Endocrine Journal 2018, 65 (11), 1147-1153

NOTE

# Pharmacokinetics of insulin disappearance after massive overdosing

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**Abstract.** Long-term glucose supplementation is required to prevent hypoglycemia after massive insulin overdosing. We fitted the blood insulin concentration-time profile to the model: $I = A \cdot \exp^{(-a\,t)} + B \cdot \exp^{(-b\,t)}$, where $I$ (μU/mL) is the serum/plasma insulin concentration, $A$ (μU/mL) and $B$ (μU/mL) are the peak insulin concentrations of each component, $a$ (time$^{-1}$) and $b$ (time$^{-1}$) are the time constants of each component, and $t$ (h) is the time elapsed from the peak of blood insulin level. Additional components were considered as needed. Patient 1 had auto-injected 600 U NovoRapid® 30Mix, and Patient 2 had auto-injected 300 U Novolet®R (regular) and 1,800 U NovoLet®N (NPH). We used the disappearance of therapeutic doses of the respective insulin in healthy individuals as controls, and we obtained parameters by Excel solver. In Patient 1, the parameter values were $A$ = 1490.04 and $a$ = 0.15 for insulin aspart and $B$ = 60.66 and $b$ = 0.04 for protaminated aspart. In Patient 2, the values were $A$ = 784.45 and $a$ = 0.38 for regular insulin and $B$ = 395.84 and $b$ = 0.03 for NPH. Compared with controls, the half-lives ($t_{1/2}$) for insulin aspart and protaminated aspart were 4 and 2 times longer, respectively, in Patient 1. In Patient 2, the $t_{1/2}$ for regular and NPH insulin were 2 and 7 times longer than those in the controls, respectively. In conclusion, the $t_{1/2}$ for insulin was elongated 2 to 7 times after massive overdosing, explaining why glucose supplementation is needed for long periods in these cases.

*Key words*: Disappearance of insulin, Kinetic analysis, Massive overdose

**SUBCUTANEOUS INJECTIONS** of excessive insulin doses in attempted suicides have been documented [1-7]. The hypoglycemic effects of insulin overdoses have been known to last long [1-7], sometimes over 10 days [6]. However, the pharmacokinetic or mechanistic bases for the sustained insulin action have not been obtained. Theoretically, subcutaneous injections of massive insulin doses may form a distinct compartment that does not exist normally. Alternatively, a massive dose may simply be metabolized or dissociates slowly. Local incision of the tissue surrounding the injection site has been tried as a treatment choice, based on the expectation that such surgery will lower the insulin level in the plasma. An assumption was that the injected insulin has stayed compartmentalized in the surrounding subcutaneous injection site [7]. We hypothesized instead that the long-term hypoglycemic effects are explained by delayed insulin disappearance from plasma after a massive dose. In this study, we performed a kinetic analysis on insulin disappearance using data from patients with insulin overdoses to test our hypothesis.

Submitted Mar. 22, 2018; Accepted Jul. 21, 2018 as EJ18-0118
Released online in J-STAGE as advance publication Sep. 4, 2018
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## Materials and Methods

### *Data acquisition and processing*

**Patient 1.** A 34-year-old man with a body mass index (BMI) 20.6 kg/m$^2$ auto-injected 300 U NovoRapid® 30Mix twice to the abdominal wall, approximately 30 min apart. NovoRapid 30Mix® is sold under the brand

name of NovoLog® Mix 70/30 in the US and NovoMix® 30 in European countries. The patient did not have diabetes mellitus; he injected the insulin prescribed for his mother. After the second injection, he became unconscious. Upon arousal, he confessed to his mother that he had injected 600 U insulin and was transferred to the Emergency Department of Aizawa Hospital. He arrived at the hospital 4 h after the initial injection according to him; his plasma glucose was 46 mg/dL and the immunoreactive insulin 1,560 μU/mL. The plasma glucose was maintained between 84 and 130 mg/dL with a glucose infusion throughout the hospital stay. He recovered uneventfully and was discharged after 6 days. The serum insulin level was determined by enzyme-linked immunoassay (Architect®, Abbott, Tokyo), with an antibody that displays 75% cross-reactivity with insulin aspart [8].

