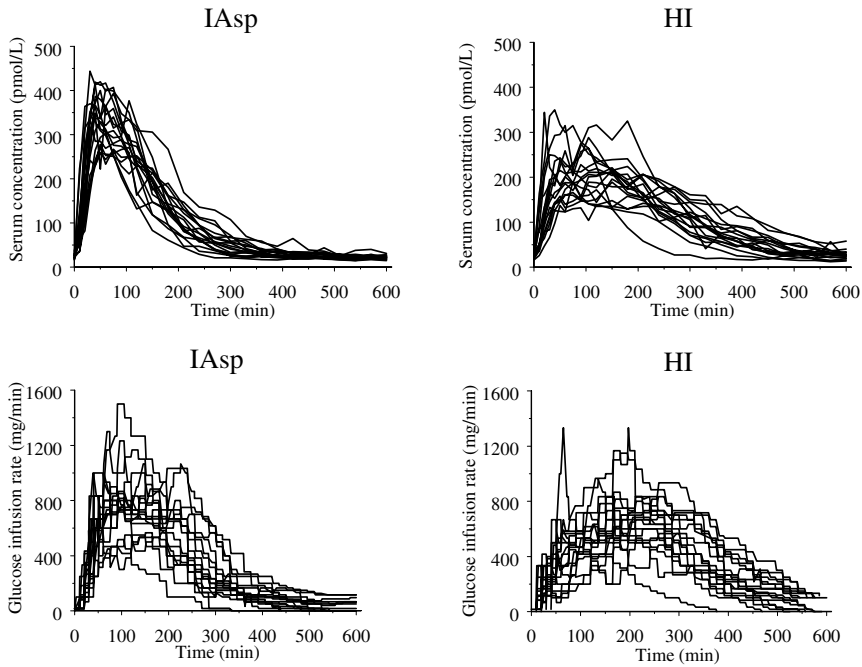

Fig. 3. Serum HI (right) and IAsp (left) concentrations and the corresponding glucose infusion rates for all subjects.

had the flexibility to describe both the IAsp and HI serum concentration time curve. PK model parameter estimates for IAsp and HI shown in Table I.

The model converged successfully in all subjects except one for the HI protocol. We have thus excluded this subject from the statistical analyses,

Table I. Mean Absorption Model (PK) Parameter Estimation Results for Insulin Aspart (IAsp) and Human Insulin (HI)[a]

Parameter values	IAsp	HI
I_b (pmol/L)	20.7 (8%)	23.2 (16%)
V_I (L)	135 (25%)	179 (71%)
a (min/pmol)	4.2×10^{-4} (23%)	6.8×10^{-4} (81%)
s (unitless)	2.1 (14%)	1.5 (28%)
k (min^{-1})	1.6×10^{-2} (23%)	1.0×10^{-2} (62%)
CL (L/min)	1.87 (N.A.)	1.67 (N.A.)

[a]Numbers in parentheses are average estimate precision expressed as percent coefficient of variation. Clearance was not estimated but derived as $CL = V_I \times k$.

