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^{125}I used for labelling of proteins in an absorption model changes the absorption rate of insulin aspart

Anders Sohoel[a,*], Anne Plum[b], Sven Frokjaer[a], Peter Thygesen[c]

[a] *Department of Pharmaceutics, The Danish University of Pharmaceutical Sciences, Copenhagen, Denmark*
[b] *Drug metabolism & Pharmacokinetics, Novo Nordisk, Denmark*
[c] *Exploratory ADME Biopharmaceutical Research Unit, Novo Nordisk, Denmark*

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Abstract

The aim of this study is to validate the ability of the disappearance model to predict absorption rates of insulin aspart in pigs. The disappearance model is used as a screening tool to estimate absorption rates after subcutaneous injections in humans or pigs especially of insulin and insulin analogues. The disappearance model measures remaining radioactivity at the injection site and therefore radioactive labelling of the insulin analogue is necessary. The labelling is done with ^{125}I. One of the assumptions for the disappearance model to be reliable is that absorption rates of the labelled and non-labelled molecules are comparable. In this study, we compared disappearance data with absorption calculated from plasma samples of insulin aspart. The calculated absorption is based on non-labelled insulin aspart. The absorption rate from the disappearance data was statistical significant ($p = 0.0028$) different from the absorption rate based on plasma samples. A control study was carried out where ^{125}I labelled insulin aspart was compared to ^{127}I (the natural non-radioactive isotope) insulin aspart. In this study, absorption rate from the disappearance data and absorption rate based on plasma samples were similar ($p = 0.63$). *Conclusion:* Iodination of insulin aspart changes the subcutaneous absorption rate.
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Keywords: Disappearance; Absorption; Insulin aspart; Iodinated insulin aspart; Deconvolution

1. Introduction

In treatment of insulin-dependent diabetes subcutaneous (s.c.) injections are the prominent route of administration in most patients. The absorption of insulin from the subcutaneous tissue plays a major role in securing sufficient plasma insulin levels and thereby securing the therapeutic efficiency of the insulin treatment. Further, patients with near-normoglycemic blood glucose have a slower progression of disease complications (The Diabetes Control and Complications Trial Research Group, 1993).

During development of new insulin analogues in the pharmaceutical industry subcutaneous absorption and absorption rate are important selection parameters. The absorption profile determines the action profile of the insulin analogues. Early in the developing phase a specific analytical method for measuring the new insulin analogue in plasma is often not available. The absorption rate based on plasma samples can therefore not be obtained. However, the disappearance of radioactively labelled insulin analogues is often used as an alternative method to compare and rank absorption rates between new insulin analogues or between new analogues and marketed insulin products.

For the disappearance model to be a reliable model the following three assumptions should be fulfilled. The labelled insulin molecule should have the same absorption kinetics as the non-labelled insulin molecule, no degradation should occur at the injection site and the externally measured radioactivity should be proportional to the actual amount of drug at the injection site remaining to be absorbed. The disappearance model has for several decades been used for testing insulin and insulin analogues in both humans (Binder, 1969; Brange et al., 1990; Kang et al., 1991; Clauson and Linde, 1995) and pigs (Deckert, 1982; Ribel et al., 1985; Markussen et al., 1996; Clausen et al., 2002).

The use of ^{125}I-insulin to predict insulin absorption has been validated before for human insulin (Deckert, 1982; Ribel et al., 1985) and bovine and porcine insulin (Binder, 1969). All

* Corresponding author. Tel.: +45 35306534; fax: +45 35306030.
E-mail address: anso@dfuni.dk (A. Sohoel).

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validations have found a good correlation between the behaviour of labelled and non-labelled insulins. Two methods have been used. In one method, the tissue containing the injected solution has been excised after a certain time and the insulin has been extracted, and the specific radioactivity was compared to the specific radioactivity of the injected insulin. In the other method, plasma profiles of radioactivity were compared to plasma profiles of insulin concentration after subcutaneous injection.

The actual absorption into the blood can be determined by measuring the insulin plasma concentrations. The absorption profile can be calculated by deconvolution of the plasma concentration profile after s.c. injection with the insulin plasma concentration profile after i.v. injection (Pedersen, 1980; Cobelli et al., 1987; Levitt, 2003).