Our aim was to perform kinetic analyses on insulin disappearance from the serum; therefore, we processed the raw data obtained during the treatment to meet our purpose. First, we set the initial blood sampling time of the patient, 4 h after the injection, as time 0 and subtracted 4 h from all subsequent time points. Second, we considered 5.5 μU/mL documented on day 5, before breakfast, as the basal, endogenous level of insulin. At that time, he was receiving a small amount of glucose (1.8 g/h, *i.e.*, 36 mL/h of 5% glucose) by peripheral vein infusion, his plasma glucose was 84 mg/dL (4.7 mmol/L), and the C-peptide immunoreactivity 1.30 ng/mL (0.43 nmol/L). Accordingly, we subtracted 5.4 μU/mL, not 5.5 μU/mL, from all insulin values of the patient, leaving the smallest value at 0.1 μU/mL (to avoid 0, for which logarithmic conversion is not feasible).

**Patient 2.** We processed the published serum insulin data [3] from a patient with BMI 30.6 kg/m$^2$ who had auto-injected 300 U regular and 1,800 U NPH insulin in a similar manner. The graph in the communication was read by SimpleDigitizer® version 3.2 (http://www.alrc.tottori-u.ac.jp/fujimaki/download/windows.html) and converted to the numerical data.

**Controls.** As the control for Patient 1, we used data from healthy men ($n$ = 8) with a mean BMI 21.7 kg/m$^2$ receiving 0.15 U/kg BW NovoRapid 30Mix® [9] (Controls 1). Increment of the plasma insulin by exogenous insulin was calculated [10] with simultaneous measurement of C-peptide immunoreactivity. We converted the graphic data into numerical data as described above (Table 1). We observed a peak level of insulin at 1 h and gradual decreases thereafter. To analyze the disappearance of insulin, we set the peak time point, 1 h, as time 0 and subtracted 1 h from all subsequent time points (Table 1).

As the control for Patient 2, we calculated the kinetics of disappearance of regular insulin and NPH insulin using data from healthy men who had received 0.1 U/kg BW regular insulin (Controls 2a, $n$ = 16) or 0.2 U/kg BW NPH insulin (Controls 2b, $n$ = 10, with a mean BMI of 21.8 kg/m$^2$). We obtained the data from the drug manufacturer's information sheet (http://image.packageinsert.jp/pdf.php?mode=1&yjcode=2492415G2020). We again converted graphic data into numerical data and processed them as described above (Table 1).

### *Kinetic analysis*

Patient 1 had auto-injected a biphasic insulin aspart 30 (NovoRapid 30Mix®) suspension, which contains soluble insulin aspart/protamine-crystalized insulin aspart at a ratio of 30/70 (Novo Nordisk A/S. NovoMix 30® EU SmPC. 2017. www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000308/WC500029441.pdf); therefore, we assumed the existence of two components on *a priori* grounds. Patient 2 had auto-injected regular insulin and NPH insulin one after the other [3], and again we assumed the existence of two components as well. On the basis of these considerations, we applied a model with three components (Eq 1 below). We stepped up our analysis to a fourth component if necessary.

$$I = A\cdot\exp^{(-a\cdot t)} + B\cdot\exp^{(-b\cdot t)} + C\cdot\exp^{(-c\cdot t)} \quad \text{Eq 1 (three-component analysis)}$$

Here, $I$ is the serum insulin concentration (μU/mL) at a given time; $A$, $B$, and $C$ are the initial insulin concentrations (μU/mL) for each component; $a$, $b$, and $c$ are the constants for time-dependent insulin reduction in each component; and $t$ is the time in hours.

We obtained the best-fit parameters for each component by Excel solver. Namely, for the three-component analysis, random numbers obtained by Excel were entered for $A$, $B$, $C$, $a$, $b$, and $c$ in Eq 1, and we applied solver with the minimum residual sum of the square ({[actual insulin value] – [the model-estimated insulin]}$^2$) as the calculation target. We repeated the solver calculation until obtaining the smallest target value. We calculated the half-life ($t_{1/2}$) of insulin, 0.693/time constant, for each component. The reproducibility of the calculation was examined by repeating the entire process for 4 times.