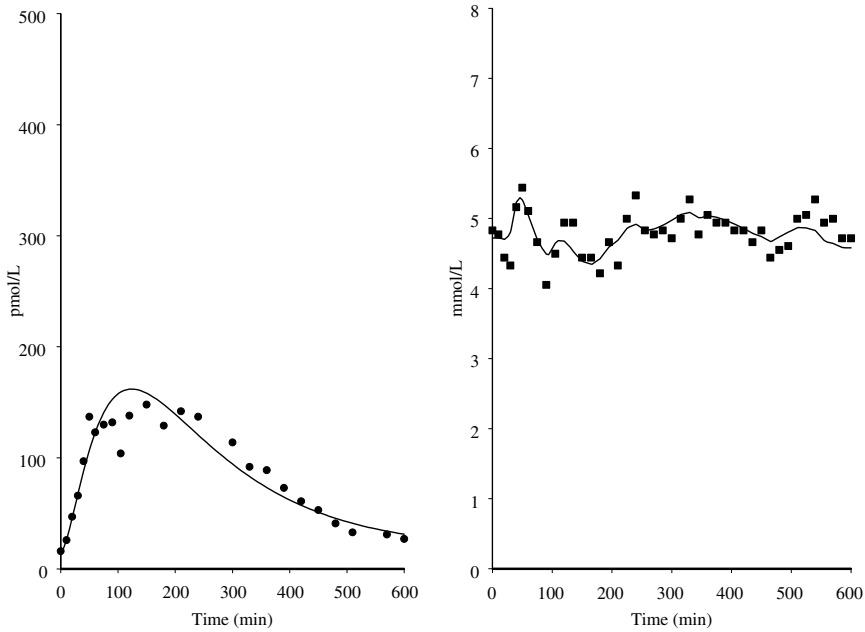

Fig. 4. Model predicted (line) and observed (black circles) serum concentrations of HI and glucose for subject No. 1.

although we report PK and PD parameter estimates for the IAsp protocol.

The corresponding PD parameter estimates for IAsp and HI are reported in Table II. A typical example of individual model fit of serum insulin and blood glucose data is shown in Fig. 4 and in Fig. 5 with HI and IAsp, respectively. The weighted residual plots for both the PK and PD model for IAsp and HI are shown in Fig. 6, the plots showed no systemic pattern, with the exception of the PK model for HI. The individually predicted vs. observed concentrations for both insulin and glucose are shown in Fig. 7.

Table II. Glucose Disappearance Model (PD) Parameter Estimation Results for Insulin Aspart (IAsp) and Human Insulin (HI)[a]

Parameter values	IAsp	HI
G_b (mmol/L)	4.7 (2%)	4.9 (2%)
V_G (L)	53 (42%)	71 (39%)
P_2 (min^{-1})	1.7×10^{-2} (34%)	1.2×10^{-2} (22%)
S_G (min^{-1})	2.7×10^{-2} (91%)	2.5×10^{-2} (113%)
S_I (min^{-1} $pmol^{-1}$ L)	8.7×10^{-5} (44%)	12.2×10^{-5} (41%)

[a] Numbers in parentheses are estimate precision expressed as percent coefficient of variation (%CV).

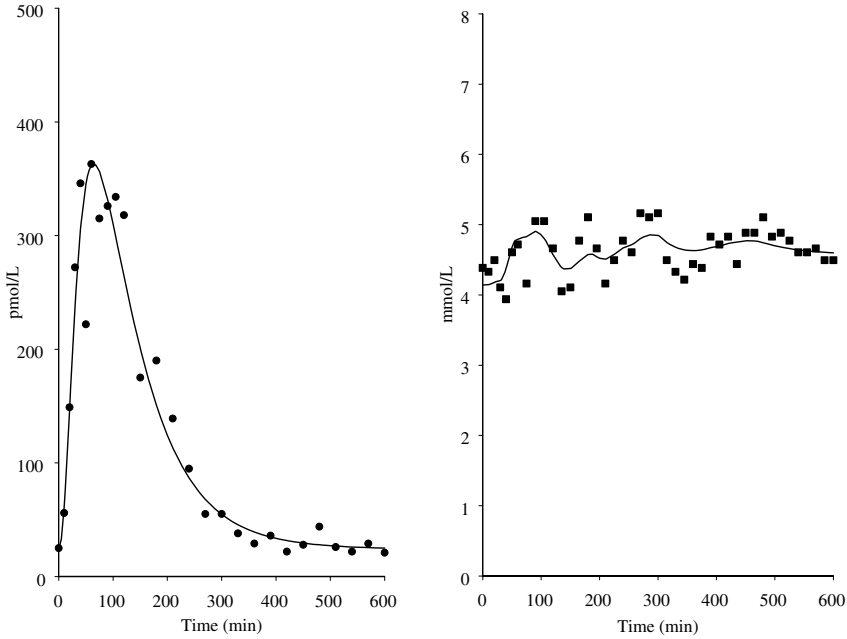

Fig. 5. Model predicted (line) and observed (black circles) serum concentrations of IAsp and glucose for subject No. 1

For two subjects in the IAsp protocol and four in the HI protocol (nos. 9, 13, 14, and 16), the PD model parameter S_G was unidentifiable on data from these subjects. Thus, in these subjects S_G was fixed to a population value, calculated as the mean value of S_G from the rest of the population. The individual PK parameter estimates were used to compare PK "parameter estimates" of IAsp and HI when performing the Wilcoxon Signed Rank test.