A preliminary study indicated a discrepancy between the actual absorption rate and the disappearance of radioactively labelled insulin at the injection site. The discrepancy indicates a difference in absorption rates between insulin and iodinated insulin.

The aim of this study is to validate the ability of the disappearance model to predict absorption rates of insulin aspart in pigs.

2. Materials and methods

In this study, s.c. absorption of iodinated insulin aspart is compared to s.c. absorption of insulin aspart. This is done by comparing the disappearance of insulin aspart by external γ-counting from the subcutaneous injection site with the absorption of insulin aspart into the blood. Disappearance is measured with a γ-counter, where only the γ-emitting ^{125}I labelled insulin aspart is measured. A small part of the insulin aspart molecules is labelled with ^{125}I. In the plasma samples both iodinated insulin aspart and insulin aspart are measured but the non-labelled insulin aspart molecules out numbers the ^{125}I-insulin aspart molecules. Therefore, if ^{125}I-insulin aspart had a different absorption profile, it could not be seen from the plasma samples. Actually, it is the disappearance of ^{125}I-insulin aspart and the absorption of insulin aspart into the blood which are compared.

To further investigate if the labelling with iodine influences absorption, an additional experiment was carried out. Instead of using ^{125}I-insulin aspart mixed with non-iodinated insulin aspart, all the insulin aspart molecules were labelled. To avoid very high emission of γ-radiation non-radioactive iodine (^{127}I) were used; the non-radioactive iodine was added the usual tracer amount of ^{125}I needed for measuring disappearance.

Insulin aspart is used in the study present due to the existence of a specific assay for measurement of insulin aspart concentration in pig plasma. Insulin aspart has the same amino acid sequence as human insulin except for the substitution of proline with aspartic acid in position B28. However it would be desirable to perform the experiment with human insulin, but at present no assay at present which can distinguish between human and porcine insulin.

2.1. Materials

^{125}I-insulin aspart labelled in the tyrosine in the A14 position was provided by Isotope chemistry at Novo Nordisk Måløv. Non-radioactive I-insulin aspart was synthesised from insulin aspart by LOPD (lactoperoxidase) plus KI, H_2O_2 (Bayse et al., 1972; Magnusson et al., 1984) and purified by HPLC. The A14 iodinated insulin aspart was separated by HPLC from insulin aspart iodinated in one of the other three tyrosine's and diiodinated insulin aspart. The HPLC system consisted of a c-18 column, 10 mm in diameter and particle size of 15 μm. The mobile phase was 36.75% ethanol, 0.05 M phosphoric acid and 0.1 M tris in water. The flow was 2.5 mL/min the oven adjusted to 40 °C and UV detection at 276 nm.

The flow fluid was collected with an auto sampler. The fractions 1.6 min before to 2.4 min after the A14 labelled top were kept. To reduce volume and remove phosphor acid and tris(2-amino-2-(hydroxymethyl)propane-1,3-diol, the amount of ethanol was diluted to 20% loaded on the HPLC column (diameter 4.6 mm) and washed out with 50% acetonitril (ACN), 0.1% trifluoroacetic acid (TFA) in water. The latter eluent was freeze dried.

2.2. Formulations

Insulin aspart was formulated as the marketed product NovoRapid® (containing: glycerol, phenol, *meta*-cresol, zinc chloride, dibasic sodium phosphate dihydrate and sodium chloride) with addition of tracer amounts of ^{125}I-insulin aspart. Two types of insulin aspart were tested: iodinated insulin aspart and non-iodinated insulin aspart. Iodinated insulin aspart is non-radioactive I-insulin aspart and a tracer amount of ^{125}I-insulin aspart. Non-iodinated insulin aspart is insulin aspart and a tracer amount of ^{125}I-insulin aspart. The formulations were 100 IU/mL or 600 nmol/mL for iodinated insulin aspart and 100 and 200 IU/mL for non-iodinated insulin aspart. The concentrations of radiation were between 1 and 2 μCi/mL.