The work described was carried out in accordance with The Code of Ethics of the World Medical Associa-

**Table 1** Data processing for analysis of insulin disappearance from the blood

| A. Raw data. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient 1: auto-injected 600 U NovoRapid 30Mix | | | | | | | | | | | | | | | | | |
| Time after injection (hours) | 4 | 20 | 24 | 42 | 54 | 66 | 90 | 114 | | | | | | | | | |
| Insulin (μU/mL) | 1,556 | 191.3 | 105.6 | 34.8 | 16.2 | 9.3 | 5.5 | 9.5 | | | | | | | | | |
| Controls 1: received 0.15 U/kg BW NovoRapid 30Mix | | | | | | | | | | | | | | | | | |
| Time after injection (hours) | 0 | 0.25 | 0.5 | 0.77 | 0.83 | 1 | 1.25 | 1.5 | 2 | 2.5 | 3 | 4 | 5 | 6 | 8 | 10 | 24 |
| Delta exogenous insulin (μU/mL) | 0 | 7.8 | 18.1 | 21.9 | 23.9 | 24.1 | 19.9 | 19.1 | 15.6 | 13.2 | 11.4 | 9.4 | 7.4 | 6.7 | 5.0 | 4.3 | 1.5 |
| Patient 2: auto-injected 300 U regular and 1,800 U NPH insulin | | | | | | | | | | | | | | | | | |
| Time after injection (hours) | 5 | 7.21 | 9.08 | 11.68 | 25.29 | 49.49 | 73.79 | 97.59 | 121.9 | | | | | | | | |
| Insulin (μU/mL) | 1,190 | 921 | 283 | 505 | 267 | 89 | 54 | 49 | 21.9 | | | | | | | | |
| Controls 2a: received 0.1 U/kg BW regular insulin | | | | | | | | | | | | | | | | | |
| Time after injection (hours) | 0 | 0.25 | 0.5 | 0.75 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 5.0 | | | | | |
| Insulin (μU/mL) | 7.7 | 18 | 25.5 | 26.5 | 27.6 | 21.9 | 18.0 | 14.6 | 12.0 | 9.4 | 8.0 | 6.3 | | | | | |
| Controls 2b: received 0.2 U/kg BW NPH insulin | | | | | | | | | | | | | | | | | |
| Time after injection (hours) | 0 | 1.25 | 1.5 | 1.75 | 2 | 2.5 | 3 | 4 | 6 | 10 | | | | | | | |
| Insulin (μU/mL) | 7.36 | 9.76 | 14.72 | 16.16 | 17.28 | 17.44 | 14.56 | 12.96 | 11.04 | 8.48 | | | | | | | |
| B. Dataset used for kinetic analysis | | | | | | | | | | | | | | | | | |
| Patient 1: auto-injected 600 U NovoRapid 30Mix | | | | | | | | | | | | | | | | | |
| Time (hours) | 0 | 16 | 20 | 38 | 50 | 62 | 86 | | | | | | | | | | |
| Delta exogenous insulin (μU/mL) | 1,550.6 | 185.9 | 100.2 | 29.4 | 10.8 | 3.9 | 0.1 | | | | | | | | | | |
| Controls 1: received 0.15 U/kg BW NovoRapid 30Mix | | | | | | | | | | | | | | | | | |
| Time (hours) | 0 | 0.25 | 0.5 | 1 | 1.5 | 2 | 3 | 4 | 5 | 7 | 9 | 23 | | | | | |
| Delta exogenous insulin (μU/mL) | 24.1 | 19.9 | 19.1 | 15.6 | 13.2 | 11.4 | 9.4 | 7.4 | 6.7 | 5.0 | 4.3 | 1.5 | | | | | |
| Patient 2: auto-injected 300 U regular and 1,800 U NPH insulin | | | | | | | | | | | | | | | | | |
| Time (hours) | 0 | 2.2 | 4.1 | 6.7 | 20.3 | 44.5 | 68.8 | 92.6 | 116.9 | | | | | | | | |
| Delta exogenous insulin (μU/mL) | 1,158.2 | 889.2 | 251.2 | 468.2 | 235.2 | 57.2 | 32.3 | 27.2 | 0.1 | | | | | | | | |
| Controls 2a: received 0.1 U/kg BW regular insulin | | | | | | | | | | | | | | | | | |
| Time (hours) | 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | | | | | | | | | | |
| Delta exogenous insulin (μU/mL) | 19.9 | 14.1 | 10.3 | 6.9 | 4.3 | 1.7 | 0.3 | | | | | | | | | | |
| Controls 2b: received 0.2 U/kg BW NPH insulin | | | | | | | | | | | | | | | | | |
| Time (hours) | 0 | 1.0 | 3.0 | 7.0 | 11.0 | | | | | | | | | | | | |
| Delta exogenous insulin (μU/mL) | 17.3 | 12.9 | 10.8 | 6.8 | 5.8 | | | | | | | | | | | | |