Parameters a, V_I, s and k were significantly different for IAsp and HI ($p < 0.006$, $p < 0.03$, $p < 0.0004$, and $p < 0.002$ respectively). Basal endogenous insulin concentration and serum IAsp and HI CL were not significantly different.

In more descriptive, terms for IAsp in comparison with HI:

1. The smaller estimate of a will result in an earlier serum insulin profile peak.
2. The higher value of s results in a more s-shaped (sigmoid) curve of the serum insulin absorption profile.
3. The lower V_I results in higher maximal serum insulin concentration.
4. The higher k results in a faster return of serum insulin to baseline values (the endogenous baseline value I_b).

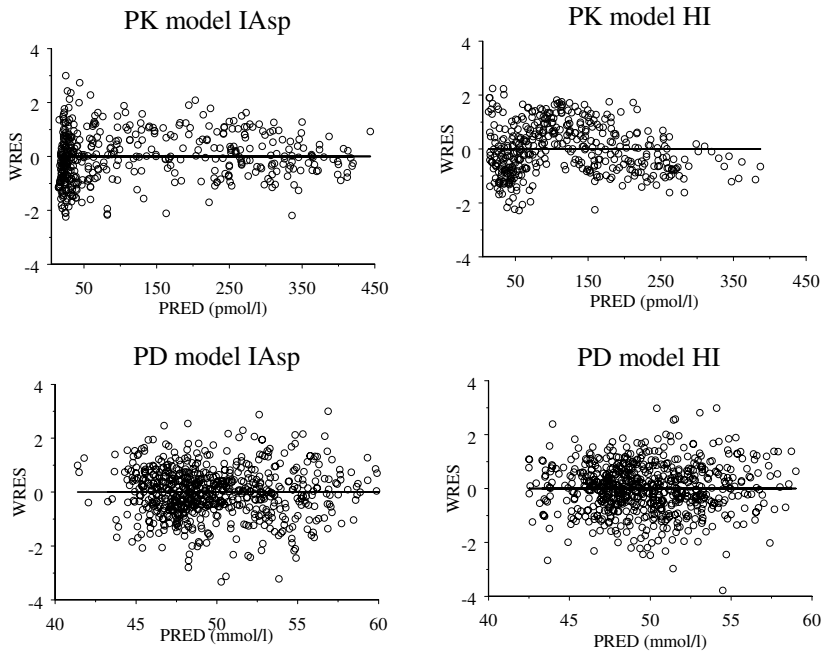

Fig. 6. Weighted residual plots for the PK model for both IAsp and HI and for the PD model for IAsp and HI.

The PD parameter estimates were mostly not significantly different for IAsp and HI, with the exception of p_2 ($p < 0.03$). The PD parameter estimates were close to those identified by the minimal model on IVGTT data: S_I and S_G for healthy male Caucasians have been estimated to be $S_I = 12.6 \pm 1.88$ (10^{-5} min^{-1} pmol^{-1} L) and $S_G = 2.6 \pm 0.008$ (10^{-2} min^{-1}) (14). Identifying the PK parameters separately and thereafter the PD parameters with fixed PK parameters did not result in significantly different results from simultaneous parameter estimation (results not shown). However, separating the PK and PD model estimation did result in improved parameter estimates (lower %CV), as would be expected.

DISCUSSION

Subcutaneous absorption of HI and analogues is a complex process influenced by many factors such as local blood flow and/or local insulin degradation (20) The rapid dissociation into monomers that are being readily absorbed gave a more pronounced peak of the serum profile of IAsp