2.3. Dose administration

Insulin aspart formulation was injected subcutaneously with a NovoPen® adjusted to 5 mm injection depth. The s.c. injections were made on the side of the neck approximately 7 cm behind the ear and 9 cm from the middle of the neck. The i.v. dose was administered trough a catheter leading to vena jugularis followed by 10 mL physiological saline. The dose of iodinated insulin aspart was 8 IU/animal or 48 nmol/animal i.v. and 60 nmol/animal s.c. The dose of non-iodinated insulin aspart was 60 nmol/animal i.v. and 120 nmol/animal s.c.

2.4. Animals

Female crossbred pigs (LYD; Land race, Yorkshire, Duroc) weighing 73–93 kg were obtained from Gundsøgård (Roskilde, Denmark). They were handled in accordance with The Danish Laboratory Animal Act (Order on Act no. 726 of 9 September 1993 of Ministry of Justice). They were housed individually

in solid concrete floor pens with beddings of straw and wood shavings. The pigs were housed individually to protect the equipment. The pigs were fed twice a day with a standard pellet diet and provided with water ad libitum. The pigs were fasted overnight before each experimental day.

2.5. Blood sampling

At least 2 days before start of the experiment an ear vein catheter leading to vena jugularis was inserted in each pig under anaesthesia. During anaesthesia a specially designed vest for carrying electronic equipment was mounted on each animal. During the actual experiment, the ear vein catheters were mounted with an extension catheter enabling the pigs to remain awake and unrestrained in the pens, not being physically disturbed during the blood sampling.

2.6. Plasma sample analysis

Blood samples were centrifuged for 10 min, 4 °C at 1200 × *g*. Plasma was removed and stored at −20 °C. Plasma insulin aspart concentrations were measured with a specific ELISA (Andersen et al., 2000). The iodinated insulin aspart in plasma was measured with the same assay. Samples with a known concentration of iodinated insulin aspart were measured in the assay to indicate if iodine might affect the assay. The measurements corresponded well with the real concentration.

2.7. Disappearance

After a s.c. injection the tracer ^{125}I-insulin aspart was measured by external γ-countering. A device for fixating the γ-counter was mounted on the pig with Tensoplast®. After injection of the s.c. dose, the γ-counter was placed above the injection site and fixed. The γ-counter was connected to a wireless transmitter and the transmitter was placed in a pocket of the pigs vest. Data were continuously transmitted to a receiver and further to a computer. Data are a continuous series of measurements of 20 s intervals with the number of counts collected by the γ-counter corrected for background radioactivity. The numbers of counts in the first interval after the injection is set to 100%, and all further of the measurements are given as a percentage of this level.

2.8. Experimental design

Intravenous and subcutaneous experiments were carried out for both iodinated insulin aspart and non-iodinated insulin aspart (see Sections 2.2 and 2.3). Disappearance data were measured in the same study as the plasma concentrations after s.c. injection. The plasma concentrations after intravenous injection were measured in a separate study but in the same animals. There was less than 1 week between the i.v. and s.c. experiments. Blood samples were collected 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90 and 120 min after i.v. injection and 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 300 and 360 min after s.c. injection.

2.9. Deconvolution

The disappearance data are compared to the absorption into the blood. The absorption is calculated by deconvolution of the plasma concentrations after s.c. injection with the plasma concentrations after i.v. injection. The formula for convolution is:

$$c(t) = \int_0^t r(t-\tau)I(\tau)\,\mathrm{d}\tau$$

where $c(t)$ is the plasma concentrations after s.c. injection, $r(t)$ the plasma concentrations after i.v. injection, $I(t)$ is the absorption of drug into the blood. When the equation is solved for $I(t)$, it is deconvolution (Pedersen, 1980; Cobelli et al., 1987; Levitt, 2003). The calculations were made in WinNonlin® (Pharsight Inc.).