Patient 1, our patient; Controls 1, the control for Patient 1; Patient 2, data for this patient was extracted from a publication [3]; Controls 2a, the control regarding regular insulin for Patient 2; Controls 2b, the control for Patient 2 regarding NPH insulin. Data for Controls were taken from the manufacturer's drug information. See Text for the details.

tion (Declaration of Helsinki).

## Results

### *The number of components*

Analysis of the data from Patients 1 and 2 revealed two significant components (Table 2). In both cases, one component was with a considerably shorter $t_{1/2}$ than the other one (see below). The third component obtained by solver calculation was virtually non-existent because "*C*" was zero (Table 2).

Analysis of the data from Controls 1 identified four components as shown in Table 2. However, Components 3 and 4 were not functioning because "C" was zero for the former and $t_{1/2}$ was as short as 0.01 h (0.6 min) for the latter. We only identified one component in Controls 2a. Although solver calculation yielded three components in the case of Controls 2b, "C" was zero for Com-

**Table 2** Parameters obtained by the kinetic analysis

| Subjects/Group | Injected Insulin | Dose (unit) | Component | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | NovoRapid30Mix | 600 | 1 | | 2 | | 3 | | 4 | |
| | | | *A* | 1,490.04 | *B* | 60.66 | *C* | 0 | — | — |
| | | | *a* | 0.15 | *b* | 0.04 | *c* | 0.45 | — | — |
| | | | $t_{1/2}$ | 4.76 | $t_{1/2}$ | 19.41 | $t_{1/2}$ | N.A. | — | — |
| Controls 1 | NovoRapid30Mix | 0.15/kg BW | 1 | | 2 | | 3 | | 4 | |
| | | | *A* | 13.97 | *B* | 8.33 | *C* | 0 | *D* | 1.80 |
| | | | *a* | 0.57 | *b* | 0.08 | *c* | 0.42 | *d* | 67.69 |
| | | | $t_{1/2}$ | 1.22 | $t_{1/2}$ | 9.16 | $t_{1/2}$ | N.A. | $t_{1/2}$ | 0.01 |
| Patient 2 | Regular and NPH | 300 R, 1,800 N | 1 | | 2 | | 3 | | 4 | |
| | | | *A* | 784.45 | *B* | 395.81 | *C* | 0 | — | — |
| | | | *a* | 0.38 | *b* | 0.03 | *c* | 0.04 | — | — |
| | | | $t_{1/2}$ | 1.83 | $t_{1/2}$ | 20.32 | $t_{1/2}$ | N.A. | — | — |
| Controls 2a | Regular | 0.1 U/kg BW | 1 | | 2 | | 3 | | 4 | |
| | | | *A* | 20.43 | — | — | — | — | — | — |
| | | | *a* | 0.79 | — | — | — | — | — | — |
| | | | $t_{1/2}$ | 0.88 | — | — | — | — | — | — |
| Controls 2b | NPH | 0.2 U/kg BW | 1 | | 2 | | 3 | | 4 | |
| | | | *A* | 1.93 | *B* | 8.15 | *C* | 0 | — | — |
| | | | *a* | 18.16 | *b* | 0.24 | *c* | 0.24 | — | — |
| | | | $t_{1/2}$ | 0.04 | $t_{1/2}$ | 2.85 | $t_{1/2}$ | N.A. | — | — |