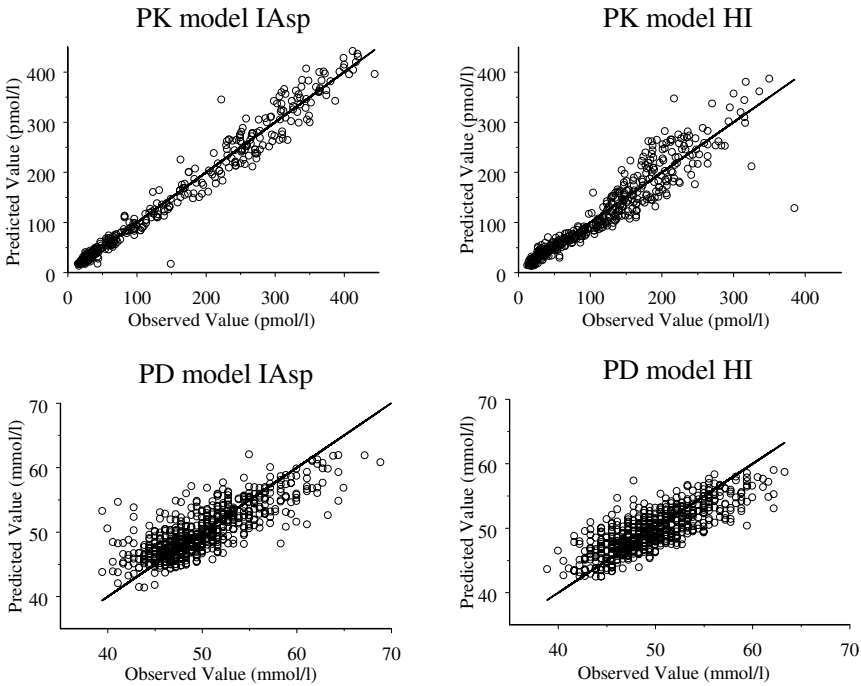

Fig. 7. Goodness of fit plots for PK model both IAsp and HI and for the PD model both IAsp and HI.

compared with HI. At any rate, the absorption model used in this study proved to be a flexible tool and a good descriptor of the absorption kinetics for both IAsp and HI as seen in Fig. 7 however the model does seem to fit IAsp data better than HI as there is some degree of systematic pattern in the weighted residual plot Fig. 6. The model of sc insulin PK have previously been used for simulation purposes (13) and to the best of our knowledge, this is the first time that this proposed sc absorption model of sc insulin PK is applied to actual data (20). The PK model resulted in reliable estimates for both IAsp and HI (as shown in Table II); however, parameter estimation, as measured by the %CV, was better for IAsp than for HI. This is possibly partly due to lower variation in serum concentrations of IAsp than for HI, and partly due to reduced model misspecification. IAsp exists at the injection site mainly as a monomer, whereas HI also exists as hexamers and dimers, for which a later dissociation into monomers is not accounted for by the proposed PK model. The significant difference in PK parameter values confirms what has been reported in previously published

studies, that IAsp has a faster absorption than HI (7,21). A high inter-subject variation (20–40%) in whole-body glucose uptake may be expected in clamp experiments (22), thus a crossover design as used in the present study is needed to obtain reproducible pharmacodynamic (i.e., GIR-profiles) results. There were no major differences in the clamp performance for IAsp and HI mean and standard deviations of the glucose profiles are plotted in Fig. 8. Judged from goodness of fit and parameter precision, the PD model provided reliable estimates of p_2, S_I, G_b, and V_G while the estimate of S_G was somewhat less reliable (the mean %CV was 91% and 113% for IAsp and HI, respectively). The unreliable estimate of S_G, together with the fact that the data for some subjects were not sufficient to ensure estimation, may indicate that the PD model is over-parameterized for this experimental design. The problems with identifying S_G may be due to the nature of the clamp experiment, where glucose is maintained at an almost constant level throughout the study and is not subject to excursions that would make it easier to separate its estimation from the other parameters. This parameter is, however, not necessarily expected to attain different values for HI and insulin analogues as the parameter expresses the ability of glucose *per se*, at