2.10. Data analysis

Data were analyzed individually for each pig. The absorption into the blood was calculated from plasma concentrations after i.v. and s.c. dosing in the same animal. The absorption into the blood was compared to the disappearance data measured in the same experiment as the s.c. plasma concentrations. The plasma concentrations after i.v. injection were fitted to one-, two- and three-compartment models, and the macro constants, for example, A, B, α and β values were used for deconvolution. A and B were corrected for differences between dose and weight of pigs of i.v. and s.c. injection according to: $A_{\mathrm{cor}} = A_{\mathrm{i.v.}} D_{\mathrm{s.c.}} \cdot W_{\mathrm{i.v.}} / D_{\mathrm{i.v.}} \cdot W_{\mathrm{s.c.}}$. The fractioned cumulative amount absorbed into the blood was subtracted from 1 to get the fraction remaining to be absorbed. The fraction remaining to be absorbed was compared to the fraction remaining at the injection site—disappearance data. The comparison of data was performed both by visual inspection and by a statistical analysis (see below).

2.11. Statistical analysis

Disappearance data and fraction remaining to be absorbed were individually fitted to first-order kinetics in WinNonlin®. The slope $-k_{10}$ of disappearance data was compared to k_{10} of fraction remaining to be absorbed with a F-test to test homogeneity of variance and a paired t-test to test if data were similar or not. Differences were considered statistically significant at p-level less than 0.05.

3. Results

3.1. Intravenous data

Plasma concentration profiles after intravenous injection were modelled with a one-, two- and three-compartment models. Analysis of i.v. concentration profile showed that a one-compartment model was too simple. The two- and three-compartment models were almost equally good for fitting the data. Therefore, it was decided that the two-compartment model

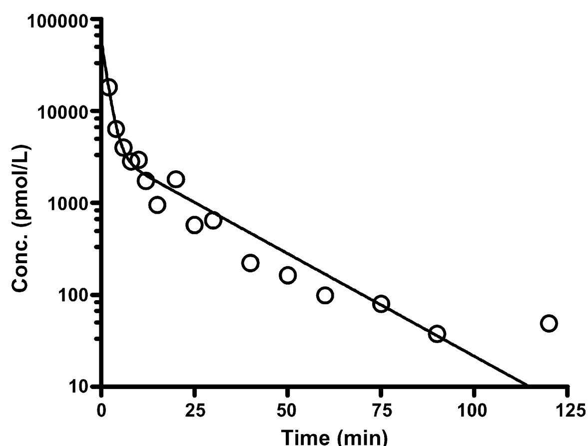

Fig. 1. Plasma concentration after i.v. injection and fit to a two-compartment model for one pig. The circles are the observed plasma concentrations and the line is the predicted plasma concentrations. The macro constants A, B, α and β from the fit were used for deconvolution.

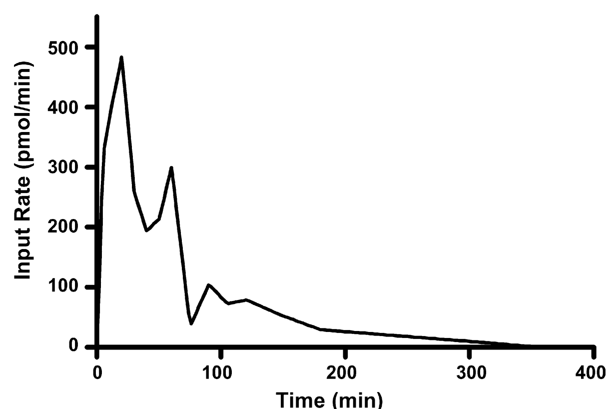

Fig. 3. Input rate calculated by deconvolution for the same pig as in Figs. 1 and 2.

was to be used, because it is the simpler of the two, and the %CV values for the two-compartment model also were considerably smaller than those for the three-compartment model due to fewer model parameters and more degrees of freedom. Furthermore a two-compartment model is also in accordance with what is seen in the literature for human insulin and porcine insulin in humans (Kobayashi et al., 1983; Nosadini et al., 1988). The averages of the %CV were less than 30% for A and B and less than 20% for α and β. A plot of the observed and the predicted plasma concentrations after intravenous injection for one pig fitted to a two-compartment model is shown in Fig. 1.