Patient 1, our patient; Controls 1, the control for Patient 1; Patient 2, data for this patient was extracted from a publication [3]; Controls 2a, the control regarding regular insulin for Patient 2; Controls 2b, the control for Patient 2 regarding NPH insulin. Data for Controls were taken from the drug manufacturer's information. The underlined components are virtually non-existent because "*C*" was zero or $t_{1/2}$ was too short. See text for the details.

ponent 3, and $t_{1/2}$ was 0.04 h (2.4 min) for Component 1. Accordingly, the number of functioning components was only 1 for Controls 2b. The concordance between the actual level of serum insulin and the estimated insulin level by the two-component model in the patients was very high ($r^2 > 0.99$ and $r^2 = 0.91$ in Patients 1 and 2, respectively) (Supplemental Fig. 1).

### *Parameter values*

We identified components with clearly distinguishable short and long $t_{1/2}$ in the patients: we considered the former as reflecting insulin aspart and regular insulin, and the latter as reflecting protaminated aspart and NPH.

$t_{1/2}$ of the serum insulin was markedly elongated in Patient 1 (Table 2), *i.e.*, 4.76 h for Component 1 and 19.41 h for Component 2; and the corresponding values for Controls 1 were 1.22 and 9.16 h. This was also the case in Patient 2 (Table 2). Namely, the $t_{1/2}$ was 1.83 and 20.32 h for Components 1 and 2, whereas the values were 0.88 and 2.85 h in Controls 2a and 2b, respectively (Table 2). The initial concentration of insulin in the components designated by italic uppercase letters was grossly elevated in the patients compared with that in the controls (Table 2). We obtained quasi-identical results regarding the parameters by repeated calculations using Excel solver for Patient 1 and Controls 1 (Supplemental Table 1). The results of the kinetic analyses for Patient 1 and Controls 1 are graphically shown in Fig. 1.

## Discussion

Large amounts of glucose infusions (20–30 g/h) are

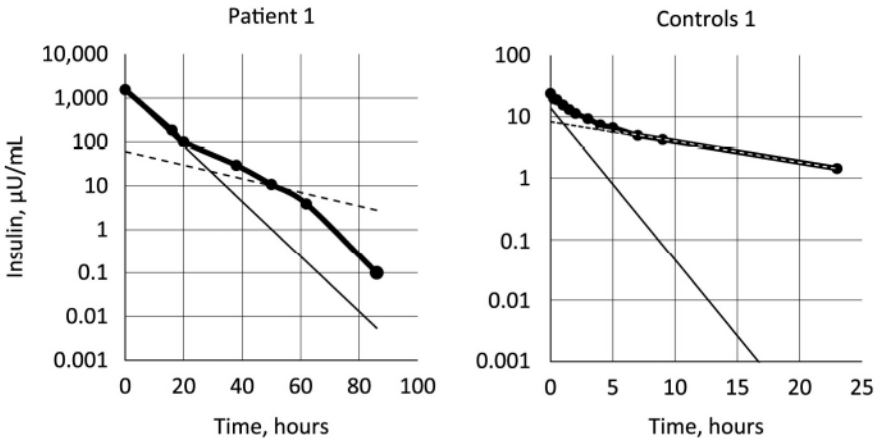



**Fig. 1** Kinetics of insulin disappearance in Patient 1 and Controls 1. Thick lines, serum or plasma concentration of insulin (Table 1 B); thin lines, regression obtained for Component 1; broken lines, regression for Component 2. Intercepts of the thin line (regression line) and the ordinate corresponded to *A* and *B*, respectively, in each Figure. Slope of the regression line was designated by *a* and *b*, respectively, in each Figure. The abscissa and the ordinate are not the same in the two graphs.

required for long periods of time [1-7] to prevent hypoglycemia in patients with subcutaneous injection of excessively large insulin doses. However, because of lack of pharmacokinetic data, the exact reason for the long-lasting hypoglycemic effect of insulin upon overdose has remained unclear. For the first time, we performed the kinetic analysis on the disappearance of insulin from the circulation in individuals with a massive overdose of premixed insulin or regular and NPH insulin injected subcutaneously. The results were compared with those obtained in healthy volunteers receiving therapeutic doses of the same insulin types.