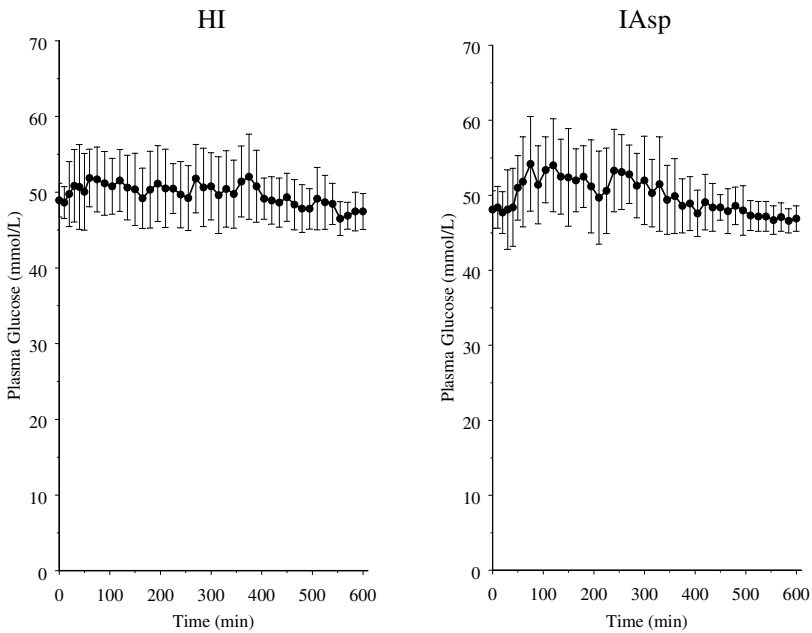

Fig. 8. Plot of the mean glucose levels (± standard deviation) during the clamp.

basal insulin, to stimulate glucose disposal and to inhibit its glucose production. Although we initially speculated that the protocol of variable glucose administration used in the clamp study might reduce the effects of the single-compartment approximation of the glucose distribution associated with Bergman's model. Making the single compartment approximation less crucial (23), this seemed not to be the case and parameter values were very similar to those estimated from IVGTT data. Thus, it seems likely that our S_I values are underestimated and our S_G values are overestimated. This, however, does not impact our comparison between IAsp and HI PK-PD performance, as the same model limitations will be present for both compounds.

The use of extended least squares as opposed to weighted least squares, which guarantees a more stable estimation process, may also account for some of the differences (weighted least squares is the method of choice when estimating minimal model parameters). Another discrepancy from the traditional use of the minimal model is that, in our analysis, G_b is an estimated parameter, and this may also reduce the precision of S_G.

The p_2 parameter was significantly higher for IAsp than for HI ($P = 0.03$), a result that suggests that the duration of insulin action on glucose disappearance is shorter for IAsp than for HI, at the same serum concentration. The difference in p_2 was only borderline statistically significant and may be a result of PK/PD-model misspecification. The "possible" difference in the PDs of IAsp compared to HI has not directly been verified by other clinical studies. However, in euglycemic clamp studies it has been shown that the time to reach maximal GIR is shorter when IAsp administered compared with HI, which seems to be related to our findings (15).

Sequential parameter estimation was performed because simultaneous parameter estimation from multiple data sets may result in biased parameter estimates if one of the models the PK or the PD is misspecified. Estimating model parameters sequentially by individually applying the IAsp or HI PK model parameters to fixed values and then estimating the glucose PD model parameters did not, however, not provide results statistically different from our previous analysis.

The major goal of the euglycemic glucose clamp study is to quantify the insulin action for different insulin preparations and/or insulin analogues. We have proposed a combined PK and PD model for the simultaneous characterization of the PK and the PD properties of HI and IAsp. The model offers assessment of the PD taking a possible PK effect into account when investigated by the clamp experiment. In contrast, the current methods of analyzing the PD properties of insulin (AUC) do not account for the PK. Hence, if an insulin analogue has different PK and PD properties compared with insulin, valuable insight on the PK and PD of the substances may be found using this new approach. In the present study we were

able to show that HI and IAsp are characterized by significantly different PK parameters, thus verifying that the sc absorption of IAsp is faster than that of HI, and we have measured their respective effect in enhancing glucose disappearance. The proposed model is a tool for data analysis of glucose clamp data and will hopefully provide a valuable framework for characterization of PK and PD properties of insulin analogues and prospective model simulations.

ACKNOWLEDGMENTS

This work was partially supported by NIH/NCRR grant 12609 and by a grant from Erhvervsfremmestyrelsen, Denmark.

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