Fig. 2 shows the plasma insulin aspart concentration profile after s.c. injection, and the profile calculated by deconvolution. The s.c. data are used for deconvolution. The calculated input rate of insulin aspart into the blood as a function of time is shown in Fig. 3. The cumulated input is shown in Fig. 4. The cumulated input is made a fraction between 0 and 1 by dividing the cumulated curve by the total amount absorbed—the last point on the cumulated curve. The fractioned curve is subtracted from 1 to get the fraction remaining to be absorbed which is compared to the disappearance data (see below).

For the non-iodinated insulin aspart, two different concentrations (100 and 200 IU/mL) of the formulation were tested with six pigs in each group. The disappearance data for the two concentrations were fitted to first-order kinetics and the k_{10} values were compared statistically. The p-values were 0.74 for F-test and 0.12 for the t-test. The p-values for the k_{10} for the fraction remaining to be absorbed were 0.16 for the F-test and 0.80 for the t-test. Since there were no significant differences between the data from the two formulations they were pooled.

3.2. Disappearance versus absorption

Disappearance data and fraction remaining to be absorbed were initially compared by visual inspection. The mean ($n = 12$) of all disappearance data for non-iodinated insulin aspart and the mean of the fraction remaining to be absorbed for non-iodinated insulin aspart were plotted as a function of time in the same graph (Fig. 5). The fraction remaining to be absorbed is decreasing

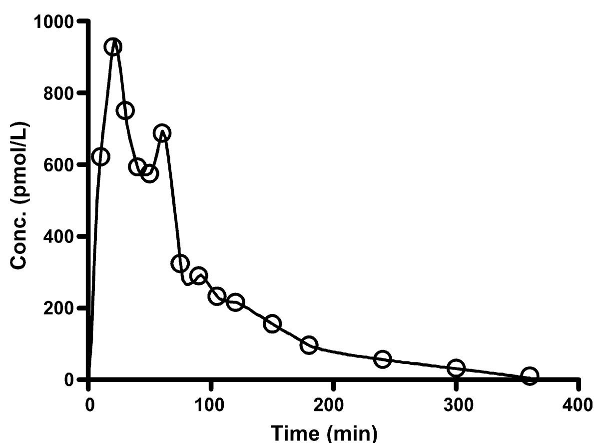

Fig. 2. Deconvolution for one pig. The circles are the observed plasma concentration after s.c. injection and the line is the concentrations profile calculated from deconvolution of the subcutaneous plasma concentrations with the intravenous plasma concentration.

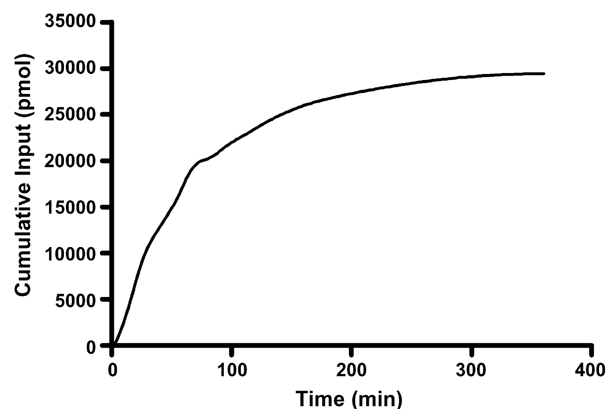

Fig. 4. The cumulative input from the same pig as previously shown. The curve shows the total amount absorbed as a function of time. All the values are divided by the total amount absorbed at the last time point to calculate the fraction absorbed. The fractions absorbed are subtracted from 1 to get the amounts remaining to be absorbed which can be directly compared to the disappearance data.