Our findings on the disappearance of serum insulin coincided well with the classic two-component model. The estimated insulin concentration by the model was correlated with the actual insulin levels with $r^2$ values >0.90 (Supplemental Fig. 1). Thus, we think our approach ruled out extra-components of insulin irrespective of the dosing and insulin preparations.

Importantly, the pharmacokinetic parameters were markedly different on the basis of dosing. Above all, the time constant for the disappearance of insulin was so small in the patient who injected the largest amount of insulin that the $t_{1/2}$ was significantly elongated. For the component representing the rapidly acting insulin (insulin aspart or regular insulin) we found the $t_{1/2}$ was elongated approximately 2 to 4 times in the patients. Also, the $t_{1/2}$ for the disappearance of intermediate insulin (protaminated aspart or NPH insulin) was about 2 to 7 times longer in the patients than in the controls. As examples, the results in Patient 1 and Controls 1 are shown in Fig. 1, in which the $t_{1/2}$ elongation in the patient can be seen. We considered that this prolongation of disappearance of insulin is causal for the long-lasting hypoglycemic effect after subcutaneous injection of massive insulin doses observed in the previous reports [1-7].

We can offer three reasons for the slowed disappearance of insulin under the massive overdose. First, the dissociation of insulin polymer to insulin monomer [11] would be slowed given the large volume of injected insulin (the large volume would impede dilution by the interstitial fluid). Insulin aspart is produced by replacing the proline at position 28 on the B chain of insulin with aspartic acid, to facilitate dissociation from hexamers into dimers and monomers upon subcutaneous injection [12]. The elongation of the $t_{1/2}$ was apparent despite such advantage of insulin aspart pharmacokinetics. Second, receptor-mediated insulin degradation would be attenuated because of saturation of insulin receptors by the injected insulin molecules. The insulin receptor gets fully saturated at an insulin concentration >10 nmol/L [13], and we found the insulin concentration in the serum of patients to be around 1,200 and 1,600 μU/mL (8.6 and 11 nmol/L, respectively). Of note, these values were obtained upon arrival to the emergency room. The insulin peak should have been higher than this level shortly after the injection of the massive insulin dose. Insulin clearance by the kidney may also have a limited capacity [14] and saturation of this process may have contributed to the accumulation and/or slow degradation of insulin in

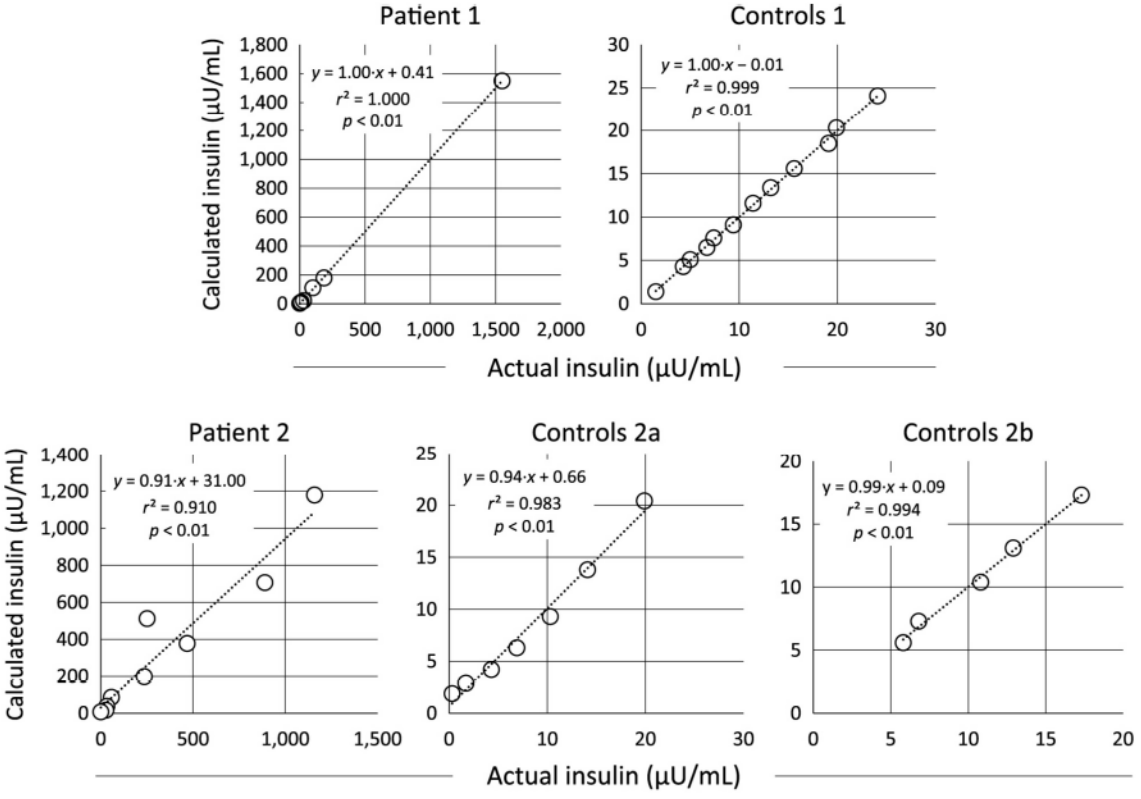
the patients. The plasma glucose levels changed during the recovery phase in Patients 1 and 2; however, the acute changes in plasma glucose do not alter the metabolic clearance rate of insulin [15]. Nonetheless, obesity in Patient 2 may have slightly contributed to the slow disappearance of insulin.

We are aware of the limitations in this study. We analyzed the data of only two patients, and the general applicability of our results cannot be confirmed. The results of kinetic analysis may be different in a case with an even higher insulin overdose such as one with 16,000 U [6]. The number of data points was relatively small, especially in the case of the patients, and the initial values obtained in the patients may have been lower than the actual peak, so that the accuracy in the parameters cannot be completely guaranteed. Despite careful confirmation of the reproducibility, the instability in the solver calculation of parameters arising from small sample sizes is of concern. The C-peptide immunoassays at each time point of blood sampling were performed only in the Controls. Finally, the subtraction of a given value of IRI from all samples may be a source of inaccuracy, especially in later periods when the exogenous insulin is closer to the basal levels.

In conclusion, for the first time, we obtained pharmacokinetic data on insulin disappearance in patients injected with a massive insulin overdose. The insulin disappearance fitted the two-component model, with considerably elongated $t_{1/2}$. The prolonged hypoglycemic effects after a massive insulin overdose by subcutaneous injection may be explained to the most part, if not completely, by the slowing down of the insulin disappearance. We found no evidence for the abnormal trapping of insulin into the extra-compartment.

## Acknowledgments

We thank Drs. H Sagesaka and T Miyakoshi for their excellent care of the patient and constructive discussion on the data.




**Supplemental Fig. 1** Comparison of actual insulin and calculated insulin in Patient 1, Controls 1, Patient 2, Controls 2a, and Controls 2b. Results of the linear regression are shown in each graph.

**Supplemental Table 1** Results of the repeated Excel solver calculation

| Individuals | Parameters | Solver calculation | | | | Mean | SD | CV |
|---|---|---|---|---|---|---|---|---|
| | | 1st calculation | 2nd calculation | 3rd calculation | 4th calculation | | | |
| Patient 1: injected 600 U NovoRapid® 30Mix | *A* | 1,490.031 | 1,490.058 | 1,490.046 | 1,490.041 | 1,490.04 | 0.01 | <0.001 |
| | *a* | 0.146 | 0.146 | 0.146 | 0.146 | 0.15 | <0.001 | <0.001 |
| | *B* | 60.669 | 60.643 | 60.656 | 60.660 | 60.66 | 0.01 | <0.001 |
| | *b* | 0.036 | 0.036 | 0.036 | 0.036 | 0.04 | <0.001 | <0.001 |
| | *C* | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 | N.A. |
| | *c* | 0.035 | 0.146 | 0.146 | 1.472 | 0.45 | 0.68 | 1.52 |
| Controls 1: received 1.5 U/kg BW NovoRapid® 30Mix | *A* | 13.974 | 13.974 | 13.974 | 13.974 | 13.97 | 0.00 | 0.00 |
| | *a* | 0.572 | 0.572 | 0.572 | 0.572 | 0.57 | 0.00 | 0.00 |
| | *B* | 8.331 | 8.331 | 8.331 | 8.331 | 8.33 | 0.00 | 0.00 |
| | *b* | 0.076 | 0.076 | 0.076 | 0.076 | 0.08 | <0.001 | <0.001 |
| | *C* | 0.000 | 0.000 | 0.000 | 0.000 | 0.00 | 0.00 | N.A. |
| | *c* | 0.818 | 0.150 | 0.154 | 0.571 | 0.42 | 0.33 | 0.78 |
| | *D* | 1.795 | 1.795 | 1.795 | 1.795 | 1.80 | <0.001 | <0.001 |
| | *d* | 70.885 | 64.248 | 63.547 | 72.081 | 67.69 | 4.42 | 0.07 |