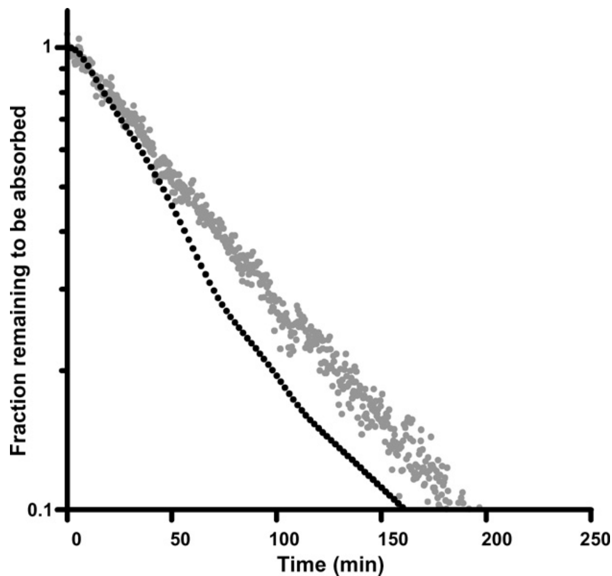

Fig. 5. Fraction remaining to be absorbed and disappearance of non-iodinated insulin aspart. The black circles are the mean fraction remaining to be absorbed calculated by deconvolution. The grey circles are the mean fraction remaining at the injection site from the disappearance data. The figure indicates that there is a difference between disappearance and fraction remaining to be absorbed and thereby a difference between ^{125}I-insulin aspart and normal insulin aspart.

faster than disappearance. The fraction remaining to be absorbed and the disappearance data are both measurements of the amount remaining at the injection site. When the fraction remaining to be absorbed is decreasing faster than disappearance of radioactive material, it means the absorption is faster for insulin aspart than for iodinated insulin aspart.

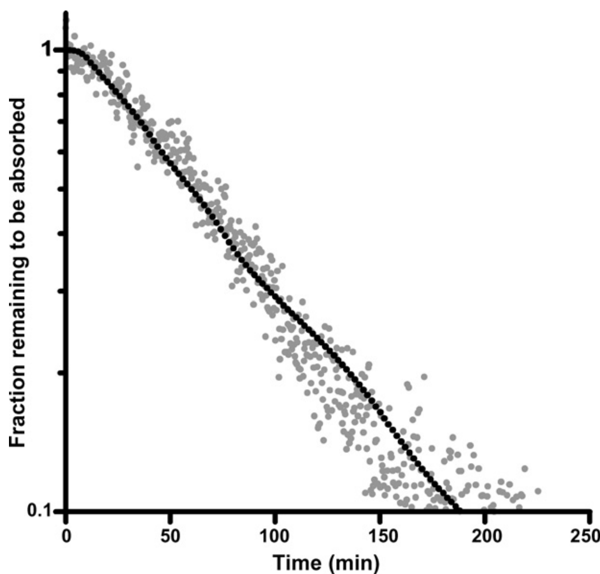

Fig. 6. Fraction remaining to be absorbed and disappearance of iodinated insulin aspart. The black circles are the mean fraction remaining to be absorbed calculated with deconvolution. The grey circles are the mean fraction remaining at the injection site from the disappearance data. The figure indicates that disappearance and fraction remaining to be absorbed are similar. The disappearance data are based on ^{125}I-insulin aspart and the absorption data are based on ^{127}I-insulin aspart (cold iodinated).

A comparable plot was made for iodinated insulin aspart ($n=7$), see Fig. 6. For iodinated insulin aspart the fraction remaining to be absorbed and disappearance were similar.

3.3. Statistical analysis

To compare disappearance and fraction remaining to be absorbed statistically, the disappearance data and the fraction remaining to be absorbed were fitted to first-order kinetics individually for each animal. All %CV for k_{10} were less than 3%. The k_{10} values were compared. The p-values from the F-test were 0.17 for non-iodinated insulin aspart and 0.34 for iodinated insulin aspart. This ensured homogeneity of variance. The p-values from the paired t-test were 0.0028 for non-iodinated insulin aspart and 0.63 for iodinated insulin aspart. The mean values of k_{10} are shown in Table 2.

4. Discussion

In this study, we have investigated the absorption of insulin aspart and iodinated insulin aspart based on the disappearance method and on measuring actual plasma insulin aspart levels in pigs. The absorption rate of iodinated insulin aspart was slower compared to that of non-iodinated insulin aspart. This could be due to either, a genuine difference in the absorption rate between iodinated and non-iodinated insulin aspart or bias related to the different methods used. To compare the to data types they were plotted in the same graph. The disappearance data and absorption data were plotted as the fraction remaining to be absorbed at the injection site versus time.