SD, standard deviation; CV, coefficient of variation. Because parameter *C* was zero and *d* was so large, implying $t_{1/2}$ was short, shaded data were considered as having no physiological significance. The mean values were used for calculation. See text for the details.

## References


1. Mork TA, Killeen CT, Patel NK, Dohnal JM, Karydes HC, *et al.* (2011) Massive insulin overdose managed by monitoring daily insulin levels. *Am J Ther* 18: e162–e166.
2. Samuels MH, Eckel RH (1989) Massive insulin overdose: detailed studies of free insulin levels and glucose requirements. *J Toxicol Clin Toxicol* 27: 157–168.
3. Matsumura M, Nakashima A, Tofuku Y (2000) Electrolyte disorders following massive insulin overdose in a patient with type 2 diabetes. *Intern Med* 39: 55–57.
4. Fasching P, Roden M, Stühlinger HG, Kurzemann S, Zeiner A, *et al.* (1994) Estimated glucose requirement following massive insulin overdose in a patient with type 1 diabetes. *Diabet Med* 11: 323–325.
5. Arem R, Zoghbi W (1985) Insulin overdose in eight patients: insulin pharmacokinetics and review of the literature. *Medicine (Baltimore)* 64: 323–332.
6. Thewjitcharoen Y, Lekpittaya N, Himathongkam T (2008) Attempted suicide by massive insulin injection: a case report and review of the literature. *J Med Assoc Thai* 91: 1920–1924.
7. Campbell IW, Ratcliffe JG (1982) Suicidal insulin overdose managed by excision of insulin injection site. *Br Med J (Clin Res Ed)* 285: 408–409.
8. Moriyama M, Hayashi N, Ohyabu C, Mukai M, Kawano S, *et al.* (2006) Performance evaluation and cross-reactivity from insulin analogs with the ARCHITECT insulin assay. *Clin Chem* 52: 1423–1426.
9. Urae A, Irie S, Tanaka T (2003) Phase I clinical study of biphasic insulin Aspart 30 (BIAsp30). *J Clin Therap Med* 19: 733–742 (in Japanese).
10. Pandyarajan V, Weiss MA (2012) Design of non-standard insulin analogs for the treatment of diabetes mellitus. *Curr Diab Rep* 12: 697–704.
11. Pierce JG, Schumitzky A (1976) Optimal impulsive control of compartment models I. Qualitative aspects. *J Optim Theory Appl* 18: 537–554.
12. Gast K, Schüler A, Wolff M, Thalhammer A, Berchtold H, *et al.* (2017) Rapid-acting and human insulins: hexamer dissociation kinetics upon dilution of the pharmaceutical formulation. *Pharm Res* 34: 2270–2286.
13. Polidori DC, Bergman RN, Chung ST, Sumner AE (2016) Hepatic and extrahepatic insulin clearance are differentially regulated: results from a model-based analysis of intravenous glucose tolerance data. *Diabetes* 65: 1556–1564.
14. Polonsky K, Jaspan J, Emmanouel D, Holmes K, Moossa AR (1983) Differences in the hepatic and renal extraction of insulin and glucagon in the dog: evidence for saturability of insulin metabolism. *Acta Endocrinol (Copenh)* 102: 420–427.
15. Thorsteinsson B, Fugleberg S, Binder C (1988) Insulin clearance from plasma in type I (insulin-dependent) diabetic patients: influence of glycaemic level. *Pharmacol Toxicol* 62: 206–209.