For the statistical comparison, the slopes of the individual first-order fits for the disappearance data and the absorption data were compared. The disappearance data were divided by their start value to get a fraction between 0 and 1. Due to the noise of disappearance data, the starting value was estimated by the data interpreter. The slopes were not affected due to all points were divided by the same value, the slopes were compared and not the intercepts. Also the data interpretation of the results did not affect the statistical analysis. The intercepts were adjusted to be around 1 for the visual inspection.

The disappearance data and the fraction remaining to be absorbed were compared. The total amount of insulin aspart was not the same for the two. The total amount of insulin in the disappearance data was the dose. The total amount of the fraction remaining at the injection site was the fraction absorbed of the dose. And as the bioavailability was different from 1, the two fractions are not equal. They were compared anyway because if disappearance data should be used as prediction for absorption, the two data sets should be proportional.

The intra-individual variability was reduced by measuring the radioactive disappearance and the plasma concentrations after the same s.c. injection. The dose was exactly the same even if some of the dose regurgitated when measured after the same injection. Also the variability from differences of the subcutaneous injection site was reduced. From a theoretical point, it is impossible to inject into the exact same type of tissue with regards to vascularisation and composition of tissue. By measur-

Table 1
Individual rate constants, k_{10} values of disappearance and fraction remaining to be absorbed for iodinated insulin aspart

Pig	Experimental day*	$k_{10\,disapp}$	$k_{10\,absorp}$
456	1	0.0120	0.0107
456	2	0.0124	0.0102
457	1	0.00957	0.0103
457	2	0.0160	0.0174
458	1	0.0130	0.0101
460	1	0.0148	0.0185
460	2	0.0161	0.0134

* To increase the number of experiments, the subcutaneously part was completed two times on different days.

ing both data types after the same injection this type of variability is reduced. The correlation of k_{10} values from the same injection was confirmed by investigating the k_{10} values. The $k_{10\,disapp}$ values varied from 0.0096 to 0.016 min^{-1}, and the $k_{10\,absorp}$ values between 0.010 and 0.019 min^{-1} for iodinated insulin aspart, but the difference was below 0.0037 between the parried k_{10} values (Table 1). From pig no. 457 it can also be seen, how different the absorption can be from two injections in the same pig. The absorption constants for day 1 and 2 were different but the absorption constants for disappearance and fraction remaining to be absorbed on the same day are anyway more similar.

The validity of using disappearance of iodinated insulin to predict absorption of insulin has been discussed before (Binder, 1969; Deckert, 1982; Berger et al., 1982; Berger and Joergens, 1985). The discussion is based on the following three assumptions: (1) iodinated insulin is absorbed like non-labelled insulin. (2) Insulin or labelled insulin is not degraded at the injection site. (3) Radioactivity counted externally is proportional to the non-absorbed amount of radioactivity (Binder, 1969). Especially the extent of degradation at the injection site has been debated. The present work is questioning the assumption that absorption of iodinated insulin and insulin is equal.

The ability of ^{125}I-insulin to predict insulin absorption has been studied before with porcine and bovine insulin (Binder, 1969) and human insulin (Deckert, 1982; Ribel et al., 1985). It has been shown that the plasma concentration time course for immunoreactive insulin and ^{125}I-insulin after s.c. injection are accepted to parallel. The relative rate of appearance of immunoreactive insulin into the blood was equal to the disappearance of ^{125}I-insulin from the subcutaneous depot (Ribel et al., 1985). The ratio between ^{125}I-insulin and insulin in excised subcutaneous tissue was also used to verify to which extent ^{125}I-insulin can be used as a predictor for insulin absorption. If ^{125}I-insulin and insulin have the same absorption constants, the ratio will be constant (Binder, 1969; Deckert, 1982). In another publication, it has been concluded that disappearance of ^{125}I-insulin is a relevant and biological sensible expression of insulin absorption, but the authors did observe an increase in the ratio of ^{125}I-insulin/insulin as a function of time (Deckert, 1982) which is to be expected if insulin absorption actually is faster than that of iodinated insulin.

The mean k_{10} values are almost equal for the three measurements of I-insulin aspart absorption, for the disappearance data for both iodinated and non-iodinated insulin aspart and the absorption of iodinated insulin aspart see Table 2. The absorption constant of iodinated insulin aspart is 20% less than the absorption constant of insulin aspart. From the experiments completed in the present work, it is evident that there is a statistical significant difference in the absorption rate of insulin aspart and iodinated insulin aspart.

Table 2
Mean k_{10} values of disappearance (disapp) and fraction remaining to be absorbed (absorp) for non-iodinated/iodinated insulin aspart

	$k_{10\,disapp}$	$k_{10\,absorp}$
Non-iodinated insulin aspart ($n = 12$)	0.0131	0.0166
Iodinated insulin aspart ($n = 7$)	0.0134	0.0129

Linear kinetics are required to perform deconvolution. The linearity of insulin kinetics has been discussed in the literature. The majority of the studies have shown linearity (Waldhausl et al., 1983; Cobelli et al., 1986; Ferrannini and Cobelli, 1987; Nosadini et al., 1988), but some studies have observed non-linear kinetics of insulin (Morishima et al., 1985; Thorsteinsson et al., 1986). In one study showing non-linear kinetics, clearance was reduced by 60% over a wide dose range (Morishima et al., 1985). These results have been challenged by others (Cobelli et al., 1986). Linearity has not been investigated in the present study. If insulin clearance is reduced with increasing dose the clearance after intravenous dose could be underestimated. Deconvolution has been performed, where the clearance obtained from the intravenous dose was increased by a factor two or three. This increase in clearance only induced a change of the calculated absorption slightly and did not change the results or conclusion in this study. The reason why a significant change of clearance does not affect the absorption rate is due to the fact that the plasma concentration profile after s.c. administration is covering a longer time span.

It could be argued from the non-iodinated insulin aspart study, that the difference between the disappearance data and the fraction remaining to be absorbed is due to degradation of ^{125}I labelled insulin aspart. After degradation, ^{125}I alone or as a fragment should be absorbed more slowly than ^{125}I-insulin aspart to explain why degradation could prolong disappearance results. However from the iodinated study, it is not possible that degradation prolongs the disappearance results. Because based on ELISA it is seen that the fraction remaining to be absorbed becomes similar to the disappearance results.

Previous publications have shown that iodinated insulin has the same absorption rate as insulin (Binder, 1969; Deckert, 1982; Ribel et al., 1985). This could be due to insulin and iodinated insulin having the same absorption or that the methods used were not sensitive enough to detect a relatively small difference in the absorption. A third explanation could be that two effects of insulin absorption are counterbalancing each other. The A14 tyrosine is involved in the hexamer formation by burial of the non-polar surface (Brange, 1994). If the iodination of the tyrosine hinders the hexamer formation, the hexamer will become less stable. A less stable hexamer will dissolve faster, and therefore, be absorbed faster (Brange et al., 1990). If the iodination

slows the absorption as seen for insulin aspart, the two effects could counterbalance each other for human insulin.

Disappearance is normally used for ranking of absorption of different compounds. Future studies will show whether the difference seen in this work only accounts for insulin aspart or if other peptides or proteins are affected by labelling with iodine as well. The observed difference in absorption for iodinated insulin aspart and insulin aspart is small. If other peptides or proteins do not show a more dramatic change of labelling, disappearance is still a valuable tool for ranking of absorption after s.c. injection in the early screening of compounds.

In conclusion, there is a statistical significant difference between the absorption rate for ^{125}I labelled insulin aspart (disappearance data) and for insulin aspart calculated from plasma samples. When ^{125}I labelled insulin aspart is compared to non-radioactive I labelled insulin aspart the absorption rate is similar. These results led to the suggestion that the absorption of iodinated insulin aspart is slower than the absorption of insulin aspart. This indicates that absorption rates of insulin analogues which are determined by the disappearance method will underestimate the true absorption rates. Further studies are necessary to elucidate if the absorption of other therapeutic proteins are affected by labelling with iodine.

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